Determination of Formalin Used in Various Fruits and Its Effects on nutritional composition.

Baha Uddin Ahammed Khasru

Roll: 0117/11

Reg: 00414

Session: 2017-2018

Semester: January - June 2019

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Food Chemistry and Quality Assurance

Department of Applied Chemistry and Chemical Technology
Faculty of Food Science and Technology
Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made.

.....

(Ms. Meherunnesa Chowdhury Sumy)

Supervisor

.....

(Md. Fahad Bin Quader)

Chairman of the examination committee

Department of Applied Chemistry and Chemical Technology

Faculty of Food Science and Technology

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

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June, 2019

Dedication

Dedicated to my beloved family and teachers

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TheAuthor

June, 2019

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List of Abbreviation

Words	Abbreviations
%	Percent
µg	Microgram
µl	Microlitre
A	Absorbance
Abs.s	Absorbance of the Standard
Abs.T	Absorbance of the Test Sample
AOAC	Association of Official Analytical Chemists
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Conc	Concentration
DCM	Dichloromethane
Dl	Deciliter
DoF	Department of Fisheries
FAO	Food and Agricultural Organization
FIQC	Fish Inspection and Quality Control
G	Gram
IFAD	International Fund for Agricultural
Mg	Milligram
mmol/L	Millimole/litre
NOSB	National Organic Standard Board
PABA	Paribesh Bachao Andolan
Ppm	Parts PerMillions
PRTC	Poultry Research&TrainingCenter
R.T	Room Temperature
Std	Standard
SD	Standard Deviation
THF	Tetra hydrofuran
USDA	United States Department of Agriculture
WHO	World health organization
WFP	World Food Programme

ABSTRACT

This experimental study was conducted to determine the formalin of the selected fruits including mango (*Mangifera indica*), malta (*Citrus x cinensis*) and papaya (*Caricapapaya*) collected from local market in Bangladesh and assess its effects on the physicochemical and nutritional parameters. It was found that local traders were used formalin to mango and malta slightly whereas formalin was not added in papaya by local traders. The lowest concentration of vitamin C were measured in mango without formalin (26.54 mg/100g), malta (49 mg/100g) and the lowest content was also found in the formalin treated mango (25.13mg/100g) and malta (38.52 mg/100g). Apparently protein, fat and carbohydrate content were found insignificantly ($P \geq 0.05$) in less amount in formalin contained in selected fruits. Like other nutrients, mineral contents of formalin found in fruits indicated fewer amounts than groups without formalin. But mineral content in mango with formalin was decreased insignificantly but in malta was not decreased significantly. So, it can be concluded that formalin influences the nutritional quality of fruits. Furthermore, it is hazardous to public health i.e. carcinogenic effects as it contains traces of arsenic and phosphorus and banned in many countries. Thus, we are at risk of short-term and long term health effects simply by eating fruits that are induced by this chemical. So, it is necessary to build awareness among fruit producers, traders and consumers against adulteration of formalin in the fruits.

Key words: Formalin, Nutritional quality, Food safety issues, Health hazard

Chapter-1

Introduction

1.1 Background

Food, one of the fundamental necessities of life, is required for growth and maintenance of our body Nutrition and health are two sides of the same coin and are therefore inseparable (Ali *et al*. 2004). It is increasingly being recognized as an indicator of development at national and international levels (FAO, IFAD and WFP, 2015). One works hard and earns to satisfy our hunger and relax later. But at the end of the day, many of us are not sure of what we eat. We may be eating a dangerous dye, sawdust, soap stone and aluminum foil and so on! Often, we invite diseases rather than good health. Nowadays foods are often adulterated (Ban *et al*., 2007). In hotels and restaurants stale and rotten foods are mixed with fresh food and served to the customers. Fish and vegetables are adulterated by putting on them chemicals and other preservatives in order to make them look fresh. Bakery and confectionery products are also prepared by using toxic substances and thus they get adulterated. Junk food contains harmful chemicals. Even fruits, milk and beverages are also adulterated (Chauhan *et al*., 2012; Dawood *et al*., 2014)).

In fact, all kinds of foods and food articles are adulterated by dishonest and greedy businessmen and shop keepers for quick and unearned profit. Adulterated foods are a serious health hazard. It is undoubtedly a social evil which can be regarded as the outcome of an interaction between a number of social, economic, technical and human behavioral factors. It is a manifestation of a sick society and can be regarded as a crime similar to other crimes like theft, burglary or murder. Like any other crime, food adulteration is expected to continue in our society as long as the existing factors which generate crime will continue (Gopalan*et al*., 2014). Food adulteration is an act of intentionally debasing the quality of food offered for sale either by the admixture or substitution of inferior substances or by removal of some valuable ingredient. Food adulteration takes into account not only the intentional addition or substitution or abstraction of substances which adversely affect nature, substances and quality of foods, but also their incidental contamination during the period of growth, harvesting,

storage, processing and distribution (Hoeberichts*et al*., 2012).

Food adulteration with poisonous chemical like formalin is widespread and regularly applied on fish, fruit, meat and milk that causes different types of cancers, asthma and skin diseases (Hossain *et al*., 2008). Coloring dyes, calcium carbide, urea, brunt engine oil and even some permitted preservatives are used in excessive amount that affect multiple organs of human body. Mostly it causes cancer like colon, peptic ulcer diseases, and chronic liver diseases including cirrhosis and liver failure, electrolyte imbalance and eventually kidney failure. Heart diseases, blood disorders and bone marrow abnormality are also detected. Chance of malignancy increases and neurological impairment or brain functions are also often compromised. Skin problems are frequently seen including allergic manifestation (Joseph *et al*., 2014).

Normally the contamination/adulteration in food is done either for financial gain or due to carelessness and lack in proper hygienic condition of processing, storing, transportation and are either Adulteration in food is normally present in its crudest form; prohibited marketing. This ultimately results that the consumer is either cheated or often become victim of diseases. Such types of adulteration are quite common in developing countries or backward countries. However, adequate precautions taken by the consumer at the time of purchase of such produce can make him alert to avoid procurement of such food (Kader, 2008).

Bangladesh faces the typical problem of using synthetic chemicals such as formalin to increase shelf life of fruits, fishes etc. In the markets of the city, an extreme percentage of summer fruit is sold which are preserved with toxic formalin such as mango, lichi, blackberry etc. Dishonest merchants are using ripening agent with food, including fruits to accelerate the ripening of climacteric fruits such as mango, banana, papaya, tomato and jackfruit. Some non-climacteric fruits are also exposed to artificial ripening agents (Kamruzzaman*et al*., 2016).

1.2 Significance of the study

Fruits play a fundamental role in nutrition and are a rich source of vitamins, minerals, dietary fibers, various important carotenoids (lycopene, β-carotene, xanthophyll etc) flavonoids, phenolic compounds and other phytochemicals (Schreiner and Huyskens-Keil, 2006).Owing to their antioxidant, anticarcinogenic and antimutagenic activities, carotenoids and other phytochemicals provide protection against chronic disease states, different types of cancers, muscular and cardiac vascular diseases and age related ailments (Kader, 2008; Vicente *et al*., 2009). Apart from regular consumption, different types of fruits have varying processing approaches for different applications. Fleshy fruits like apple, peach, pear, pineapple, watermelon and mango are commercially valuable as human food, eaten fresh and as jams, marmalade and other products. Fruits are also used in manufactured foods like cookies, muffins, yogurt, ketchup, puree, sauces, soup, salad, ice cream, cakes and many more.

Bangladesh is a tropical country with many fruit varieties throughout the year. The total availability of fruit per person per day is 200 g, which is much higher than the current consumption of 79 g per day per person in Bangladesh (BBS, 2014). About 5,118 thousand metric tons of fruit are produced each year in Bangladesh (BBS, 2017) and post-harvest losses of fruit and vegetables vary between 19-40% at different levels of the supply cycle. These costs of spoilages about 3000-3500 cror taka per year (Hassan, 2010) . To overcome this problem, different chemicals are used in an unofficial and illegal way; however, most of these chemicals are very harmful to human health. Formalin is one of the most common examples.

Formalin, a 40% solution of formaldehyde in water, is very poisonous and can cause various diseases such as cancer (Fischer, 2005). Traders often use this chemical as a preservative to make fruits and vegetables fresh for a longer period (Dhareshwar and Stella, 2008; Gatesoupe, 2002). The environmental group Paribesh Bachao Andolan (PABA) reported terrible results on the use of formalin in various foods in Dhaka. The group found that 100% of spaghetti and 90% of noodles were contaminated with formalin and other harmful chemical preservatives. In addition, 100% of citrus fruits, 95% of grapes, 91% of bananas, 82% of mango, 77% of dates, 60% of eggplants, 59% of apples and 20% of cucumbers have a certain amount of formalin or other chemicals during random sampling (Rahman et al., 2015; Guha and Bhuiyan, 2007).

This alarming situation is even worse for farmers and fruit traders, as they do not have other techniques to reduce spoilage or preserve the unsold product. Research continues to develop profitable preservation techniques and the delay in fruit ripening appears to be a promising alternative used in several countries (Watkins, 2008; Hayes, 2005).

1.3 Aim and specific objectives of the study:

1.3.1 Aim of the study:

- The overall aim of the study is to determine and assess the effects of formalin on the quality of mango, malta and papaya.

1.3.2 Specific objectives of the study

- To determine the formalin in ripened mango, malta and papaya which are collected from the selected study area.
- To assess the proximate composition (moisture, protein, fat, ash, carbohydrate) in mango and malta in which formalin is found.
- To find out effect of formalin on vitamin C content of selected fruits.
- To compare the nutritional composition between naturally ripened fruits and formalin found fruits.

Chapter 2

Review of Literature

2.1 Basic information about fruits

Being part of a balanced diet, fruits play a vital role in human nutrition by providing the necessary growth regulating factors essential for maintaining normal health. Increasing consumption of fruits and vegetables significantly reduces the incidence of chronic diseases such as cancer, cardiovascular disease and other age related diseases (Prakash et al., 2012). Fruits offer protection against free radicals that damage lipids, proteins and nucleic acids. Polyphenols, carotenoids (provitamin A), vitamins C and E present in fruits have antioxidant activity and the elimination of free radicals and play an important role in the prevention of many diseases (Prakash *et al*., 2012).

The fruits have provided a series of trace elements that protect the cell from oxidative cell damage as these minerals are the cofactor of enzymes. Zinc, copper and manganese are necessary for superoxide dismutase both in the cytosol and in the mitochondria. Iron is a component of catalase, a hemoprotein that catalyzes the decomposition of hydrogen peroxide (Machlin and Bendich, 1987). Small amounts of micronutrients (minerals and vitamins) are necessary for good physical condition along with energy, fat and protein. Sodium, potassium, iron, calcium and many trace elements together with vitamins and antioxidants are vital for the body. Fruits and vegetables, in particular the leaves, have considerable quantities of calcium, iron and potassium together with vitamin C (Bhattacharjee*et al*., 2007).

2.2 Mango production and nutritive value

Fruits are considered as ‘protective food’ and they play a significant role in human diet through the supply of required vitamins and minerals. Among the fruits, mango (*Mangiferaindica*) is one of the best tropical fruit in the world market due to its excellent flavor, attractive fragrance, beautiful colour, delicious taste and health giving properties; hence it is popularly called as “King of fruits” or “Apple of tropics”. The mango tree is from the anacardiaceae family and it was disseminated all over the world in the beginning of the sixteenth century, and there are currently around a thousand known varieties of mango (FAO, 2002).The global production of mango in 2010 was estimated to be about 35 million tons, accounting for nearly 50%

of world tropical fruit production (FAOSTAT, 2012). In Bangladesh 12,88,315 metric tons' mango is produced with in an area of 61,997 acres (BBS,2017).

The fruit of the mango is fleshy, generally sweet and varies considerably in size, shape, color, flavor and composition (FAO, 2002). Its characteristic orange yellow color is due to the presence of carotenoids. Like other tropical fruits, mango is seasonal, with a relatively short post-harvest shelf life due to its perishable nature. Mango is an excellent source of bioactive compounds such as provitamin A carotenoid, vitamin C and phenolic compounds, as well as dietary fibers (Lemmens*et al*., 2013; Pott et al., 2003; Sogi*et al*., 2012;), essential for nutrition and human health. It is known that these compounds have antioxidant properties and are essential for vision. It is also an excellent source of vitamin B6 (pyridoxine), vitamin C. Vitamin C develops resistance to infectious agents and also eliminates harmful free oxygen radicals. Vitamin B-6 or pyridoxine is necessary for the production of GABA hormone in the brain.

Table 2.1 Nutritive value of Mango (Mangiferaindica) per 100gm

Nutrients	**Nutrient value**
Energy	60 Kcal
Carbohydrates	14.98 g
Protein	0.82 g
Total Fat	0.38 g
Dietary Fiber	1.60 g
Folates	43 µg
Niacin	0.669 mg
Pyridoxine (vitamin B-6)	0.119 mg
Riboflavin	0.038 mg
Thiamin	0.028 mg
Vitamin C	36.4 mg
Vitamin A	1082 IU
Vitamin E	0.90 mg
Vitamin K	4.2 µg
Sodium	1 mg
Potassium	168mg

Calcium	11 mg
Phosphorus	14 mg
Iron	0.16 mg
Magnesium	10 mg
Zinc	0.09 mg

(Source: United States Department of Agriculture National Nutrient data base, 2018)

2.3 Malta production and nutritive value:

Malta (*Citrus x cinensis*) is one of the most popular fruits in Bangladesh. It is being cultivated commercially in different areas of Bangladesh such as Jessore, Thakurgaon etc. with a small amount of total production of 1000 metric tons. The majority of malta is imported. Bangladesh is the 7th importer of malta. A total of about 22 million kg maltas enters Bangladesh through sea and land ports. About 600000 kg maltas are sold and bought everyday (BBS, 2018). Malta is also a source of bioactive compounds such as phenolic compounds, dietary fiber which also strengthens stomach. Fresh malta is an excellent source of vitamins and minerals. 100 g of fruit contain 53.2 mg of vitamin C necessary for the synthesis of collagen in the body. It also contains small amount Vitamin-A (provides 11 micrograms per 100 g) and β-carotene levels (USDA, 2018).

Malta contains powerful natural antioxidant, folate, dietary fibre and other bioactive components, like carotenoids and flavonoids that prevent cancer and degenerative diseases (Ejaz*et al*., 2006).Citrus flavonoids contain compounds with anti-inflammatory activity due to the presence of regulatory enzymes (protein kinase C, phosphodiesterase, phospholipase, lipoxygenase, and cyclooxygenase) that control the formation of the biological mediators, responsible for the activation of endothelial cells and specialized cells involved in inflammation. Flavonoid inhibition of the immune and inflammation responses can be associated with their inhibition of these enzymes (Tripoli *et al*., 2007). Citrus flavonoids of malta can prevent câncer through selective cytotoxicity, anti-proliferative actions and apoptosis (Elangovan*et al*., 1994; Hirano *et al*., 1994). Flavonoids are antimutagenic, thus protects the DNA from damage by their ability to absorb ultraviolet light (Stapleton and Walbot*et al*., 1994). They neutralize free radicals that promote mutations when they are generated near DNA. This has been shown in mice body irradiated with c-ray (Shimoi *et al*., 1994).

Table 2.2 Nutritive value of Malta (Citrus x cinensis) per 100gm

Nutrients	Nutrient value
Energy	197 kJ (47 kcal)
Carbohydrates	11.75 g
Sugars	9.35 g
Dietary Fiber	2.4 g
Fat	0.12 g
Protein	0.94 g
Vitamin A equiv.	11 µg
Thiamine (B1)	0.087 mg
Riboflavin (B2)	0.04 mg
Niacin (B3)	0.282 mg
Pantothenic acid (B5)	0.25 mg
Vitamin B6	0.06 mg
Folate (B9)	30µg
Choline	8.4 mg
Vitamin C	53.2 mg
Vitamin E	0.18 mg
Calcium	40 mg
Iron	0.1 mg
Magnesium	10 mg
Manganese	0.025 mg
Phosphorus	14 mg
Potassium	181 mg
Zinc	0.07 mg
Water	86.75 mg

(**Source:** United States Department of Agriculture National Nutrient data base, 2018)

2.4 Papaya production and nutritive value

Papaya (*Carica papaya*) is one of the most important and popular fruits in Bangladesh with a considerable total production of 135655 metric tons produced in an area of 18,366 acres (BBS, 2018).It is an important horticultural commodity worldwide and plays a key role in the human diet.Fresh papaya has few calories. However, it is a source of several unique compounds that promote health, minerals

and vitamins that are essential for optimal health. 100 g of fruit provide about 43 calories; equivalent to that of apples (USDA, 2018). It does not contain saturated fats or cholesterol. However, it is a rich source of soluble and insoluble dietary fiber like pectin. The fruit is a rich source for different types of enzymes. Papain, vegetable pepsin present in good amount in unripe fruit is an excellent aid to digestion, which helps to digest the protein in food at acid, alkaline and neutral medium. The celiac disease patients, who cannot digest the wheat protein gliandin, can tolerate it, if it is treated with crude papain, papaya has the property of tenderizing meat. This knowledge is being put to use by cooking meat with raw papaya to make it tender and digestible (CSIR, 1992; Marotta F *et al.,* 2006).

Fresh papaya is an excellent source of vitamins and minerals. 100 g of fruit contain 60.9mg or 101% of vitamin C necessary for the synthesis of collagen in the body. It also contains good amount Vitamin-A (provides 950 IU per 100 g) and β-carotene which reduce cancer risks (USDA, 2018). The fermented papaya fruit is a promising nutraceutical as an antioxidant. It improves the antioxidant defence in elderly patients even without any overt antioxidant deficiency state at the dose of 9 g/day orally. The papaya lipase, a hydrolase enzyme tightly bonded to the water insoluble fraction of crude papain, is considered as a "naturally immobilized" biocatalyst (Moratta F *et al*., 2006). Papaya markedly increases iron (Fe) absorption from rice meal, which was measured in parous Indian women, using the erythrocyteutilization of radioactive Fe method. The black seeds edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper. In some parts of Asia the young leaves of papaya are steamed and eaten like spinach (Dominguez De Maria P *et al*., 2006).

Table 2.3 Nutritive value of papaya (Carica papaya) per 100 gm

Nutrients	**Nutrient value**
Energy	179 kJ (43 kcal)
Carbohydrates	10.82g
Sugars	7.82 g
Dietary Fiber	1.7 g
Fat	0.26 g

Protein	0.47 g
Vitamin A equiv.	47 µg
Beta-Carotene	274 µg
Thiamine (B1)	0.023 mg
Riboflavin (B2)	0.027 mg
Pantothenic acid (B5)	0.191 mg
Folate (B9)	38 µg
Vitamin C	62 mg
Vitamin E	0.3 mg
Vitamin K	2.6 µg
Iron	0.25 mg
Magnesium	21 mg
Manganese	0.04 mg
Phosphorus	10 mg
Potassium	182 mg
Zinc	0.08 mg
Water	88 g
Lycopene	1828 µg

(Source: United States Department of Agriculture National Nutrient data base, 2018)

2.5 Health hazards of formalin

Formalin is a chemical so harmful that its manipulators are not safe. Formalin has a bad effect on the eyes and nose. The eyes are more sensitive to formaldehyde exposure (formalin). The eyes, nose and throat are irritated by formaldehyde vapors at low levels of about 0.3 parts of formaldehyde per million parts of air (0.3 ppm). The lowest level at which many people can start to smell formaldehyde is around 0.05 ppm. Exposure from its gas or steam may cause irritation to the eyes, nose and respiratory tract, causing eye tearing, sneezing, headache and throat burning, coughing and difficulty breathing (Uddin *et al*., 2011). Formalin may appear as symptoms of nausea, vomiting and diarrhoea with bloody stools, blood from the urine, difficulty in breathing etc. When formaldehyde enters the body, it becomes formic acid, increases the acidity of the blood and causes difficulty in breathing. But its long term use has a delayed effect, such as damage to the kidneys, liver, brain,

bone marrow and defense of the body and fetus in pregnant women (Yeasmin*et al*., 2010b).

2.6 Status of the use of formalin in Bangladesh

Formaldehyde is a toxic material that can kill bacteria and viruses, as well as damage human cells. Food manufacturers sometimes add formaldehyde to foods like fish, meat, milk, etc. extend its useful life (Jaman*et al*., 2015). Many common foods like fruits naturally contain small amounts of formaldehyde. However, the excess of formaldehyde has been reported in many fruits as adulterated by different channels during marketing. Available reports suggest that fish dealers sometimes add or spray formalin into fish as they are transported to the internal marketing chain to prevent deterioration and increase shelf life (Yeasmin et al., 2010b). Studies conducted in different markets in the city of Dhaka (Hossain et al., 2008; Haque and Mohsin, 2009) and MymensinghSadar (Yeasmin et al., 2010a) has rationalized the incidence of formalin addition. A preliminary study conducted by the Department of Fisheries (DoF) Inspection and Quality Control Office (FIQC) reported that the fish available on the domestic market is contaminated with formalin, which varies between 0.5% -1 % (Yeasmin*et al*., 2010a). She also studied formalin treatment significantly increasing fish life, but reducing protein solubility and fish quality of food.

Mannan*et al*. (2006) reported that formalin is used uncontrolled in our country for the storage of basic products such as raw, semi-processed and prepared foods, including milk. Most users and consumers ignore the negative effect of this chemical reputation already acquired in this regard. According to Alano (2007), formalin and other preservatives are considered harmful because they act as free radicals that lead to oxidative stress, which has been implicated in a growing list of diseases, from cataracts to cancer.

Chapter-3

Materials and Methods

3.1 Location of the experimental area

The experiment was conducted during Marchto August 2019 in the laboratory of Department of Applied Chemistry and Chemical technology,Modern Food Testing Laboratory (Chattogram City Corporation), Poultry Research and Training Center of Chittagong Veterinary and Animal Sciences University (CVASU). The study was conducted for a period of six month from 1st March,2019 to 30th August,2019.

3.2 Sample collection

Mangos and Maltas were collected from five districts Rajshahi, Dhaka, Feni, Chittagong, Rangamati respectively for the laboratory analysis and papayas are

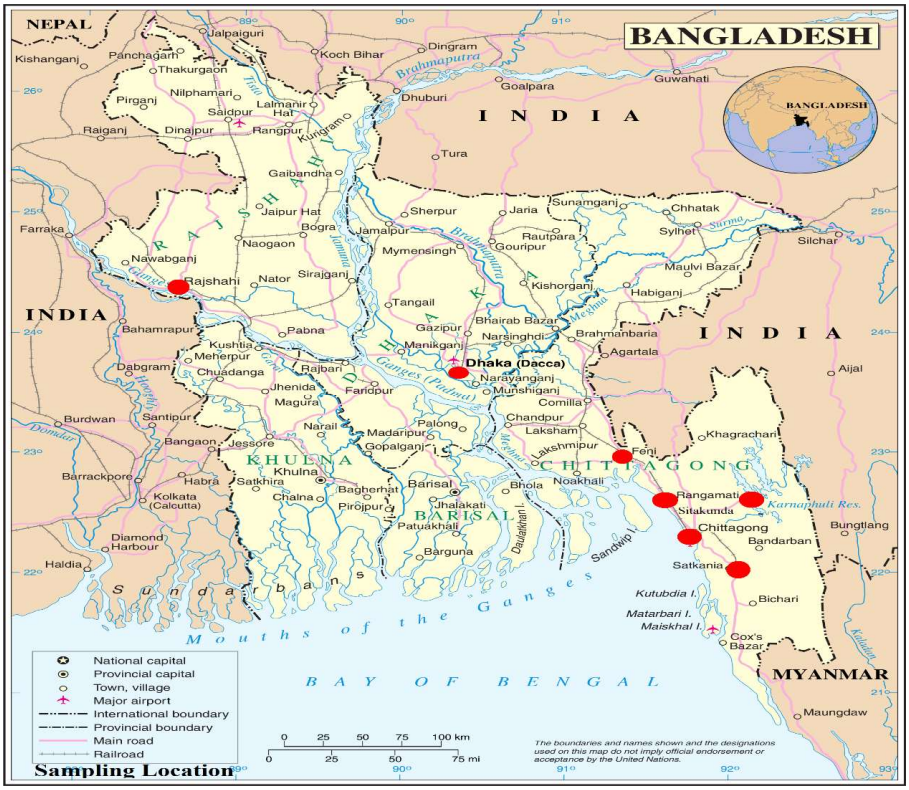

Figure 3.1: Mango, malta and papaya collection from different parts of Bangladesh

collected from five places of the district of Chittagong such as Feni, Sitakunda, Wireless moor of Khulshi Thana, Reazuddin Bazar of Kotwali Thana andSatkaniafor the laboratory analysis. From each market of each district or place 20 samples of mangoes, 20 of maltas, 20 of papayas were collected from five wholesellers randomly. 10 wholesale dealers were visited for each market and observed their preprocessing and processing their mangoes, maltas and papayasbefore marketing. So, total 150 numbers of samples were collected from five respected districts of Bangladesh.

3.3 Experimental design

Selected mangos, papayas and Maltas were divided into three experimental groups. After that each selected group is divided into five experimental groups based on regions for formalin test. Two to four samples were taken from each group for determination of physicochemical and nutritional composition. Each experiment was replicated for three times.

3.4 Formalin detection procedure

At first, the samples were taken and dipped into water for half hour. Then 10ml of water solution was taken in a test-tube using a dropper. Then one and two drops of phenyl hydrazine solution are added into test tube. It was mixed properly by stirring. After solution was added into it, if the yellow precipitation, the presence of formalin will be ensured. On the other hand, if the color of the solution remains sun changed or green, it indicates that there is no presence of formalin in the sample.

3.5 Determination of Total Soluble Solids

Total soluble solid contents were recorded with the help of a hand refractometer. Crushed fruit pulp was placed on the prism of the refractometer and readings were observed through the eye piece. For accurate measurement the readings taken were corrected for temperature variations to 20°C and results expressed as °Brix (Ranganna, 1991).

3.6 Determination of titratable acidity

A known weight of the fruit sample was crushed and taken in a 100 ml volumetric flask and the volume was made up by adding distilled water. After filtration, 10 ml of the filtrate was taken in a separate conical flask and titrated against 0.1 N sodium hydroxide using phenolphthalein as an indicator. The end point was determined by the appearance of a faint pink colour. Titratable acidity was calculated (Ranganna, 1991).

Calculation: The titratable acidity was determined by using the following calculations

$$\text{Titratable acidity (\%)} = \frac{T \times N \times V1 \times E}{V2 \times W \times 1000} \times 100$$

Where,

T = Titre value

N= Normality of NaOH

V 1 = Volume made up

V 2 = Volume of sample taken

E=Equivalent weight of acid

W=Weight of sample

3.7 Proximate composition analysis

Moisture, protein, fat, ash, crude fiber and carbohydrate contents of experimental groups were measured in triplicate according to AOAC methods. The moisture was measured by oven drying at 105^{o}C to constant weight (AOAC, 2016). The crude protein content was measured by the kjeldahl procedure (6.25 x N). For animal food, the protein factor is 6.25 and above. For animal food, protein factor is 6.25 and above. For plant origin food, it is below 6.25 because of presence of nitrogen from non-protein compounds. Total fat was extracted by the AOAC (2016) method using the soxhlet system. Ash was measured gravimetrically in a furnace by heating at 550^{o}C to constant weight (AOAC, 2016).

3.7.1 Moisture determination

At first weight of empty crucibles were dried for 1 hour at 100^{o}C and 5 gm of sample was placed on it. Then the crucible was placed in an air oven (thermostatically

controlled) and dried at room temperature of 100 to 105^{o}C for 24 hours. After drying, the crucible was removed from the oven and cooled in desiccators. It was then weighted with cover glass. The crucible was again placed in the oven, dried for 30 minutes, took out of the dryer, cooled in desiccators and weighed. Drying, cooling and weighing were repeated until the two constant weights were same.

Calculation

From this weight the percentage of moisture in food samples was calculated as follows:

$$\% \text{ Moisture content} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

3.7.2 Protein determination

Reagents used

A. Concentrated sulphuric acid (nitrogen free)
B. Digestion mixture
i. Potassium sulphate = 100g
ii. Copper sulphate = 10g
iii. Selenium di-oxide = 2.5g
C. Boric acid solution = 2% solution in water
D. Alkali solution = 400g sodium hydroxide in water and dilute to 1 liter.
E. Mixed indicator solution = Bromocresol; 0.1g and Methyl red: 2g dissolved in 250 ml ethyl alcohol
F. Standard HCL: 0.1 N

For estimation of protein, the steps were followed:

Digestion: 2g sample, 3 g digestion mixture and 25 ml H2S04was taken in aKjeldahl digestion flask. It was heated for 4 hours in a kjeldahl digestion and distillation apparatus. The digestion was completed when the color of the substance was pale yellow.

Distillation: After digestion 100 ml water ,100 ml 40% NaOH and glass blitzwere added to kjeldahl flask which containing about 10ml 2% boric acid and 2-3 drops

mixed indicator. About 100ml distillate was collected just before the distillation was stopped. The receiving flask was moved so that the tip of the distillating tube of the distillate. Some distillate was collected in this way to make sure the condenser tube was free from traces of ammonia.

Titration: The ammonia collected was titrated with 0.1 N HCl solutions andtitre value was recorded.

Calculation

The calculation of the percent of protein in the sample using protein factor 6.25.

$$\% \text{ Nitrogen} = \frac{(Ts - Tb) \times N \text{ of HCI} \times 14 \times \text{Volume made up the digest} \times 100}{\text{Wt of sample(gm)} \times \text{Aliquot of the digest taken} \times 100}$$

Where,

Ts = Titre volume of the sample (ml)

Tb = Titre volume of the blank (ml)

% Protein = Nitrogen × Protein factor

3.7.3 Fat Determination

The dried sample was transferred to a thimble and plugged the top of the thimble with a wood of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet apparatus. Approximately 75ml or more of anhydrous petroleum ether was poured through the sample in the tube into the flask. Top of the fat extraction tube was attached to the condenser. The sample was extracted for 16 hours or longer on a water bath at 70-80^{o}c. At the end of the extraction period, the thimble from the apparatus was removed and distilled of the petroleum ether by allowing it or collected in Soxhlet tube. The petroleum ether by allowing it or collected in Soxhlet tube. The petroleum ether was poured off when its volume, was nearly full. When the petroleum ether had reached small volume, was nearly full. When the petroleum ether had reached small, it was purer into a small, dry (previously weighed) beaker through a small funnel containing plug cotton. The flask was rinsed and filtered thoroughly using petroleum ether. The petroleum ether was evaporated on steam bath at low

temperature and was then dried at 100°c for 1hour, cooled and weighed. The deference in the weight gave the ether soluble materials present in the sample.

Calculation

The percent of crude fat was expressed as follows:

$$\% \text{ Crude fat} = \frac{\text{Wt of petroleum ether soluable material}}{\text{Wt of sample taken}} \times 100$$

3.7.4 Ash Determination

The oven dried sample was taken in a muffle furnace at 600°C for 4 hours after charging over an electric heater. The difference between oven dried matter and final weight represented the ash, which was expressed in percentage.

Calculation

It was calculated using the following formula

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Initial weight of dry matter}} \times 100$$

3.7.5 Determination of total carbohydrate

The carbohydrate content was determined by calculating the difference of Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a sum total of the other proximate components. Hence it was calculated using the formula below: % CHO = 100% - % (Protein + Fat + Fibre + Ash + Moisture content).

3.7.6 Crude Fiber Determination

Reagents required

A. 0.2 N sulphuric acidsolution

B. 10.0% Potassium sulphatesolution

Procedure

A 2 g sample was taken and transferred to the digestion flask with approximately 0.5 g asbestos. 200 ml of boiling sulphuric acid solution was

added and immediately was connected the digestion flask with leibig condenser and was boiled briskly for 30 min. During digestion care was taken to keep material remaining on the sides of the digestion flask without contact with solution. After completed the boiling, the flask was removed and filtrated through line in a fluted funnel and washed with boiling water until the washing are no longer acid. Sodium hydroxide solution was heated to boiling under reflux condenser and washed the residue from acid digestion back into the flask with 200 ml of boiling sodium hydroxide solution and connected the flask with reflux condenser and boiled for exactly 30 minutes. After 30 minute of boiling, the flask was removed and immediately filtered through filtering cloth in a fluted funnel washed with water and potassium sulphate solution. The residue was returned to the digestion flask thoroughly washing all residues from cloth will water. Then it was filtered into die Gooch crucible was prepared with thin but a packed layer of ignited asbestos. After washing of the residue in the Gooch crucible with boiling water, washing was repeated with approximately 15 ml of alcohol. The crucible with the contents was dried at °C to constant weight and then cooled in a desiccator and weighed. The contents were ignited of the crucible in an electric muffle furnace at dull red heat °C) until carbonaceous is destroyed (approximately 20 min). Then it was cooled in desiccators and again weighed.

Calculation

The loss in weight represents crude fiber

$$\% \text{ Crude fiber} = \frac{\text{Loss in weight noted}}{\text{Weight of sample taken}} \times 100$$

3.8Analysis of vitamin C

Vitamin C was analyzed by UV visible spectrophotometric method, as described by Rahman *et al*., (2007). A Shimadzu spectrophotometer with a pair of 1 cm quartz cells was used.

Chemicals and reagents required

1. Standard vitamin C (ascorbic acid) solution

2. 5% Metaphosphoric acid-10% acetic acid: 15g of solid metaphosphoric acid were dissolved in a mixture of 40 ml of glacial acetic acid and 450 ml of distilled water in a 500 ml volumetric flask. The solution was filtered and collected.
3. 10% Thiourea solution,
4. 2,4-Dinitrophenyl- hydrazine solution,
5. 85% Sulphuric acid.

Preparation of standard vitamin C (Ascorbic acid) solution

Stock standard solution containing 0.5 mg/ml of ascorbic acid was prepared in water by dissolving 0.05 g of ascorbic acid in 100 ml of water and stored in a glass stoppered bottle. Solutions of variable concentrations were prepared by diluting the stock solution in water.

Sample preparation

10 g blended sample was homogenized with about 50 ml of 5% metaphosphoric acid-10% acetic acid solution. Then it was quantitatively transferred into a 100 ml volumetric flask and was shaken gently until a homogeneous dispersion was obtained. Then it was diluted up to the mark by the 5% metaphosphoric acid-10% acetic acidsolution. Then the solution was filtered and the clear filtrate was collected for the determination of vitamin C in that sample.

Estimation of vitamin C procedure

Bromine water was added to the filtered sample solution to oxidize the ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea was added to it to remove the excess bromine and thus the clear solution was obtained.

Standard solutions of ascorbic acid (5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm) were prepared from 500-ppm stock solution of ascorbic acid by proper dilution. Then 1 ml of 2, 4- DNPH solution was added thoroughly with all standards and also with the oxidized ascorbic acid. For completion of the reaction, all the standards, samples and blank solution were kept at 37oc temperature for 3 hours in a water bath (thermostatic). After this incubation all of those were cooled in an ice bath and treated

with 5 ml of 85% H2SO4 with constant stirring. As a result, a colored solution was obtained whose absorbance was taken at 521 nm.

Reactions

a. Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.

b. L-dehydroascorbic acid reacts with 2,4- dinitrophenylhydrazine and produces an osazone which on treatment with 85% H2SO4 forms red colored solution.

3.9 Determination of mineral content in the experimental date fruits samples

The mineral content of date fruits was measured by atomic absorption spectrophotometry.

Atomic spectroscopy

This technique is applicable to most gas phase elements over a wide range of concentrations and involves detecting, measuring and analyzing radiation that is either absorbed or emitted from the atoms or ions of the element of interest (McMahon, 2007). It involves three techniques: Absorption, emission and fluorescence. In all the above, the sample is decomposed by intense heat into hot gases consisting of free atoms and ions of the element of interest (McMahon, 2007). As atoms are the simple stand purest form of matter and cannot rotate or vibrate as a molecule does when subjected to high energy radiation, electrons within the atom undergo transitions. The high energy radiation is commonly produced by

a. Flame in flame atomic absorption spectroscopy (FAAS)

b. Electro-thermal furnace in flameless graphite furnace atomic absorption spectroscopy (GFAAS)

c. Plasma in inductively coupled plasma-optical emission spectroscopy(ICPOES)

d. X-ray in X-ray fluorescence spectroscopy (XRF) ((LajunenandPaavo, 2007))

The above four belong to one of three major types of atomic spectroscopy namely absorption, emission and fluorescence (LajunenandPaavo, 2007).

Process of determination of mineral contents from sample by AAS

An atom is made-up of positively charged nucleus surrounded by a number of negatively charged particles necessary to provide neutrality. These atoms occupy discrete energy levels but it is possible for an electron to be moved from one level to another by introduction of energy. Such transitions will only occur if the available energy is equal to the difference between the two levels. Energy levels and the energies associated with electron transitions are unique for each element. When light (energy) of a characteristic wavelength enters an analytical system, outer shell electrons of corresponding atoms within the light path will be excited as energy is absorbed. The amount of light transmitted through the system from a source to the detector will be less. The loss of light is proportional to the number of atoms. The measurement of the radiation transmitted (using Beer-Lambert's law) in such a transition form the basis of AAS. Beer- Lambert's law relates absorbance, to the concentration of metallic atoms in the atom cell, c as follows

$\text{Log } T^{-1} = a\ b\ c$

Where

a is the absorptivity in grams per litre-centimetre

b is the atom width in centimeters

c is the concentration of atoms

3.10 Statistical analysis

Data were compiled in MS Excel. Raw data related to Moisture, Protein, Fat, Carbohydrate, Energy Content, Ash, Minerals, were tested for normality by using normal probability plot and analyzed for one-way ANOVA by using STATA (2017). Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was accepted at $p \leq 0.05$ for F-test.

Chapter – 4

Results

Fruits are in general rich sources of vitamins, minerals and phyto chemicals, as well as dietary fiber and polyphenols (Khoo*et al*., 2011; Masibo and He, 2009; Slavin and Lloyd, 2012). Formalin has negative effects on nutritional composition of fruits. The data recorded on mango, maltas and papayas during the course of investigation have been presented in this chapter along with appropriate table, figures and illustrations.

4.1 Detection of formalin in various fruits

A total of 25 samples of mango were tested for the experiment (Table 4.1) among which 16% samples of mango exhibits presence of formalin. In addition, 44% samples of malta shows positive result among 25 samples of malta. Furthermore, there is not found the presence of formalin in a total number of 25 samples of papaya.

Table 4.1: Determination of formalin in mango, malta and papaya by screening test

Type of fruits	Districts /place	Number of sample	Total No. of sample	Positive	Negative	Formalin Percentage (%)
Mango	Rajshahi	5	25	0	0	0
	Dhaka	5		2	3	40
	Feni	5		1	4	20
	Chittagong	5		1	4	20
	Rangamati	5		0	5	0
Malta	Rajshahi	5	25	2	3	40
	Dhaka	5		2	3	40
	Feni	5		3	2	60
	Chittagong	5		2	3	40
	Rangamati	5		2	3	40
Papaya	Feni	5	25	0	0	0
	Sitakunda	5		0	0	0
	Wireless	5		0	0	0
	Reazuddin Bazar	5		0	0	0
	Satkania	5		0	0	0

4.2Effect of formalin on physicochemical parameter of mango and malta

Table 4.2: Comparison on physicochemical parameter between fruits without and with Formalin

Sample	Variable	Without Formalin	With Formalin	p-value	Level of significance
Mango	TSS (°B)	15.83±1.04	13.67±0.58	0.16	NS
	Titratable acidity (%)	0.43±0.03	0.39±0.02	0.16	NS
Malta	TSS (°B)	10.83±0.76	9.67±0.76	0.31	NS
	Titratable acidity (%)	0.47±0.01	0.46±0.01	0.41	NS

[A]Results are means ± Standard deviation (SD) of triplicate

[B]NS=Not Significant

Table 4.2 represents the changes in TSS and titratable acidity of formalin found in mango and malta compared to formalin found in mango and malta. The TSS and titratable acidity of mango without formalin were 15.83±1.04°B and 0.43±0.3% respectively .In case of malta without formalin, the TSS and titratable acidity were 10.83±0.07°B and 0.47±0.01% respectively.

There was no significant ($P<0.05$) difference in the TSS and titratable acidity in mango and pineapple with formalin.

4.3Effect of formalin on proximate composition of mango and malta

Table 4.3 Comparison in proximate composition between fruits without and with Formalin

Sample	Variable (g/100g)	Without Formalin	With Formalin	p-value	Level of significance
Mango	Moisture	81.61±0.68	86.35±0.68	0.02	<0.05
	Protein	0.39±0.02	0.36±0.01	0.157	NS
	Fat	0.23±0.02	0.20±0.02	0.157	NS
	Carbohydrate	15.48±0.78	11.45±0.81	0.157	NS
	Ash	0.47±0.07	0.24±0.12	0.04	<0.05
	Crude fiber	1.61±0.02	1.58±0.02	0.157	NS
Malta	Moisture	83.65±0.21	86.61±0.13	0.00	<0.05
	Protein	0.85±0.05	0.67±0.08	0.306	NS
	Fat	0.07±0.01	0.06±0.01	0.572	NS
	Carbohydrate	13.83±1.27	10.26±1.15	0.306	NS
	Ash	0.22±0.03	0.08±0.04	0.010	<0.05
	Crude fiber	1.87±0.15	1.75±0.05	0.406	NS

[A]Results are means ± Standard deviation (SD) of triplicate

[B]NS=Not Significant; P< 0.05= Significant

Table 4.3 represents the changes in moisture, protein, fat, carbohydrate, ash of mango and malta after finding formalin. There was significant (P<0.05) difference in the moisture and ash in formalin found in mango and malta.

4.4Effect of formalin on vitamin C content of mango and malta

The result of vitamin C of mango without formalin along with mango with formalin were shown in the following figure 4.1. There was significant (P<0.05) difference in the vitamin C content of the selected fruits.

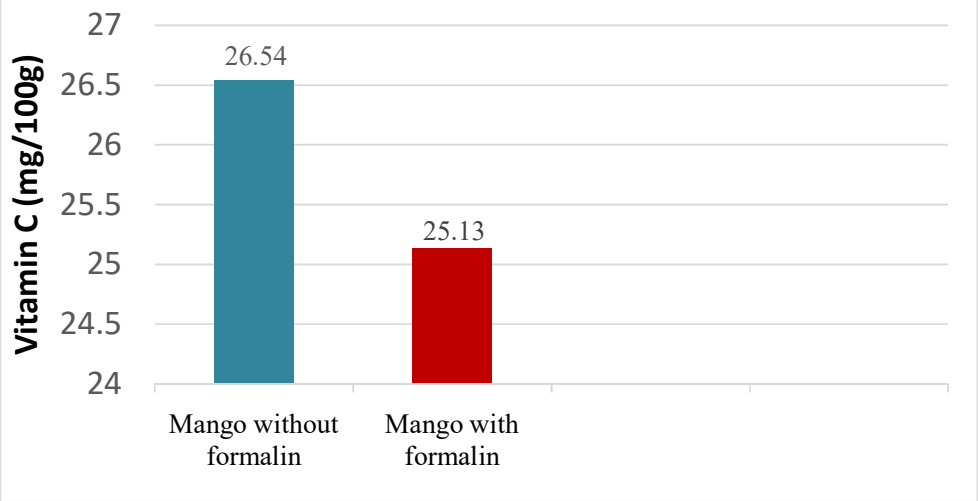

Figure 4.1: Comparison of vitamin C contents (mg/100g) between mango without and with formalin

The result of vitamin C of malta without and with formalin were shown in the following figure 4.1. There was not significant difference in the vitamin C content.

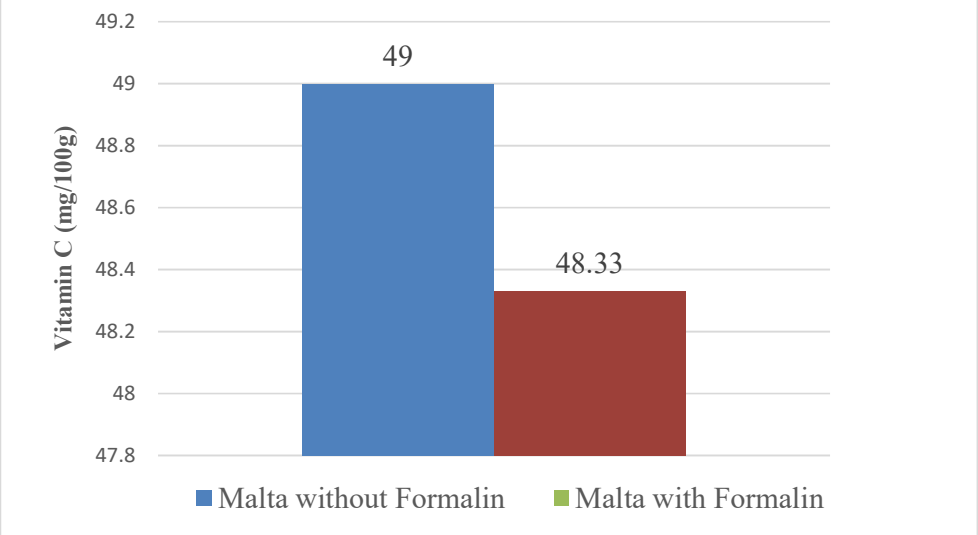

Figure 4.2: Comparison of vitamin C contents (mg/100g) between Malta without and with formalin

4.5 Effect of formalin on mineral contents of mango and malta

Table 4.4 Comparison of mineral contents between fruits without and with Formalin

Sample	Variable (mg/100 g)	Without Formalin	With Formalin	p-value	Level of significance
Mango	K	157.62±0.36	155.26±0.29	0.001	<0.05
	Ca	12.31±0.45	11.40±0.14	0.031	<0.05
	Mg	0.33±0.04	0.24±0.03	0.039	<0.05
Malta	K	176.33±1.53	174.67±2.08	0.306	NS
	Ca	37.17±1.26	36.67±1.53	0.406	NS
	Mg	0.88±0.08	0.78±0.10	0.406	NS

[A]Results are means ± Standard deviation (SD) of triplicate

[B] P< 0.05= Significant

Table 4.4 represents the changes in K, Ca and Mg of mango and malta after finding formalin. The K, Ca and Mg of mango without formalin were 157.62±0.36 mg/100g, 12.31±0.45 mg/100g and 0.33±0.04 mg/100grespectively. In case of naturally ripened pineapple the K, Ca and Mg value was 176.33±1.53mg/100g, 37.17±1.26mg/100g and0.88±0.08mg/100g respectively.

There was significant (P<0.05) difference in the K, Ca and Mg in mango with formalin but not significant in malta.

Chapter-5

Discussion

Fruits are in general rich sources of vitamins, minerals and phyto chemicals, as well as dietary fiber and polyphenols (Khoo et al., 2011; Masibo and He, 2009; Slavin and Lloyd, 2012). Formalin has health hazards and negative effects on nutritional composition of fruits. The data recorded on mango, pineapple and tomato during the course of investigation have been presented in this chapter along with appropriate table, figures and illustrations.

5.1 Detection of formalin of mango, malta and papaya by screening test

A total of 25 samples of mangoes were tested for the experiment (Table 1) among which 16 % of mango was found to be slightly affected by formalin. Formalin was not found in Rajshahi and Rangamati town. Formalin was found in one out of five sample at local market at town of Dhaka, Chittagong, Feni.

A total of 25 samples of maltas were tested for the experiment (table 1) among which 44% of mango was found to be slightly affected by formalin. Formalin was found in two out of five sample at local market at town of Rajshahi, Dhaka, Chittagong and Rangamati but 3 out of 5 sample at Rangamati.

A total of 25samples of papayas were tested for the experiment (table 1) among which formalin was not found i.e. formalin was not present in papaya at local market of Feni, Sitakunda, Wireless, Reazuddinbazar, Wireless and Satkania.

5.2 Effect of formalin on physicochemical parameter of mango and malta

In the present study, the TSS and titratable acidity content of formalin not found mango and formalin found mango as well as formalin not found malta and formalin found malta was represented at table 4.2.

The TSS content of formalin not found mango and formalin found mango were 15.83±1.04°B and 13.67±0.58°B found respectively. In case of malta, TSS content in formalin not found and formalin found were 10.83±0.76°B and 9.67±0.76°B respectively. The differences in TSS content were not significant ($P>0.05$).

The titratable acidity content of formalin not found mango and formalin found mango were 0.43±0.03% and 0.39±0.02% found respectively. In case of malta, titratable acidity content in formalin not found and formalin found were 0.47±0.01% and 0.46±0.01% respectively. Titratable acidity content was decreases in formalin found mango and malta than not-found but not significantly.

5.3 Effect of formalin on nutritional composition of mango and malta

The moisture content was increased in formalin found in mango and malta; other proximate compositions were decreased in formalin found in mango and malta presented in tables (4.3). The results were significant ($P<0.05$) for the moisture and ash in formalin found in mango and malta but carbohydrate, protein, fat, crude fiber content were decreased apparently ($P>0.05$).

The moisture content was significantly increased in formalin found in mango (86.35±0.68%) than not found (81.61±0.68%) and ash content significantly decreased in formalin found in mango (0.24±0.12) than not found (0.47±0.07). The content of protein, fat, carbohydrate, crude fiber was reduced in formalin treated mango (0.39±0.02 g/100g, 0.23±0.02 g/100g, 15.48±0.78g/100g and 1.61±0.02g/100g respectively) than not found (1.61±0.02 g/100g, 0.23±0.02 g/100g,15.48±0.78g/100g, 1.61±0.02 g/100g respectively).

In case of malta, the moisture content was significantly increased in formalin found (86.61±0.13%) than not found (83.65±0.21%) and ash content significantly decreased in formalin found (0.08±0.04 g/100g) than not found (0.22±0.03 g/100g). The content of protein, fat, carbohydrate, crude fiber was decreased in formalin found in malta (0.67 ± 0.08 g/100g, 0.06 ± 0.01g/100g, 10.26 ± 1.15g/100g,1.75 ± 0.05g/100g respectively) than not found (0.85±0.05g/100g, 0.07±0.01g/100g, 13.83±1.27 g/100g and 1.87±0.15 g/100g respectively).

Vitamin C was decreased significantly in mango and malta after finding formalin that was graphically represented at figure 4.2. In this research, vitamin C content were 26.54 mg/100g and 25.13 mg/100g respectively in formalin not found and formalin found in mango. This result is similar with Majaji and javannavar, (2018) who observed less amount of vitamin C in chemically treated (ethephone) mango than control. In case of malta, vitamin C content was found that 49 mg/100g without

formalin and 48.33 mg/100g with formalin. The result which is found from this study is similar with the is similar with Izundu*et al.,* (2016) who found higher vitamin C content in control than those pineapples treated by chemical agents.

Effects of formalin on mineral contents of mango and pineapple were shown on table 4.4.The analysis of data revealed that finding formalin significantly lower the mineral contents in mango and pineapple than not finding. In mango, the content of potassium calcium and magnesium were significantly reduced in formalin found (1.12±0.01 mg/100g, 155.26±0.29 mg/100g, 11.40±0.14 mg/100g, 0.24±0.03 mg/100g respectively) than without formalin (1.92±0.09 mg/100g, 157.62±0.36 mg/100g, 12.31±0.45 mg/100g, 0.33±0.04 mg/100g respectively). In malta, the content of potassium calcium and magnesium were significantly reduced in formalin found in (174.67±2.08 mg/100g,36.67±1.53 mg/100g,0.78±0.10 mg/100g, respectively) than without formalin (176.33±1.53mg/100g, 37.17±1.26mg/100g, 0.88±0.08mg/100g, respectively). This result is similar to Alano*et al*. (2007) who explained that minerals are oxidized due to formalin treatment.

Chapter-6

Conclusion

Fruits are inevitable to human development and remedy for various diseases highly valued in human diet mainly for vitamins and minerals which are highly valued in human diet mainly for vitamins and minerals. But now a day some dishonest and fraud growers as well as traders in Bangladesh are commercially using some chemicals for increasing shelf life and attractive skin color of fruits. So, the present study is conducted to assess the effects of formalin on nutritional value of mango, pineapple and tomato. This study showed that formalin is present in mango and malta but not in papaya and formalin in mango and malta induce significant negative alterations of the fruits. Apart from this, most of the nutrients are significantly found in low in amount in formalin treated mango and malta. Furthermore, eating of formalin treated fruits is most harmful and responsible for many life-threatening diseases in human beings. Considering its hazardous aspects, the use of formalin must be strictly monitored and controlled. It is not solely the responsibility of the government; the people must also become aware and avoid consuming contaminated fruits. It requires the combined involvement of the government agencies, policymakers, fruit-sellers, farmers, scientists and consumers for an effective solution to this matter. The guilty must be punished to prevent further spread of such harmful practice. Mass awareness and social resistance are the most effective deterrents to such dangerous activities.

Chapter-7

Recommendations and Future Perspectives

Formalin is used in fruits in recent years. This chemical is health hazardous. The national and international laws and regulations available to prohibit or control this chemical is also reported.

Recent study is conducted to investigate the effects of formalin on the nutritive value of mango, Malta and papaya. The research work needs to be carried out for other fruits available in markets especially for off season. Continuous research along with formalin is crucial to analyze effect of other chemicals like preservatives, insecticides and fungicides on the nutritional composition of different kinds of fruits and their hazardous effects on human health.

Now, fruit traders pick unripe fruits & use certain methods to increase the shelf life of them. It is important to develop new and better technique of application, which are environmentally safe, not harmful for human health and prevents direct contact of the substance with the fruits.

Although different laws and acts have been enacted to prohibit the illegal use of harmful artificial ripening agents in Bangladesh, the temptation for using formalin is evident among the farmers and the vendors because of the high demand of seasonal fruits and possible economic loss during the distribution. To resolve the problem from the root, the related technical and economic issues have to be addressed from the government level.

However, some limitations have in this study. Other vitamins and minerals such as thiamin, zinc, sodium, riboflavin, copper, iron etc. we're not analyzed in this study. Studies of potential carcinogenic hazards associated with fruits were not identified.

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Brief Biography

Baha Uddin Ahammed khasru passed the Secondary School Certificate Examination in 2007 and then Higher Secondary Certificate Examination in 2010. He also received Bachelor of Food Science and Technology (BFST) degree from Faculty of Food Science and Technology in 2015, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong. Now, he is a candidate for the MS degree in Food Chemistry and Quality Assurance under the Department of Applied Chemistry and Chemical Technology faculty of Food Science and Technology.

His research interests are in the areas of Product development, improving nutritional value of food, reduction of malnutrition among children.