Analysis of the Concentration and Formation Behavior of Naturally Occurring Formaldehyde Content in Food

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Abstract*—In recent years, in spite of being legally prohibited, formaldehyde is reported to be widely used as a food preservative to increase the shelf life of fruits and fishes in tropical countries. The hot and humid weather of the tropical countries tends to quickly deteriorate fruits, vegetables, fishes, meat and other food items. Formaldehyde is detrimental to human health and reported as a human carcinogenic. To prohibit formaldehyde application in foods, the regulatory bodies often conduct on the spot analytical tests to detect artificially added formaldehyde in food items. However, formaldehyde is ubiquitous in the environment and is present in many animal and plant species as a product of their normal metabolism. Formaldehyde can be found naturally in food items including fruits, vegetables, meats, marine fishes and crustaceans. The formation and concentration of natural occurring formaldehyde may vary according to food types and conditions. The naturally occurring formaldehyde may interfere in the detection of artificially added formaldehyde in foods. It is therefore important to study the concentration and formation mechanism of naturally occurring formaldehyde in food items. The objective of this study is to determine naturally occurring formaldehyde levels in different fruits, vegetables and (cow) milk samples. In addition, time dynamic behavior of the formation of endogenous formaldehyde content in food sample (banana sample; AAB genome of *Musa Spp.*) was studied in this study.

***Index Terms*—formaldehyde, methylated compounds, cancer Risk, formation dynamics**

I. Introduction

Formaldehyde is a flammable, highly reactive and readily polymerizing colorless gas at normal temperature and pressure. It has a pungent, distinct odor and may cause a burning sensation to the eyes, nose, and lungs at high concentrations [1], [2]. Formalin, a formaldehyde-water solution (40vol% or 37wt%), is a colorless liquid which is used as a biological preservative [3]. Recently, it has been reported that formalin is widely used in different countries as a chemical preservative for fruits and fishes [3]-[10]. There are direct and indirect health hazards associated with formaldehyde consumption. Consumption of formalin on a regular basis can be injurious to the nervous system, kidney and liver, and may cause asthma, pulmonary damage and even cancer [5], [11]-[13]. The use of formaldehyde as a food preservative is prohibited in most of the countries [5], [14]. To restrict the use of formaldehyde as a food preservative, the regulatory bodies often collect food samples from local markets to perform on the spot analysis, or to send it to the nearby analytical laboratory for the qualitative and quantitative analysis of formaldehyde added in food items [6], [15].

However, formaldehyde is naturally present in a wide variety of food items, such as: fruits and vegetables, meats, fish, crustacean and dried mushroom, as a common metabolic by-product [16]. In biological systems, formaldehyde is generated from different methylated compounds by demethylases, and from serine-glysine interconversion catalyzed by pyridoxal phosphate [17]. Naturally occurring formaldehyde content also varies according to the food items and food conditions. The presence of naturally occurring formaldehyde in food items may interfere in detecting artificially added formaldehyde in foods. Therefore, it is important to quantify the naturally occurring formaldehyde content in food items to estimate external formaldehyde dosage. This study aims to determine and quantify naturally occurring formaldehyde content in food items such as fruits, vegetables and milk. The formaldehyde contents of fresh fruits, vegetables and milk samples were determined using Nash's reagent and spectrophotometer [7], [8], [18]. In addition, the time dynamic behavior of naturally occurring formaldehyde formation in the food sample was analyzed. This study will help consumers and regulatory agencies by providing baseline values of naturally occurring formaldehyde contents in foods, and also will help them to understand the dynamic behavior of formaldehyde formation in foods.

II. Possible Health Hazards of Formaldehyde Consumption

The maximum allowable limit of formaldehyde in food is 100 ppm [6]. If consumed at a higher concentration, formaldehyde may cause damage to the gastro intestinal (GI) tract, kidney, liver and lungs, and may lead to cancer [2]-[19]. Formaldehyde, when ingested, exerts an irritant action upon mucous membranes, and after prolonged use

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appears to cause inflammatory changes in the liver and also in the kidneys, where a portion of it is excreted [20]. Furthermore, there are sufficient evidence linking formaldehyde with nasopharyngeal cancer [5], [21]. The international Agency for Research on Cancer (IARC) has classified formaldehyde as well as formalin as a Group 1 carcinogenic (Carcinogenic agents with sufficient evidence in humans) [3], [22]. Table I describes different health hazards caused by formaldehyde consumption.

TABLE I. HAZARDOUS EFFECTS OF FORMALDEHYDE CONSUMPTION [2], [3], [5], [13], [19]

Ingestion	Excessive ingestion can cause - • Severe pain with inflammation, ulceration and necrosis of the mucous membranes lining almost every internal organ; • Nausea; • Vomiting blood; • Diarrhea with bloody stool; • Blood from urine; • Gastrointestinal lesions; • Acidosis; • Vertigo and circulation failure; • Systemic effects include; • Metabolic acidosis; • CNS depression and coma; • Respiratory distress; • Renal failure; • Liver failure; • Cancer and tumor development; • Irreversible neurotoxicity
Cancer	The Department of Health and Human Services (DHHS) and the International Agency for Research on Cancer (IARC) have characterized formaldehyde as a human carcinogen based on studies of inhalation exposure in humans and laboratory animals. Formaldehyde has been linked to: • Nasopharyngeal cancer; • Gastrointestinal cancer; • Possible links to brain cancer and leukemia

III. MATERIALS AND METHODOLOGY

A. Sample Collection

Fruit and vegetable samples were collected from local market and fruit orchards in Dhaka, Bangladesh. Pure cow milk sample was collected from a dairy firm in Mymensingh, Bangladesh. Different UTH (cow) milk samples (AARONG, MILK-VITA, IGLOO and PRAN), and powdered (cow) milk samples (DANO, MARKS and DIPLOMA) were collected from local grocery shops in Dhaka, Bangladesh.

B. Chemicals and Reagents

Reagent grade ammonium acetate, acetic acid, potassium hydroxide, nitric acid (Merck, Germany) andacetyl acetone (Loba-chemie, India) was used in the experiments. Ultra-high purity de-ionized water (18.2MΩ.cm, Purite, UK) was used for dilution. Whatman 42 filter paper was used to filter the sample solutions. Nash's Reagent was used as an indicator to detect the absorbance (415nm) of formaldehyde in sample solutions [7]. Nash's reagent was prepared by diluting 18.75g ammonium acetate in a 100ml erlenmeyer flask with an addition of 0.38ml of acetic acid and 0.25ml of acetyle acetone. The total volume was made 62.5ml by adding de-ionized water. Nash's Reagent is light sensitive, hence, it was kept in an air tight dark-glass reagent bottle at room temperature [7, 10]. Fresh Nash's reagent was prepared for every experiment. 0.1N potassium hydroxide and 0.1N nitric acid were used to adjust the pH of the distillate to be in range of 6.0 to 6.5 [7]. A calibrated Hanna Pocket Sized pH Meter Hi96107 was used to check the pH and a Shimadzu UV-VIS 2600 spectrophotometer was used to measure the absorbance.

C. Sample Preparation and Formaldehyde detection

Fruit and vegetable samples were peeled off, cut into small pieces, and blended with water in 1:10 ratio. Using a clean cloth as sieve; the fruit juice was separated from the residual solids and then filtered using Whatman 42 filter paper. UTH milk samples were used directly and powdered milk samples were prepared by diluting solid powder with water in 1:2 ratio; the milk sample was then filtered. The filtrate (fruits, vegetables and milk) was diluted 100 times. The pH of the diluted samples was kept in around 6-6.5 [7, 10]. 5 ml of controlled pH sample was taken in a test tube and equal amount of Nash reagent was applied. The mixture was kept at 60 ℃ in a water bath for 10-15min and then cooled down to room temperature. The formaldehyde content was then measured using a spectrophotometer.

IV. RESULTS AND DISCUSSIONS

A. Naturally Occurring Formaldehyde Content in Fruits and Vegetable Samples

Experimental results of naturally occurring formaldehyde contents in different fruit and vegetable samples are presented in Table II and Fig. 1. The experimental results of banana, grape, apple, pear, carrot, radish, cucumber and tomato were found compatible with the reported values [23]. No reported data of natural occurring formaldehyde were found for pomegranate, pomelo fruit, pineapple, ripe papaya, green papaya, and lemon. The experimental results provide the baseline data for the above food items.

B. Formaldehyde Content of Milk Samples

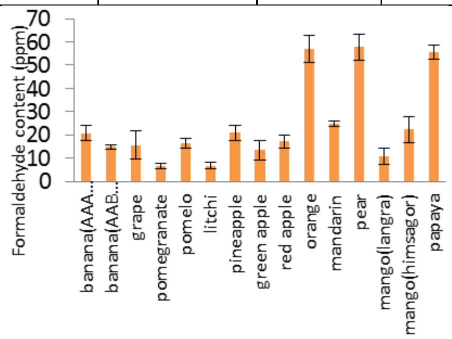
Table III and Fig. 2 represent the formaldehyde contents found in pure cow milk sample and other UTH and powdered (cow) milk brands. The experimental results for pure cow milk (5.28±3.47ppm) was compatible with reported values (3.3 ppm) [23], [24]. The experimental results show that formaldehyde content in UTH milk and powdered milk samples were high compared to that of pure milk (58.79 to 187.75 ppm).The possible reason for higher formaldehyde content in commercial milk samples are dosing of formaldehyde during milk processing, preservation and/or packaging to improve the shelf life, or, conversion of milk ingredient to primary aldehyde during milk processing [25]-[27].

C. Time Dynamic Behavior of Formaldehyde formation in Foods

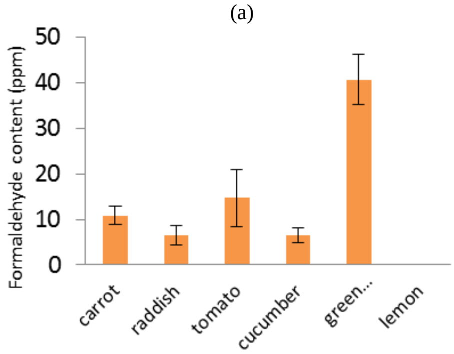
The time dynamic behavior of the formation of formaldehyde naturally in foods was investigated in this study. To understand the time dynamic behavior, the formaldehyde content and pH of banana samples (AAB genome of *Musa Spp.*) were measured for 3 days. Fig. 3 represents the time dynamic behavior of endogenous formaldehyde content in banana samples (AAB genome of *Musa Spp.*).

TABLE II. NATURALLY OCCURRING FORMALDEHYDE CONTENT OF DIFFERENT FOOD ITEMS (ST. DEV. FOR N=5)

Fruit Items		**Vegetable Items**	
Samples	**Formaldehyde content (ppm)**	**Samples**	**Formaldehyde content (ppm)**
Banana (AAA genome of *Musa Spp.*)	20.68 ±3.17	Carrot	10.86 ±2.05
Banana (AAB genome of *Musa Spp.*)	14.77±1.08	Radish	6.44 ±2.19
Grape	15.68 ±6.23	Tomato	14.67 ±6.21
Pomegranate	6.65 ±1.05	Cucumber	6.44 ±1.64
Pomelo fruit	16.33 ±2.24	Green papaya	40.65±5.57
Litchi	6.67 ±1.39	Lemon	0
Pineapple	20.83 ±3.05		
Green apple	13.41 ±4.05		
Red apple	17.18 ±2.69		
Orange	56.89 ±5.72		
Mandarin orange	58.34 ±3.94		
Pear	57.69 ±5.47		
Mango (Langra)	10.8 ±3.31		
Mango(Himsagor)	22.4 ±5.64		
Papaya	55.7±3.03		



(a)



(b)

Figure 1. Naturally occurring formaldehyde content of different fruits and vegetable items; (a) fruits, and (b) vegetables (error bars for n=5 samples)

TABLE II I. FORMALDEHYDE CONCENTRATION IN MILK SAMPLES (ST. DEV. FOR N=5)

Samples	**Formaldehyde content (ppm)**
Pure Milk	5.28 ±3.47
DANO Powder Milk	129.38 ±3.13
DIPLOMA Powder Milk	194.08±6.64
MARKS Powder Milk	90.65±3.54
PRAN UTH milk	69.94 ±7.13
IGLOO UTH milk	187.75 ±3.11
AARONG UTH milk	64.68 ±5.01
MILK VITA UTH milk	58.79±6.64

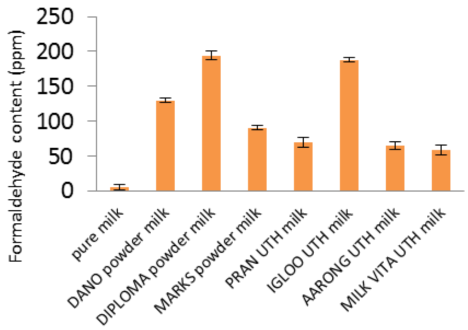


Figure 2. Formaldehyde content of milk samples (error bars for n=5 samples)

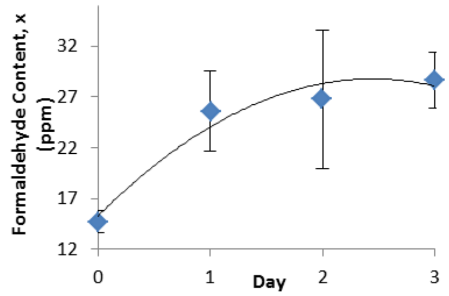


Figure 3. Time dynamic behavior of naturally occurring formaldehyde content in Banana (error bars for n=5 samples)

It was found that the formaldehyde content in banana samples gradually increased with time (Fig. 3). The possible reason of the gradual increase of formaldehyde content is the formation of *S*-adenosyl-L-methionine (SAM) during banana ripening, which is associated with endogenous formaldehyde production [17], [28]. Banana is a climacteric fruit, which is associated the with increase in respiration and ethylene production during the ripening process [29]. SAM, a major methyl donor in cells, is associated with the biosynthesis of ethylene [30], [31]; it has been reported that, during ripening process, the SAM level increases in climacteric fruits [30]. In addition, the pH value of banana sample changes from 4.5 (t = 0) to 5.2 (t = 3 days) during the ripening process, which indicates a decrease in acid content [32]. Formaldehyde formation from methylated compounds can be represented by the following reaction (equation 1) [33]:

$$Methylated\ compounds \xrightarrow{Demethylase} Formaldehyde \tag{1}$$

$t = 0$	a	0
$t = t$	$a\text{-}x$	x

The order of the kinetics of the above reaction (Equation 1) can be described as [33]:

$$\text{First order: } 2.303\times\log(a-x) = -Kt + c \quad (2)$$

$$\text{Second order: } 1/(a-x) = Kt + c \quad (3)$$

The experimental results of Fig. 3 were plotted following the kinetic equations (Equations 2 and 3); it was found that the endogenous formaldehyde formation in banana followed second order kinetics (Equation 3) with a rate constant (K) 0.2332 $mole^{-1}day^{-1}$ (per mole of methylated compound per day) (Fig. 4).

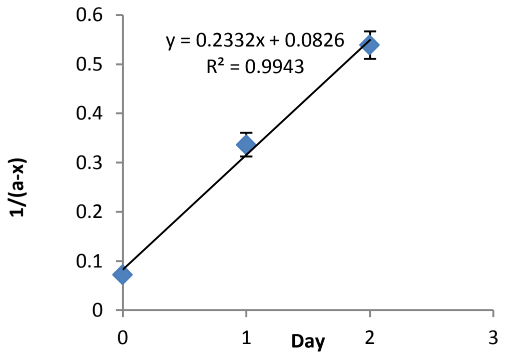


Figure 4. Kinetics of naturally occurring formaldehyde formation in Banana (error bars for n=5 samples)

V. Conclusion

In the tropical countries, formaldehyde is reported to be used as a food preservative. However, because of possible health issues, using formaldehyde as a food preservative is prohibited in most of the countries. Formaldehyde is naturally present in food items such as fruits, vegetables and milk. Therefore, it is important to know the concentration of naturally occurring formaldehyde in food items to determine any external formaldehyde dosage in food samples. This study offers baseline data of formaldehyde content naturally found in different food items. In this study, the naturally occurring formaldehyde content of fifteen fruit samples, six vegetable samples and eight milk samples were analyzed. According to the European Food Safety Authority (EFSA) the daily exposure to formaldehyde from food should not exceed 100 mg/kg (100 ppm) food per day [6]. Formaldehyde content of fruit samples ranged between 6 to 58 ppm; for vegetables the range was in between 0 (zero) to 41 ppm; and for pure cow milk sample the formaldehyde concentration was about 5 ppm. These values are in accordance with other published results, and less than the maximum daily exposure set by EFSA [6], [23]. However, the formaldehyde content of the seven commercial (cow) milk samples were found higher than that of pure (cow) milk sample; in three commercial milk samples the formaldehyde level exceeded the daily exposure limit set by EFSA. Addition and/or formation of formaldehyde during milk processing and preservation are the possible reasons to have high formaldehyde concentrations in commercially available milk samples. Further study is required to analyze the possible reasons to have high formaldehyde concentration in commercial milk samples and associated health effects. In addition, the time dynamic behavior of the formation of endogenous formaldehyde in banana samples was analyzed. The reaction kinetics showed that the endogenous formaldehyde production in banana sample follows second order reaction kinetics, and is likely to be related to the concentration of methylated compounds present in the banana sample. Methylated compounds such as *S*-adenosyl-L-methionine (SAM) play an important role in many biochemical reactions in plants, such as: the biosynthesis of polyamines and plant hormone ethylene [30], [31]. However, the dynamics and the key factors of the formation of formaldehyde in other food items could be different depending on the molecular structure and aging pattern of the food items.

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