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Analytical Methods

Determination of formaldehyde in food and feed by an in-house validated HPLC method

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ABSTRACT

Formalin is carcinogenic and is detrimental to public health. The illegal addition of formalin (37% formaldehyde and 14% methanol) to foods to extend their shelf-life is considered to be a common practice in Bangladesh. The lack of accurate methods and the ubiquitous presence of formaldehyde in foods make the detection of illegally added formalin challenging. With the aim of helping regulatory authorities, a sensitive high performance liquid chromatography method was validated for the quantitative determination of formaldehyde in mango, fish and milk. The method was *fit-for-purpose* and showed good analytical performance in terms of specificity, linearity, precision, recovery and robustness. The expanded uncertainty was <35%.

The validated method was applied to screen samples of fruits, vegetables, fresh fish, milk and fish feed collected from different local markets in Dhaka, Bangladesh. Levels of formaldehyde in food samples were compared with published data. The applicability of the method in different food matrices might mean it has potential as a reference standard method.

1. Introduction

Food contamination and food adulteration are significant problems in Bangladesh (Ali, 2013; Noman & Atahar, 2013). A lack of strong regulatory controls, weak infrastructure for transport, storage and refrigeration and increasing consumer demand for fresh produce have led to an increase in fraudulent practices to increase shelf-life of food products. Food adulteration can have a detrimental impact on the health of a population, as adulterants can lead to developmental defects, chronic diseases, or death. Children, in particular, are more vulnerable to unsafe food, and is a major cause of child mortality (United Nations (UN), 2012).

Formaldehyde (HCHO) is a common air pollutant and a gas at ambient temperature. In its liquid form as formalin (35–40% aqueous solution stabilized with methanol), it is widely used in the manufacture of household products such as paint, furniture laminates and cleaning fluids. It is a proven carcinogen and, therefore, detrimental to public health (International Agency for Research on Cancer (IARC), 2004). In Bangladesh and South-East Asian countries, formalin has been reported to be added fraudulently to foods to extend shelf-life (Riaz, Moin, Tasbira, Naz, & Kumar, 2011). On occasions, tonnes of fruits and vegetables allegedly adulterated with formalin have been destroyed by authorities to protect consumers. There is no scientific evidence in the country corroborating the actual presence of this adulterant in foods and, generally, colorimetric qualitative tests are used during inspections. However, as formaldehyde is naturally present at varied concentrations in foods, its qualitative detection is not conclusive evidence of adulteration. To date, formalin adulteration in Bangladesh has only been evidenced by media reports.

The lack of accurate *fit-for-purpose* methods to determine formaldehyde in food and the pervasiveness of formaldehyde in nature make the detection of illegally added formalin challenging. Moreover, formaldehyde content in fresh food products varies with development stages and environmental factors. Formaldehyde occurs naturally in free and bound forms. Formaldehyde can bind reversible arginine, tyrosine and lysine protein residues yielding methylol groups, Schiff bases, methylene bridges and imidazolidinone adducts. Primarily, free formaldehyde is of toxicological interest and it is the compound measured as a potential adulterant (Metz et al., 2006; Rehbein, 1987).

The presence of formaldehyde as a breakdown product of hexamethylenetetramine is permitted in cheeses in Europe to a maximum residue limit (MRL) of 25 mg/kg (Directive 95/2/EC). Formaldehyde has also been permitted as preservative in gelling additives up to 50 mg/kg (Directive 2009/10/EC). Given the great

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variability of formaldehyde in foods, a more general MRL has not been set. The use of formaldehyde as preservative for feed is still under discussion at the European level (European Commission (EC), 2002, European Food Safety Authority (EFSA), 2014b) although a concentration of 2.5 g/kg is already permitted in the USA (United States Environmental Protection Agency (USEPA), 2010). According to the European Food Safety Authority (EFSA) (2014a), daily exposure to formaldehyde from food of animal and plant origin should not exceed 100 mg/kg food per day. Average dietary exposure is estimated to be about 11 mg/kg food per person per day (Agence Francaise de Securite Sanitaire des Aliments (AFSSA), 2004).

The official method for the determination of formaldehyde in foodstuffs is based on a colorimetric reaction where sample distillates are mixed with sulfuric acid yielding a purple color if formaldehyde is present. The intensity of the color is proportional to formaldehyde concentration and can be measured by UV spectrophotometer (AOAC 931-08, 1931). Titrations and acetylcholine have also been used to detect and quantify relatively the presence of formaldehyde in foods (European Pharmacopoeia 6.0 method., 2008; Lee, Su, & Chang, 1984). Currently, a colorimetric-based kit is being used during inspections in Bangladesh to detect adulteration with formaldehyde (Noordiana, Fatimah, & Farhn, 2011; Riaz et al., 2011). Drawbacks of this and other colorimetric methods are their poor specificity, selectivity, prolonged analysis times and highly acidic conditions, which together lead to over-reporting and/or false positives (Bicking, Cooke, Kawahara, & Longbottom, 1998).

Other techniques, such as LC and GC, have been proven to be more selective and accurate in determining formaldehyde in water (Tomkins, McMahon, & Caldwell, 1989), mushrooms (Claeys et al., 2009), milk (Kaminski, Atwal, & Mahadevan, 1993), fish (Jianrong, Junli, & Lifang, 2007; Tai-Sheng, Tzu-Chun, Ching-Chuan, & Hwui-Mei, 2013) and shrimps (Radford & Dalsis, 1982). There are a number of methods and extraction procedures available in the literature, which emphasizes the need of a harmonized reference method with broad applicability. To support regulatory authorities, the present study aimed to optimize and validate an HPLC method for the accurate determination of formaldehyde in food products. The applicability of the method was demonstrated in three different matrices: milk, mango and fish. A range of food products collected from local Dhaka markets were further screened for formaldehyde content.

2. Materials and methods

2.1. Chemicals

Solvents were of analytical grade (SIGMA–Aldrich, Buchs SG, Switzerland). 2,4 dinitrophenylhydrazine (2,4 DNPH) was purchased from Merck (Darmstadt, Germany). Formaldehyde in water certified reference material (CRM) (4815 mg/L) was from SIGMA–Aldrich (Buchs SG, Switzerland).

2.2. Formaldehyde solution

The certified value for formaldehyde in water CRM was 47.5 mg/L ± 8.91 (mean ± st. dev.) with an expanded uncertainty of 1.82, (k = 2.23). A stock solution of formaldehyde in water (500 mg/L) was prepared using deionized water. A matrix free calibration curve was prepared at six concentrations: 1, 2, 5, 25, 50 and 100 mg/L. For matrix matched calibrations, matrix samples (mango, fish and milk) were spiked before extraction at 1, 2, 5, 25, 50 and 100 mg/L. To calculate the bias of the method, a stock solution of formaldehyde CRM at 47.5 mg/L concentration was prepared following the instructions provided.

2.3. 2,4 dinitrophenylhydrazine working solution

2,4 DNPH was recrystallized prior to use. Recrystallization was performed by dissolving 10 g of 2,4 DNPH in 100 mL in hot analytical grade acetonitrile to form a saturated solution. After complete dissolution, the solution was cooled to room temperature, capped in a brown bottle and stored overnight at 4 °C for crystallization. The crystals were collected by vacuum filtration. 150 mg of 2,4 DNPH crystals were accurately weighed, dissolved in 49.5 mL of acetonitrile and mixed with 0.5 mL of phosphoric acid (85%).

2.4. Derivatization kinetics and sample preparation

Derivatization kinetics followed the procedure described by Claeys et al. (2009) but was slightly modified. Edible parts of the food; fruit flesh and fish fillets were used for the analysis. For derivatization kinetics, mango samples were ground, homogenized and spiked with 10 mg/L of formaldehyde standard. To sample aliquots of 5 g, 5 mL of acetonitrile were added, and the sample vortexed and then sonicated for 30 min. The samples were centrifuged at 5000 rpm for 5 min and the supernatant was passed through a 90 mm diameter Whatman® 541 (Hardened Ashless) filter paper (SIGMA–Aldrich, Buchs SG, Switzerland). Two and half milliliter of 2,4 DNPH was added to the extract and mixed well. Samples were incubated at 40 °C for 30, 60, 90 and 120 min in a shaking water bath (model BS-11, Oxon, UK). Formaldehyde was quantitatively converted to its Schiff base in 60 min. In all experiments, derivatization time was set to 60 min. After incubation, the acetonitrile layer was collected, membrane filtered (0.45 μm) and injected into the HPLC.

2.5. High performance liquid chromatography conditions

Analyses were performed on a C_{18} Luna column (25 cm × 4.6 mm id., 5 μm particle size), (Phenomenex, Utrecht, The Netherlands) using a HPLC (model SPD-M20A) coupled to a photodiode array detector (both manufactured by Shimadzu, Kyoto, Japan). The wavelength was set to 355 nm and the oven temperature at 30 °C. Separation was achieved using isocratic elution with a mixture of water/methanol (35:65, v/v). The flow rate was 1.0 mL/min and the injection volume 20 μL. The total run time was 12 min.

2.6. Method validation

The method was validated in terms of specificity, linearity, range, limit of detection (LOD), limit of quantification (LOQ), repeatability, intermediate precision and robustness. The specificity of the method was tested by injecting reagent blank (2,4 DNPH and phosphoric acid), sample blank and formaldehyde solution individually. For linearity the determination coefficient (R^2) was calculated from the responses of 0.1, 1, 2, 5, 25, 50 and 100 mg/L standards. The limit of detection was calculated by the expression 3.3 $s_{y/x}$/slope, based on the assumption that, the standard deviation of the signal of a solution with a concentration near to the blank is roughly the standard deviation of y-residuals ($s_{y/x}$). General, there is a normal distribution of 5% of occurring error type a or b and the curve intercepts at zero. The quantification limit was estimated by the expression 10 $s_{y/x}$/slope (Miller & Miller, 1993). For repeatability and recovery studies, 5 samples of each of the matrices were spiked at nominal concentrations at the LOQ, 2xLOQ and 5xLOQ levels and extracted by the method described in Section 2.3. Recoveries were expressed in % and repeatability as

the standard deviation (s_r) and relative standard deviation (%) (RSD_r). The repeatability limit (r) was calculated for a coverage factor of 99.9% (using the expression, $r = 2.8 \times s_r$). Intermediate precision was the standard deviation (s_{ip}) and relative standard deviation (%) (RSD_{ip}) obtained from measuring six independent sample replicates spiked at 3 different levels on 3 different days (IUPAC, 2002).The trueness of a method is the expression of how close the mean of a set of results is to the true value. The quantitative expression of trueness is bias and it was calculated using:

$$|c_m - c_{CRM}| \leqslant 2 \cdot \sqrt{u_m^2 + u_{CRM}^2}$$

where C_m is the measured value; C_{CRM} is certified value, u_m is uncertainty of the measurand, and u_{CRM} is uncertainty of the certified reference material.

The u_m was calculated using:

$$u_m = s_r/\sqrt{n}$$

where s_r is the standard deviation of the replicate measurements and; n is the number of measurements (10).

The robustness of the method was tested by analyzing a formaldehyde standard solution of 25 mg/L after applying minor changes to the analytical method. The column temperature was set to 33 °C and 37 °C, the mobile phase to pH 5.5 and 6.5 and the percentage of methanol in the mobile phase to 55% and 65%.

2.7. Measurement of uncertainty

Uncertainty was estimated based on intra-laboratory data following EURACHEM. (1993) guidelines. Uncertainty from stock standard solution ($u_{stock\ sol.}$) is not covered by the uncertainties from regression, precision and recovery and was studied separately. Four sources contributed to the uncertainty of stock solutions: the certified reference material, the balance, the volumetric flask and micropipettes. CRM uncertainty was calculated by taking into account the expanded uncertainty as per certificate ($U = 1.82$), with a coverage factor of $k = 2.23$ (99% confidence interval) and considering a triangular distribution.

Three main factors contributed to the balance uncertainty, the accuracy of a EW 62000-2NM balance is ±0.02 with a coverage factor of $k = 2$ and a rectangular distribution; balance repeatability (obtained by weighing 10 times a standard mass of 1 g) and balance trueness (comparing the measured mass with the nominal mass).

Five main factors contributed to the uncertainty of the volumetric flask; the tolerance given by the manufacturer's certificate considering a triangular distribution; the temperature given by the expansion coefficient of an aqueous solution: 2.1×10^{-4}; the dilatation coefficient, $100 \times 4 \times 2.1 \times 10^{-4}$ and assuming a rectangular distribution; the repeatability, calculated by filling and weighting of a 10 mL volumetric flask, ten times; and trueness, comparing the average of the measured volume to the certified value.

Three uncertainties were attributed to the micropipettes: the uncertainty due to the micropipette as per the certificate, considering a rectangular distribution, micropipette repeatability, and; micropipette trueness. The overall uncertainty contribution from the stock solution was estimated by the following expression:

$$u_{stock\ sol.} = \sqrt{u_{CRM}^2 + u_{balance}^2 + u_{micropipette}^2 + u_{v.flask}^2}$$

The uncertainty of the regression (u_{reg}) was estimated as follows:

$$u_{reg} = \frac{s_{x/y}}{m} \cdot \sqrt{\frac{1}{n_{rep}} + \frac{1}{n_{cal}} + \frac{(x_{pred} - \bar{x})^2}{\sum (x_i - \bar{x})^2}}$$

where $s_{x/y}$ is the residual standard deviation; m, is the slope; $n_{rep,}$ is the replicate analysis for the spiked sample (5), $n_{cal,}$ is the number of measurements for calibration; $x_{pred,}$ is the predicted concentration for unknown; x_i, is the concentration of individual calibration standards, and; x, is the mean concentration of calibration standards.

All uncertainties including those related to recovery (u_{rec}) and intermediate precision (u_{ip}) were expressed as relative standard deviation (RSD) as a percentage.

The combined uncertainty was calculated as follows:

$$u_c = \sqrt{u_{stock\ sol.} + u_{rec}^2 + u_{ip}^2 + u_{reg}^2}$$

The expanded uncertainty (U) was calculated as $U = ku_c$ where $k = 2$ is the coverage factor, for a 95% confidence level.

2.8. Collection of food products

Samples of mango, milk and fish were purchased from Dhaka local markets, processed (non-edible parts removed) and ground up using a Waring® Laboratory grinder (Dynamic Corp. of America, USA). Samples, which were not directly analyzed, were stored at −20 °C until analysis. Samples of fruits (18), vegetables (21), milk (5), fish (5) and fish feed (14) were collected from different markets from four areas of Dhaka city, namely, Nakhalpara, Gulshan, Mohakhali and Gazipur.

2.9. Formaldehyde intake from the Bangladeshi diet

The approximate formaldehyde intake in the Bangladeshi diet was calculated assuming the levels of formaldehyde estimated in the commodity survey and the foods mostly consumed in Bangladesh (Food, 2013): rice, 464 gram/day/person (g/d/p), fish, 50.3 g/d/p, leafy vegetables, 36 g/d/p, non-leafy vegetables, 131 g/d/p, fruits, 45 g/d/p and milk, 32 g/d/p.

2.10. Statistical analysis

In comparisons performed during robustness and sample screening, at least three repetitions were performed and results were tested for statistical significance using Microsoft Excel t-test for two samples assuming unequal variances. Differences were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Method validation

There was no interference between the matrix and the HCHO-2,4 DNPH derivatized product. The peaks of derivatized samples were identified by comparing retention times and UV–visible spectra with those of 2,4-DNPH and derivatized standard. The retention times of 2,4 DNPH and HCHO-2,4 DNPH were 5 min and 10.5 min, respectively (Fig. 1). In most cases, the food matrices contained formaldehyde and it was necessary to subtract blanks. The method was selective for the analysis of formaldehyde in different food commodities.

A standard calibration curve was built using known amounts of formaldehyde in the concentration range from 1 to 100 mg/L. The limit of detection was 0.39 mg/L, which was lower than the 2 mg/kg reported by Tai-Sheng et al. (2013) using HPLC and the 10 mg/kg reported by Lee et al. (1984) using a UV spectrophotometer. However, the detection limit was higher than the 0.05 mg/kg reported by Radford and Dalsis (1982) for water using HPLC. The regression square coefficient (R^2), LODs and LOQs for matrix free and matrix-matched calibrations are presented in Table 1. The LOD and LOQ for fish samples were greater than those for mango

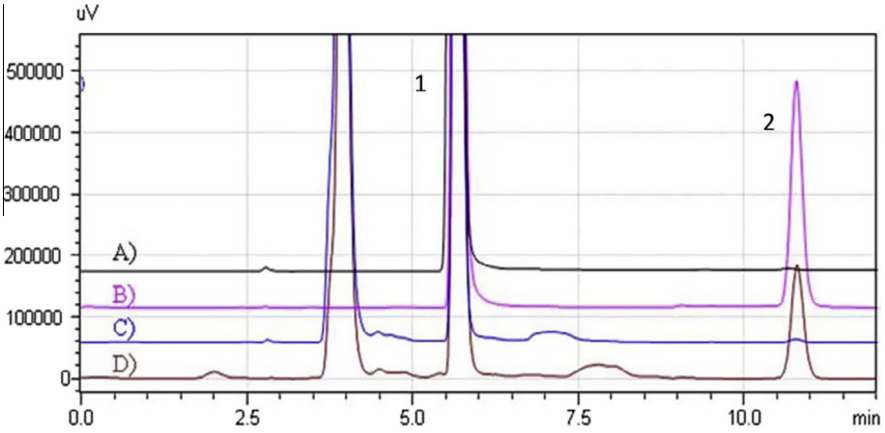

Fig. 1. HPLC chromatogram: (A) 2,4 DNPH; (B) 10 mg/L derivatized standard (HCHO-DNPH); (C) derivatised milk sample; (D) derivatised milk sample (spiked with 10 mg/L formaldehyde). 1) 2,4 DNPH, 2) HCHO-2,4-DNPH.

and milk due to a higher content of natural formaldehyde from the enzymatic reduction of trimethylamine oxide to dimethylamine (Nielsen & Jorgensen, 2004; Sotelo, Pineiro, & Perez-Martin, 1995).

The trueness of the method was tested with the analytical CRM. The value from the subtraction of the Certified Value from the measured value was <2 u_c, which indicates that the method was not biased. The average recoveries and RSD are presented in Table 2. The average recoveries were >80%.

RSD values for repeatability and intermediate precision were <11% and <15%, respectively (Table 2).

All validation parameters were within the acceptance performance criteria recommended by AOAC Official Methods of Analysis (2012).

Temperature was a critical parameter in the method. At 33 °C, the formaldehyde peak co-eluted with other matrix compounds giving a greater peak area ($p > 0.05$). Temperatures between 35 °C and 40 °C provided optimal results. Changes in pH and percentage of methanol in the mobile phase shifted the formaldehyde retention time peak, but did not affect peak areas ($p < 0.05$).

3.2. Measurement of uncertainty budget

The relative standard uncertainties from the stock solution, regression, recovery, precision, combined and expanded uncertainties, all expressed as relative standard deviation are presented in Table 2.

As shown in the uncertainty histogram for milk in Fig. 2, the major contribution to uncertainty was intermediate precision as it includes random errors from many variables. The maximum expanded uncertainty (at 95% confidence level) was the uncertainty of the method and it was found to be less than 35%.

3.3. Determination of formaldehyde in different foods

The applicability of the method to a variety of foods was demonstrated by screening samples of fruits, vegetables, fresh fish, milk and fish feed collected from different local markets in Dhaka, Bangladesh. Background levels of formaldehyde were found in all the commodities tested (Fig. 3). In most cases, formaldehyde content was within or below the natural levels reported by other authors (Table 3). Environmental pollution and intrinsic metabolism in the foods might explain these differences. Formaldehyde content in the samples did not exceed 15 mg/kg. In some cases more than five samples from different markets were analyzed. The formaldehyde range was 1.83–1.93 mg/kg for apples, 1.83–4.62 mg/kg for mangos, 1.77–10.48 mg/kg for mango juices and 0.76–2.67 mg/kg for milk.

This study provides data on the formaldehyde content of fruits and vegetables such as mango, litchi, cucumber, capsicum and dates which have not previously been reported (Fig. 3). These results demonstrate the presence of formaldehyde in foods. Only reliable quantitative methods can give the actual formaldehyde content in food, but in absence of MRLs, whether formaldehyde is a natural present or adulterant cannot be concluded.

Comparisons between fresh and commercial products might be another approach to verify formaldehyde adulteration. To this end, freshly caught fish species from a family farm and fish from the market were analyzed. Content of formaldehyde in fresh and non-fresh fish was not significantly different ($p > 0.05$) (Fig. 4). According to Tunhun, Kanont, Chaiyawat, and Raksakulthai (1996), the natural content of formaldehyde in fish flesh from rake-gilled mackerel (0.68 mg/kg) was 7.68 mg/kg after dipping the fish samples for 10 min in a formaldehyde solution of 1000 mL/L (units as described by the authors). They also reported

Table 1
Linearity, limit of detection, limit of quantification and range of the method.

Method validation parameters				
Parameter	Matrix-free	Matrix-matched		
		Mango	Milk	Fish
Linear equation	$y = 488{,}092x + 4440.9$	$y = 209{,}862x + 189{,}675$	$y = 202{,}380 + 52{,}100$	$y = 27507.17 + 125966.7$
R^2	0.99	0.99	0.99	0.99
LOD [mg/L]	0.39	0.32	0.67	1.75
LOQ [mg/L]	1.30	1.08	2.23	5.83
Range [mg/L]	1.30–100	1.08–100	1.0–100	5.0–100

Table 2
Recovery, repeatability, intermediate precision and uncertainty of the method.

	Method validation parameters								
	Mango (n = 6)			Milk (n = 6)			Fish (n = 6)		
True value/spiked level [mg/L]	1	2	10	1	2	10	5	10	25
Recovery [%]	115.56	114.79	99.82	93.29	83.25	93.69	105.36	102.66	91.25
Repeatability s_r [mg/L]	0.12	0.08	0.64	0.14	0.17	0.63	0.35	0.68	0.12
Repeatability RSD_r* [%]	10.59	3.52	6.44	7.29	10.43	6.81	6.72	6.65	0.56
Repeatability limit, r [mg/L][1]	0.94	0.22	1.79	0.39	0.47	1.76	0.98	1.90	0.33
Intermediate precision s_{ip} [mg/L]	0.14	0.29	0.96	0.11	0.17	0.95	0.18	0.33	2.99
Intermediate precision RSD_{ip} [%]	14.15	14.13	8.69	12.62	9.00	9.32	3.93	3.75	12.06
Stock solution uncertainty, $u_{stock\ sol.}$	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70	2.70
Precision uncertainty, u_{prec}	12.63	9.32	9.00	14.51	14.13	8.69	3.93	3.73	12.06
Recovery uncertainty, u_{rec}	10.97	5.54	8.77	5.41	6.49	6.10	6.72	6.08	0.45
Regression uncertainty, u_{reg}	4.79	2.94	0.64	4.39	1.79	0.65	7.81	3.16	1.23
Combined uncertainty, u_c [%]	16.94	11.17	12.85	15.72	15.78	10.95	11.35	8.25	12.43
Expanded uncertainty, U [%]	33.89	22.35	25.70	31.44	31.56	21.90	22.70	16.51	24.85

* RSD: relative standard deviation.
[1] Repeatability limit r, is the value below which the absolute difference between two single test results obtained under repeatability conditions may be expected to lie with a probability of 95%. This limit is obtained as (ISO 57125:1994): $r = 2.8\ S_r$.

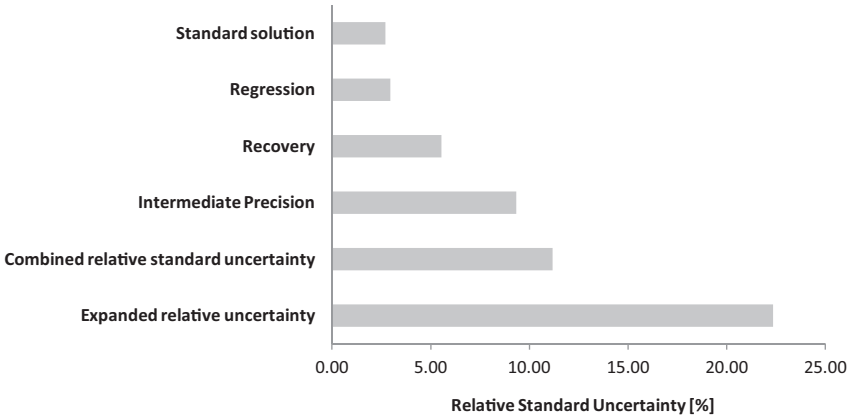

Fig. 2. Uncertainty histogram of milk samples spiked with formaldehyde standard at 2 mg/L.

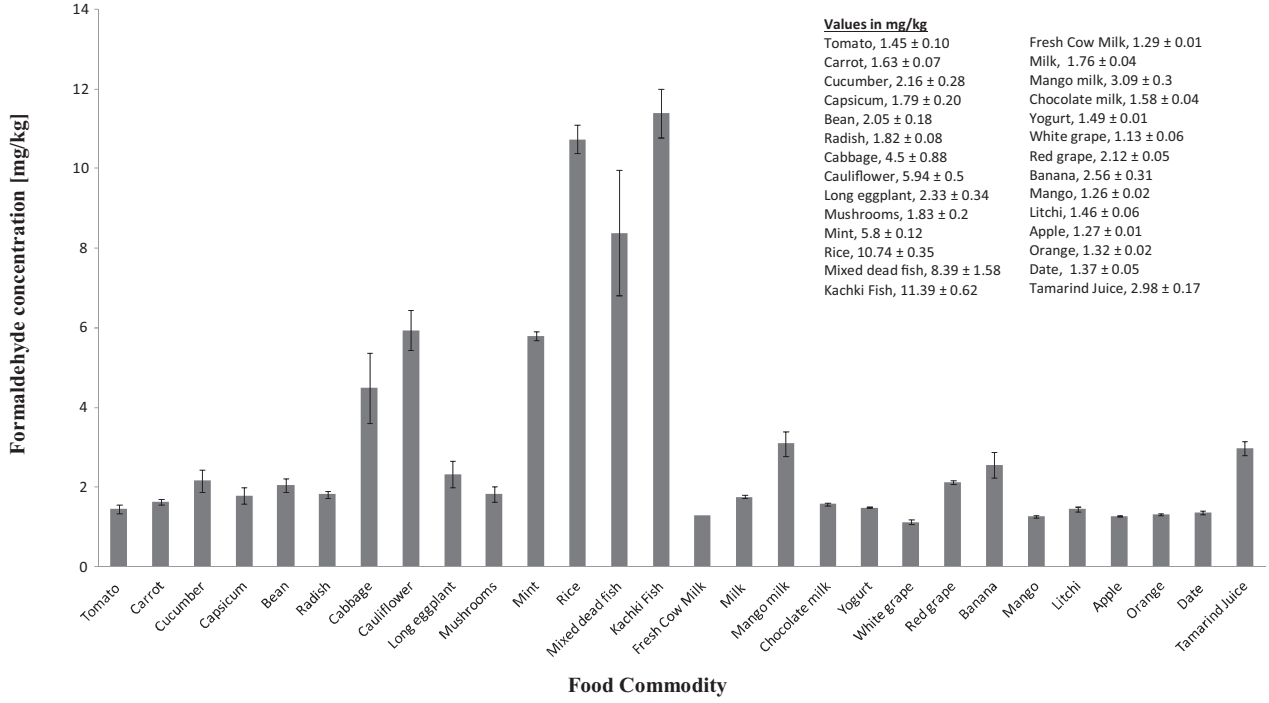

Fig. 3. Formaldehyde content (expressed as mean ± std. dev) determined in different food products.

Table 3
Background levels of formaldehyde in food.

Commodity	Formaldehyde content [mg/kg]	Methodology	References
Fruits and vegetables			
Pear	60	Colorimetric	Möhler and Denbsky (1970)
Apple	17.3	Colorimetric	Tsuchiya, Hayashi, Onodera, and Hasegawa (1975)
Banana	16.3	Unknown	Yau (2008)
Cabbage	4.7	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
Carrot	6.7	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
Cauliflower	4.7	Colorimetric	International Programme on Chemical Safety (IPCS), 1989
Green onion	13.3	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
Spinach	3.3	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
Tomato	5.7	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
White radish	3.7	Colorimetric	Tsuchiya, Hayashi, Onodera and Hasegawa (1975)
Shiitake mushroom	100–320	Colorimetric	Mason, Sykes, Panton, and Rippon (2004)
Milk and dairy products			
Goat's milk	1	Colorimetric	Mills, Sharry, Cook, and Scott (1972)
Cow's milk	3.3	Colorimetric	Möhler and Denbsky (1970)
Fresh milk	0.027	HPLC	Kaminski et al. (1993)
Commercial milk	0.164	HPLC	Kaminski et al. (1993)
Cheese	3.3	Colorimetric	Möhler and Denbsky (1970)
Meat products			
Pig,	20	Colorimetric	Florence and Milner (1981)
Poultry meat	5.7	Colorimetric	Möhler and Denbsky (1970)
Fish and crustaceans			
Fresh water/sea fish	8.8/2.38–2.95	Colorimetric	Möhler and Denbsky (1970), Aminah, Zailina, and Fatimah (2013)
Frozen cod	20	Colorimetric	Möhler and Denbsky (1970)
Shrimp	0.39–1.44	HPLC	Rehbein (1986), Radford and Dalsis (1982)
Crustaceans	1–60	Colorimetric	Cantoni, Milva, and Bazzani (1987)
Dried squid	35.3	HPLC	Jianrong et al. (2007)
Squid	19.3/26.6	GC/HPLC	Tai-Sheng et al. (2013)
Beverages			
Water	0.1 mg/L	HPLC	Mason et al. (2004)
Soft drink	7.4–8.7	HPLC-MS	Lawrence and Iyengar (1983)
Coffee	3.4–4.5	GC	Hayashi, Reece, and Shibamoto (1986)
Instant coffee	10–16.3	GC	Hayashi et al. (1986)

a pungent irritant smell when samples were dipped in a formaldehyde solution of 2000 mL/L or higher (Tunhun et al., 1996). In their study, they demonstrated that formaldehyde levels in treated fish increased after dipping them in formaldehyde solutions. However levels were far below the levels reported as natural in other fish species (Table 3). The naturally high levels of formaldehyde in fish complicate the accurate detection of illegally added formaldehyde. It is also known that formaldehyde concentration increases naturally during post-mortem in fish and crustaceans due to the enzymatic reduction of trimethylamine oxide to formaldehyde and dimethylamine (Nielsen & Jorgensen, 2004; Sotelo et al., 1995).

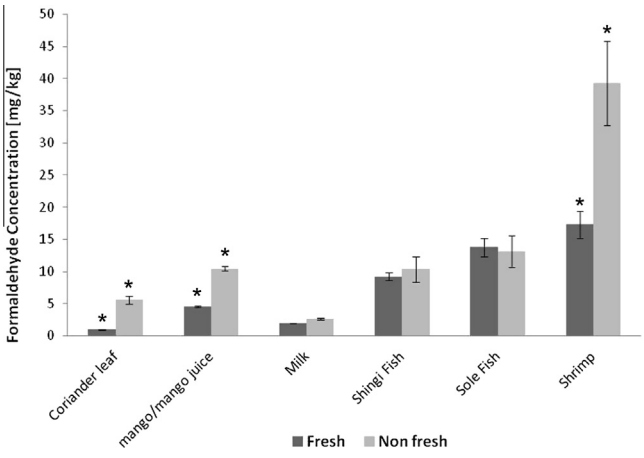

Fig. 4. Comparison of the formaldehyde content (mean ± std. dev) of fresh/non fresh food products ($n = 3$). Samples were considered statistically different when $p < 0.05$ using t-test for two samples assuming unequal variances (*).

Formaldehyde content of milk collected from a family farm and from a supermarket was determined and compared. The content in formaldehyde in both samples was not significantly different ($p > 0.05$). In contrast, the content of formaldehyde found in fresh and non-fresh shrimps was significantly different ($p < 0.05$) (Fig. 4). Content of formaldehyde found in shrimps from Bangladeshi markets were higher than levels reported by Radford and Dalsis (1982) (Table 3). It is unclear whether the high levels of formaldehyde found in shrimps and fish are due to environmental pollution, metabolism, carry over from feed or adulteration.

The formaldehyde levels found in this brief survey were below the natural values published elsewhere (Table 3). These works were, however, mainly based on colorimetric methods that are considered non-specific (Bicking et al., 1998). No clear case of adulteration could be concluded.

Formaldehyde content in fourteen different fish feed samples was analyzed and was found to range from 21.11 to 66.09 mg/kg. Three samples contained high levels of formaldehyde 107.70 ± 0.52, 127.86 ± 1.85 and 150.24 ± 5.50 mg/kg, and one of the commercial fish feed pellets had a formaldehyde content of 890.68 ± 18.66 mg/kg. Although levels were high, they were below 2.5 g/kg, which is the level permitted by the United States Environmental Protection Agency (2010). According to European Food Safety Authority (2014b), the carryover of formaldehyde from feed to meat appears to be negligible. It is unknown whether carryover of formaldehyde from feed to fish/shrimp flesh occurs. If there is carry over, this might explain the higher levels of formaldehyde found in fish and shrimp.

Table 4
Intake of formaldehyde derived from the Bangladeshi diet.

Food Items	Formaldehyde content [mg/kg][a]	Average consumption [g/p/d][b]	Intake	
			mg/day[c]	mg/kg body weight day
Cereals (rice)	10.74	464	4.98	0.083
Fish	26.2	50.3	1.3	0.021
Leafy vegetables	5	36	0.18	0.003
Non-leafy vegetables	2.5	131	0.32	0.005
Fruits	3.08	45	0.14	0.002
Milk	3.0	32	0.09	0.0015
Total Consumption of formaldehyde per day (mg)			7.01	
Formaldehyde intake per body weight (adult 60 kg) (mg/kg/d)				0.12

[a] Average of the formaldehyde concentration detected in different commodities (see Fig. 3), it is assumed that the preparation of food did not reduce the formaldehyde concentration.
[b] Average dietary consumption of the Bangladeshi population (Food, 2013).
[c] Calculated deterministically; the body weight was assumed to be 60 kg.

3.4. Formaldehyde exposure in the Bangladeshi diet

Human exposure to formaldehyde was calculated taking into account the most representative foodstuffs of the Bangladeshi diet and assuming the average formaldehyde levels estimated in this work. The formaldehyde intake was estimated to be 7.01 mg/kg food or 0.12 mg/kg b.w. per day (Table 4). This level of exposure to formaldehyde from the average diet in adults is well below maximum limits suggested by EFSA (<100 mg/kg food per day) and 11 mg/kg food per person per day (AFSSA, 2004). There appears to be no health risk associated with the consumption of formaldehyde present in foods.

4. Conclusion

In this work, a HPLC method was validated for the determination of formaldehyde in three different food matrices (mango, milk and fish), and the overall uncertainty budget was measured. The method is *fit-for-purpose* and would be suitable as reference method to estimate formaldehyde in different foodstuffs. One of the main advantages of this method is its applicability to a range of food matrices. The method is specific, linear, precise and robust. The method was used to conduct a limited market survey including a range of fruits, vegetables, milk, fish and fish feed. Levels of formaldehyde found in the samples were lower than those reported in other works. This study provides further data on the formaldehyde content of other food products not analyzed previously. These and levels reported in other works (Table 3) could be used by policy-makers or inspectors to regulate adulteration. However, naturally high levels of formaldehyde in some food commodities, such as fish or mushrooms, make discern between natural and illegally added formaldehyde difficult.

Strict inspections at the retailer level and all along the supply chain need to be undertaken to prevent the misuse of this hazardous chemical in food. Inspectors could target areas where food is stored or sold. Additional law enforcement during production, transport, storage, import, use and sales of hazardous chemicals is needed to avoid the misuse of these substances in foods.

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