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Concentration and formation behavior of naturally occurring formaldehyde in foods

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Abstract

Background: In recent years, formaldehyde is reported to be widely used as a food preservative to increase the shelf life of fruits and fishes in tropical countries. Formaldehyde is detrimental to human health. Hence, use of formaldehyde as a food preservative is legally prohibited in most of the countries. To regulate 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. This 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.

Results: In this study, the formaldehyde contents of food samples were determined using spectrophotometric technique. The naturally occurring formaldehyde contents of a wide range of fruit, vegetable, milk, poultry, mutton and meat samples were determined. In addition, formaldehyde contents of processed food items, such as: cooked beef and poultry, beverages, and commercially available UHT milk and powdered milk samples, were also assessed and analyzed. The naturally occurring formaldehyde contents of fruit, vegetable, milk, poultry, mutton and meat samples were found up to 58.3, 40.6, 5.2, 8.2, 15.2 and 8.5 ppm, respectively. Formaldehyde contents of commercially available UHT milk, powdered milk, beverages, cooked beef and poultry were found up to 187.7, 194.1, 21.7, 4.3 and 4.0 ppm, respectively. This study also analyzed the time dynamic behavior of the formation of endogenous formaldehyde content of banana (AAB genome of *Musa* spp.), mandarin and beef.

Conclusions: The experimental results provide a baseline data of natural occurring formaldehyde content of the analyzed food items. The formation behavior of formaldehyde may vary according to food types, storage temperature, storing time, and aging pattern of the food items. The findings of this study will be useful for the consumers, researchers, legal authorities and other stakeholders working on food safety and preservation.

Keywords: Formaldehyde, Methylated compounds, Natural formation, Health risk, Formation kinetics

Background

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 eyes, nose, and lungs at high concentrations [1–3]. Formalin, an aqueous solution of formaldehyde (37–40 wt%), is a colorless liquid which is used as a biological preservative [4]. Recently, it has been reported that formalin is widely used in different tropical countries as an artificial preservative for fruits, vegetables and fishes [4–13]. There are direct and indirect health hazards associated with formaldehyde and formalin 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 cancer [6, 14–16]. The use of formaldehyde as a food preservative is prohibited in most of the countries [6, 13, 17–21]. 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 food samples to the nearby analytical laboratory for the qualitative and

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quantitative analysis of formaldehyde in food items [7, 19, 22]. However, formaldehyde is naturally produced in a wide variety of food items, such as: fruits and vegetables, meats, fish, crustacean and dried mushroom as a common metabolic by-product [23]. In biological systems, formaldehyde is generated from different methylated compounds by demethylases, and from interconversion of glycine and serine that is catalyzed by pyridoxal phosphate [24]. Naturally occurring formaldehyde content also varies according to food types and food conditions.

The presence of naturally occurring formaldehyde may interfere in detecting artificially added formaldehyde in foods. Thus, it is important to quantify the naturally occurring formaldehyde content in foods to estimate external formaldehyde dosage. Limited scientific information is available regarding the levels of naturally occurring formaldehyde in foods [25]. This study aims to identify and quantify naturally occurring formaldehyde content in a wide range of food items such as: fruits, vegetables, milk and meats. In addition, formaldehyde contents of processed food items, such as: cooked beef and poultry, beverages, and commercially available UHT and powdered milk samples, were also assessed and analyzed. The formaldehyde contents of food samples were determined using spectrophotometric technique [8, 9, 26]. The time dynamic behavior of naturally occurring formaldehyde formation in the food samples was also analyzed in this experimental study. This study will help consumers, nutritionists, scientists, legal authorities and other stakeholders by providing a baseline data of naturally occurring formaldehyde contents in foods, and also to help them understanding the dynamic behavior of formaldehyde formation in foods.

Possible health hazards of formaldehyde consumption

There is no set standard for the daily intake of formaldehyde from food; however, the World Health Organization (WHO) estimated it to be in the range of 1.5–14 mg/d (mean 7.75 mg/d) for an average adult [25], and according to the European Food Safety Authority (EFSA), the daily oral exposure to formaldehyde from the total diet should not exceed 100 mg formaldehyde per day [7, 27]. If consumed at a higher concentration, formaldehyde may cause damage to the GI tract, kidney, liver and lungs, and may lead to cancer [2, 4, 6, 16, 28]. Formaldehyde, when ingested, exerts an irritant action upon mucous membranes and may cause inflammatory changes in the liver and kidneys [29]. In addition, there is evidence linking formaldehyde with nasopharyngeal cancer [6, 30]. The international Agency for Research on Cancer (IARC) has classified formaldehyde (as well as formalin) as a Group 1 carcinogen [4, 31]. Table 1 describes different health hazards caused by formaldehyde consumption.

Table 1 Hazardous effects of formaldehyde [2, 4, 6, 16, 25]

Interactions	Possible health hazards
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 and Irreversible neurotoxicity
Inhalation	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 and Possible links to brain cancer and leukemia

Methods

Sample collection

Fresh fruit, vegetable and meat samples were collected from local markets (Dhaka). Pure milk sample was collected from the local dairy firm (Mymensingh). Different UTH (cow) milk samples (AARONG, MILK-VITA, IGLOO and PRAN), powdered (cow) milk samples (DANO,MARKS and DIPLOMA) and beverage samples (Instant and brewed coffee, COCA COLA, CLEMON and VITA MALT) were collected from local grocery shops (Dhaka).

Chemicals and reagents

Reagent grade 37% formaldehyde solution (Merck KGaA, Germany), ammonium acetate (Merck, Germany), acetic acid (Merck, Germany), potassium hydroxide (Merck, Germany), nitric acid (Merck, Germany), acetyl acetone (Loba-chemie, India) and trichloroacetic acid (EMPLURA grade, Merck, Germany) were used in the experimental study. Ultra-high purity de-ionized water (18.2 MΩ.cm, Purite, UK) was used for dilution and solution preparation. Whatman 42 filter paper was used to filter the sample solutions. In this study, freshly prepared 10% (wt%) trichloroacetic acid (TCA) was used for extraction of formaldehyde from meat samples. Freshly prepared Nash reagent was used as an indicator to detect the absorbance (415 nm) of formaldehyde in sample solutions [8]. Nash reagent is light sensitive and was kept in an air tight dark-glass reagent bottle at room temperature [8, 11]. 0.1 N potassium hydroxide and 0.1 N nitric acid were used to adjust the pH (6.0–6.5) of the distillate [8]. A pH meter (HANNA Instruments, USA, HI2211) was used to check the pH, and a Shimadzu UV–VIS 2600 spectrophotometer was used to measure the absorbance.

Sample preparation

Fruit and vegetable samples were peeled off, cut into small pieces, and blended with water in 1:10 ratio; the juice was separated from the residual solids using a clean cloth as sieve and then filtered using Whatman 42 filter paper. Fresh milk, UTH milk and beverage samples (except instant and brewed coffee) were used without filtration. Powdered milk samples and coffee (instant and brewed) samples were prepared by diluting the solid with water in 1:2 ratio; followed by filtration using Whatman 42 filter paper. The filtrates of fruit, vegetable, milk and coffee samples were diluted to 100 times. The pH of the diluted samples was kept within 6–6.5 [8, 11]. For the preparation of meat and lever samples, 10 g of each sample was cut into small pieces and blended with equal weight of water. After fine blending, the weight of the sample was taken and equal amount of 10% TCA was added. The sample was kept for homogenization [32]. After homogenization was done, it was filtered through Whatman 42 filter paper.

Time dynamic study

The time dynamic behavior of naturally occurring formaldehyde in foods was investigated in this study. To understand the time dynamic behavior of natural formation of formaldehyde, the formaldehyde contents of banana (AAB genome of *Musa* spp.) and mandarin samples were measured for 3 days; for beef sample, formaldehyde contents were measured for 8 weeks. During this study, beef sample was kept in frozen storage for 8 weeks (at a temperature of − 5 °C) while banana and mandarin samples were kept in a normal refrigerator for 3 days at a temperature of 4 °C. Sample preparation of the frozen items and the measurement of formaldehyde contents were carried out at room temperature following the same process mentioned in the previous section.

Detection method

The pH of freshly prepared diluted fruit, vegetable, milk and beverage samples was adjusted between 6 and 6.5 (with potassium hydroxide or nitric acid) [8, 11]. For the meat samples, 5 ml sample of the TCA extract was added to 5 ml of water and then adjusted to pH between 6 and 6.5. The solution volume was made up to 25 ml with water. Then, 5 ml of ready samples was added to 5 ml of Nash Reagent followed by heating in a water bath for 10–15 min at 60 °C, and cooling under running tap water. The formaldehyde contents of the above samples were measured using spectrophotometer (Shimadzu UVVIS 2600).

For the spectrophotometric detection of formaldehyde content, a calibration curve was generated by plotting absorbance of known formaldehyde concentration (0–10 ppm) prepared from a stock solution of 37% formaldehyde. Formaldehyde solutions of known concentrations (0–10 ppm) were added to Nash reagent to get the respective absorbance reading at 415 nm. The curve obtained by plotting formaldehyde concentrations in aqueous solutions against absorbance is shown in Fig. 1.

Results and discussions

Naturally occurring formaldehyde content in fruits and vegetable samples

Experimental results of naturally occurring formaldehyde contents of different fruit and vegetable samples are presented in Table 2 and Fig. 2. The experimental results of banana, grape, apple, pear, plum, beetroot, cabbage, cauliflower, potato, onion, kohlrabi, carrot, radish, cucumber and tomato were found compatible with results reported by Centre for Food Safety, Hong Kong [30]. No reported data of naturally occurring formaldehyde were found for pomegranate, pomelo fruit, pineapple, ripe papaya, sapodilla, guava, olive, amla, bangi fruit, green papaya, plantain and lemon; therefore, the experimental results provide the baseline data for the above food items.

Formaldehyde content of milk samples

Table 3 and Fig. 3 represent the experimentally obtained results of formaldehyde contents in pure (cow) milk, commercial UTH and powdered (cow) milk samples. The experimental results for pure cow milk (5.2 ± 3.5 ppm) were compatible with reported value (3.3 ppm) [30, 33]. The experimental results show that formaldehyde

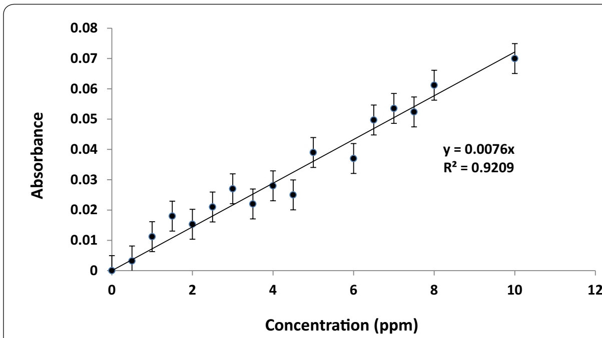

Fig. 1 Calibration curve showing the relation between absorbance and concentration of formaldehyde in aqueous solutions at 415 nm (error bars indicate SD for $n = 5$ samples)

Table 2 Naturally occurring formaldehyde contents in different fruits and vegetables (SD for *n* = 5 samples)

Serial no.	Fruit items		Vegetable items	
	Samples	**Formaldehyde content (ppm; avg ± SD)**	**Samples**	**Formaldehyde content (ppm; avg ± SD)**
1	Banana (AAA genome of *Musa* spp.)	20.7 ± 3.1	Carrot (*Daucus carota*)	10.8 ± 2.1
2	Banana (AAB genome of *Musa* spp.)	14.8 ± 1.1	Radish (*Raphanus sativas*)	6.4 ± 2.2
3	Grape (black; *Vitis vinifera*)	15.7 ± 6.2	Tomato (*Solanum lycopersicum*)	14.7 ± 6.2
4	Pomegranate (*Punica granatum*)	6.7 ± 1.1	Cucumber (*Cucumis sativus*)	6.4 ± 1.6
5	Pomelo fruit (*Citrus maxima*)	16.3 ± 2.2	Green papaya (*Carica papaya*)	40.6 ± 5.5
6	Litchi (*Litchie chinensis*)	6.7 ± 1.3	Lemon (*Citrus limon*)	Below detection limit
7	Pineapple (*Ananas comosus*)	20.8 ± 3.0	Beetroot (*Beta vulgaris*)	37.5 ± 2.1
8	Green apple (*Malus domesticus*)	13.4 ± 4.0	Cabbage (*Brassica oleracia var.capitata*)	8.1 ± 6.3
9	Red apple (*Malus domesticus*)	17.2 ± 2.6	Cauliflower (*Brassica oleracia var.botrytis*)	30.8 ± 4.5
10	Orange (*Citrus sinensis*)	56.9 ± 5.7	Potato (*Solanum tuberosum*)	16.5 ± 3.8
11	Mandarin orange (*Citrus reticulata*)	58.3 ± 3.9	Plantain (*Musa paradisiacal*)	38.9 ± 3.3
12	Pear (*Pyrus communis*)	57.7 ± 5.5	Onion (*Allium cepa*)	10.5 ± 5.5
13	Mango (Langra; *Mangifera indica*)	10.8 ± 3.3	Kohlrabi (*Brassica oleracia gongylodes*)	36.2 ± 5.4
14	Mango (Himsagor; *Mangifera indica*)	22.4 ± 5.6		
15	Ripe papaya (*Carica papaya*)	55.7 ± 3.0		
16	Guava (*Psidium guajava*)	33.3 ± 3.2		
17	Sapodilla (*Manilkara zapota*)	11.5 ± 3.2		
18	Olive (*Olea europaea*)	56.6 ± 4.2		
19	Amla (*Phyllanthus emblica*)	8.7 ± 1.2		
20	Plum (*Prunus domestica*)	10.3 ± 1.0		
21	Bangi fruit (*Cucumis melo*)	15.1 ± 2.8		

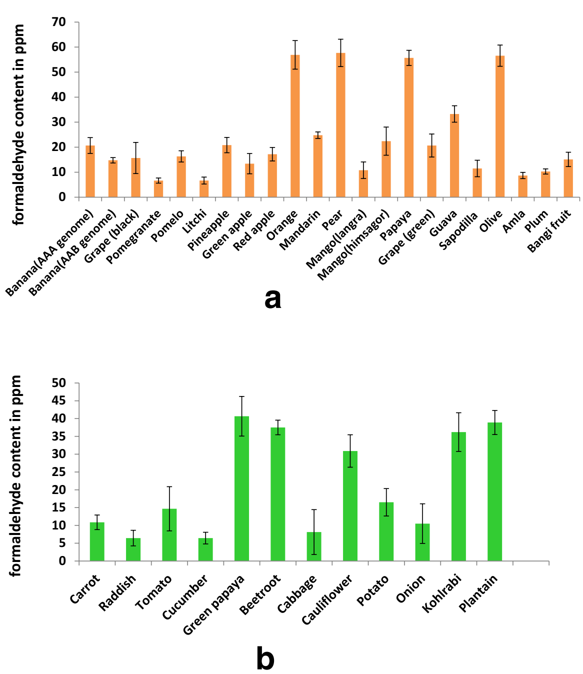

Fig. 2 Naturally occurring formaldehyde contents of different fruits and vegetable items; **a** fruits, and **b** vegetables (*n* = 5 samples, error bar indicates SD)

Table 3 Formaldehyde concentrations in different milk samples (SD for *n* = 5 samples)

Serial no.	Samples	Formaldehyde content (ppm; avg ± SD)
1	Pure milk	5.2 ± 3.5
2	DANO powder milk	129.3 ± 3.3
3	DIPLOMA powder milk	194.1 ± 6.6
4	MARKS powder milk	90.6 ± 3.5
5	PRAN UTH milk	69.9 ± 7.1
6	IGLOO UTH milk	187.7 ± 3.1
7	AARONG UTH milk	64.6 ± 5.1
8	MILK VITA UTH milk	58.7 ± 6.6

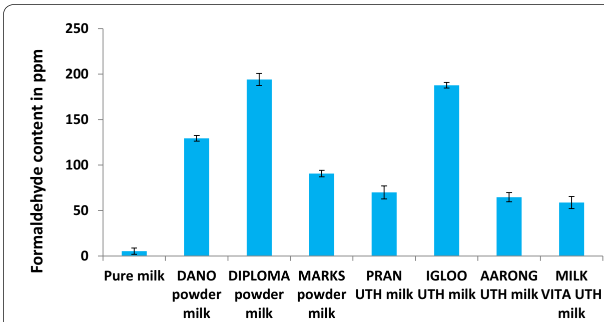

Fig. 3 Formaldehyde content of milk samples (*n* = 5 samples, error bar indicates SD)

content in UTH milk and powdered milk samples were higher (58.7–187.7 ppm) than that of pure milk sample. Possible explanations 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 [34–36].

Formaldehyde content of meat samples

Figure 4 and Table 4 represent experimentally obtained results for naturally occurring formaldehyde in meat and lever samples. Values obtained for poultry (8.2 ± 1.0) and beef (8.5 ± 0.6) are slightly higher than the reported values, which are 2.5–5.7 and 4.6, respectively [30]. Generally formaldehyde is introduced in ruminant feeds either as a preservative agent or as a reagent used to dietary components from ruminal degradation [33]. It is reported that significantly higher concentration of formaldehyde was obtained from the fresh muscle tissue of calves consuming 0.10% formalin-treated whey, whereas the muscle tissue of controlled calves or those consuming whey containing 0.05% formalin exhibited lower formaldehyde concentration [37]. Therefore, formalin-treated diet could be a probable reason of obtaining comparatively higher concentration of formaldehyde than the reported values. There was no reported value found for formaldehyde content in mutton. Hence, the experimental result serves as baseline data for mutton.

In cases of cooked poultry and beef, a significant drop in formaldehyde concentration was observed (Table 4, Fig. 4). Poultry and beef samples were minced and cooked in water for 6 min at 70 °C. Reduction in formaldehyde content in cooked samples could be due to the volatile characteristics of formaldehyde at high temperature (50 °C or above) [38, 39].

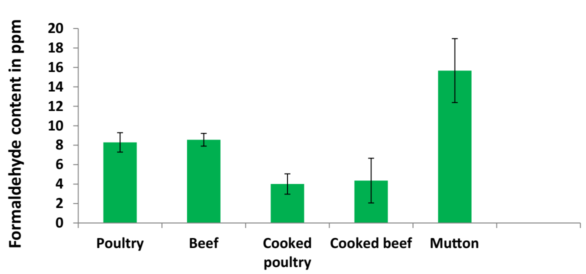

Fig. 4 Formaldehyde content of meat samples ($n = 5$ samples, error bar indicates SD)

Table 4 Formaldehyde concentrations in meat samples (SD for $n = 5$ samples)

Serial no.	Samples	Formaldehyde content (ppm; avg ± SD)
1	Poultry	8.2 ± 1.0
2	Beef	8.5 ± 0.6
3	Cooked poultry	4.0 ± 1.1
4	Cooked beef	4.3 ± 2.3
5	Mutton	15.2 ± 3.2

Formaldehyde content of beverage samples

Figure 5 and Table 5 represent the formaldehyde levels found in different beverages. The results obtained were compatible with the reported data [30]. Formaldehyde was found at slightly higher in instant coffee than in brewed coffee. This slightly greater value suggests that formaldehyde might escape from coffee during brewing [40]. There was no literature value found for formaldehyde content in malt beverages.

Time dynamic behavior of formaldehyde formation in foods

During the investigation of time dynamic behavior of naturally occurring formaldehyde in foods, banana (AAB genome of *Musa* spp.), mandarin and beef samples were analyzed. Beef sample was kept in frozen storage for eight weeks (at a temperature of − 5 °C) while banana and mandarin samples were kept in a normal refrigerator for 3 days at a temperature of 4 °C. The formaldehyde contents were measured at room temperature. Figure 6 represents the time dynamic behavior of endogenous formaldehyde contents in banana (AAB genome of *Musa* spp.), mandarin and beef samples.

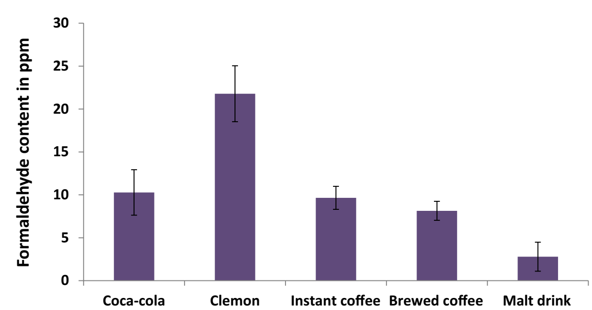

Fig. 5 Formaldehyde content of beverage samples ($n = 5$ samples, error bar indicates SD)

Table 5 Formaldehyde concentrations in beverage samples (SD for $n = 5$ samples)

Serial no.	Samples	Formaldehyde content (ppm; avg ± SD)
1	Coca-cola	10.2 ± 2.6
2	Clemon (transparent)	21.7 ± 3.2
3	Instant coffee	9.6 ± 1.3
4	Brewed coffee	8.1 ± 1.1
5	Malt drink (VITA MALT)	2.7 ± 1.6

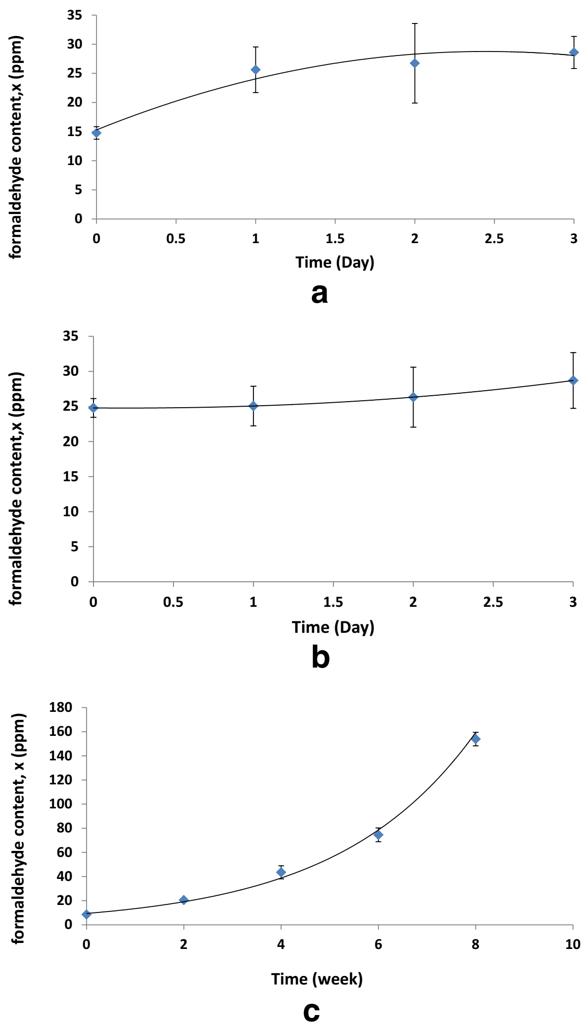

Fig. 6 Time dynamic behavior of naturally occurring formaldehyde content in **a** banana (AAB genome of *Musa* spp.), **b** mandarin and **c** beef ($n = 5$ samples, error bar indicates SD)

It was found that the formaldehyde content in banana sample gradually increased with time (Fig. 6a). The possible reason of the gradual increase in formaldehyde content is the formation of *S*-adenosyl-L-methionine (SAM) during banana ripening, which is associated with endogenous formaldehyde production [24, 41]. Ethylene is produced during the ripening process of banana [42]. SAM, a major methyl donor in cells, is associated with the biosynthesis of ethylene [43, 44]. It has been reported that, during ripening process, the SAM level increases in climacteric fruits [43]. In addition, the pH value of banana sample changes from 4.5 (at $t = 0$) to 5.2 (at $t = 3$ days) during the ripening process, which indicates a decrease in acid content [45].

Mandarin sample also exhibited gradual increase in formaldehyde content with time (Fig. 6b). The possible explanation could be the continuous formation of formaldehyde in acidic condition [46]. Mandarin is a non-climacteric and strongly acidic fruit. So in this acidic condition, acid hydrolysis of N-, O- and S-methoxy compounds takes place and increases the formaldehyde content gradually [47]. Other potential precursors of formaldehyde formation are various sulfur compounds present in fruits, for example, 1,2,4-trithiolane, 1,2,4,5-tetrathiane and dimethyl disulfide which can also undergo degradation to form formaldehyde [48].

A slow increase in formaldehyde content was observed in frozen beef sample (Fig. 6c). Formaldehyde accumulation during the frozen storage of meat could be a possible reason [30, 49]. Formaldehyde might be formed during the aging and deterioration of flesh [50]. It was reported that proteins of muscle undergo chemical and physical changes during frozen storage which may result in, loss of quality, change in flavor, odor and color; most of which changes are caused by the production of formaldehyde in the muscle [51]. It was also reported that the accumulation of formaldehyde and the resulting deterioration of different meat products during frozen storage are primarily caused by the enzymatic activity of trimethylamine oxide aldolase (TMAOase) [49]. The amount of formaldehyde formed depends mainly on the temperature of frozen storage and storing time [52].

Formaldehyde formation from methylated compounds can be represented by the following reaction (Eq. 1) [53]:

$$\text{Methylated compounds} \xrightarrow{\text{Demethylase}} \text{Formaldehyde} \quad (1)$$

$$\begin{array}{lll} \text{At } t = 0 & a & 0 \\ \text{At } t = t & a{-}x & x \end{array}$$

The order of the kinetics of the above reaction (Eq. 1) can be described as [53]:

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

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

The experimental results of Fig. 6 were plotted following the kinetic equations (Eqs. 2 and 3); it was found that the endogenous formaldehyde formation in banana, mandarin and beef followed second-order kinetics (Eq. 3) with a rate constant (K) 0.2332, 0.083 $ppm^{-1}day^{-1}$ and 0.010 $ppm^{-1}week^{-1}$ (0.0014 $ppm^{-1}day^{-1}$), respectively (Fig. 7a–c). Table 6 represents the rate constants obtained from graphs in tabulated form.

Conclusion

Formaldehyde is naturally present in different food items. It is important to know the concentration of naturally occurring formaldehyde in foods to determine any

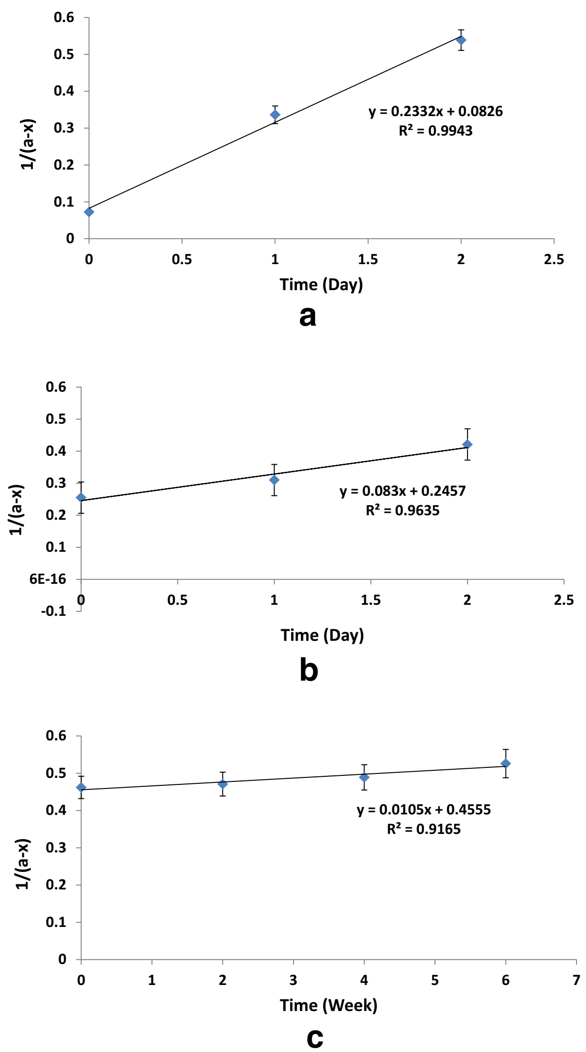

Fig. 7 Kinetics of naturally occurring formaldehyde formation in **a** Banana (AAB genome of *Musa* spp.), **b** mandarin and **c** beef ($n = 5$ samples, error bar indicates SD)

Table 6 Rate constants for banana, mandarin and beef samples

Serial no.	Samples	Rate constant (K; $ppm^{-1}day^{-1}$)
1	Banana (AAB genome of *Musa* spp.)	0.2332
2	Mandarin	0.083
3	Beef	0.0014

external formaldehyde dosage. This study offers baseline data of formaldehyde content naturally found in a wide range of food items: fruits, vegetables, milk and meats. Formaldehyde contents of processed food items, such as: commercially available UHT and powdered milk samples, beverages, and cooked poultry and beef, were also assessed and analyzed. The formaldehyde concentrations of cooked meat samples were found lower than those of fresh meats. The formaldehyde contents of the commercially available milk samples (cow) were found higher than that of pure milk sample. Addition or formation of formaldehyde during milk processing and preservation could be the possible reasons to have high formaldehyde concentrations in commercially available milk samples. Higher formaldehyde concentrations in commercial milk samples are alarming since young population is the major consumer of them. Further study is required to identify the sources of high formaldehyde contents in the commercially available milk items and associated health effects. In addition, the time dynamic behavior of the formation of endogenous formaldehyde in banana, mandarin and beef samples were analyzed. This study demonstrated that the endogenous formaldehyde formation process in banana, mandarin and beef samples followed second-order reaction kinetics. However, the formation behavior of formaldehyde may vary according to food types, storage temperature, storing time and aging pattern of the food items. The above understanding will be useful for the consumers, researchers, legal authorities and other stakeholders working on food safety and preservation.

Authors' contributions
FN carried out a major part of the literature review, experimental research and drafted the manuscript. MNI helped experimental sections and to revise the manuscript. MSK conceived the study, supervised the research project, coauthored and supervised manuscript preparation, and helped to finalize the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
Not applicable.

Consent for publication
The authors confirm that the content of the manuscript has not been published or submitted for publication elsewhere.

Ethical approval and consent to participate
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