DETERMINATION OF FORMALDEHYDE IN COMMERCIAL NOODLES COLLECTED FROM DHAKA CITY, BANGLADESH, BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract
In modern food technology, chemical preservation has become a common practice which is swelling up day by day. As a preservative formaldehyde is used in noodles, tofu, fruits and much another foodstuff to prolong their shelf-life and attractiveness not only in the Asian country but also all over the world. An experimental study for the level of formaldehyde concentration in three different brand noodles available in three different bazaars in Dhaka city of Bangladesh was determined by high performance liquid chromatography. Handmade noodles sample was used as the control to compare the range of formaldehyde content with commercial brand noodles. A Luna 5 µm C18 100 Å column (250×4.60 mm) was used for the chromatographic analysis. Chromatographic separation was accomplished by the extraction of acetonitrile derivatized with 2, 4- Dinitrophenylhydrazene and H3PO4 (85%) and the chromatograms were recorded at 355nm. The mobile phase was made up of 50% acetonitrile. The limit of detection and quantification for formaldehyde was 1.94mg/100gm and 5.88mg/100gm respectively. Results of the determination showed that the range of formaldehyde concentration (mg/100g) varies from 1.94mg/100g to 2.43mg/100g for brand 1 noodles, 1.74mg/100g to 1.88mg/100g for brand 2 noodles and 1.72mg/100g to 2.70mg/100g for brand 3 noodles in three different bazaars whereas the concentration was found as 1.69mg/100g for handmade noodles.

Keywords: Chemicals, Formaldehyde, Noodles, Preservatives

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1. INTRODUCTION

The chemical formula for formaldehyde is HCHO. In the solid state, it is known as "paraformaldehyde." At room temperature, it is an odorless and colorless gas that retains a distinct scent (Varela, 1995). Formaldehyde is immeasurably soluble in water and easily absorbed in the upper respiratory tract. It is also found in the human body in the slight and harmless amount which is produced naturally. It is classified as poisonous by the World Health Organization and by the U.S. EPA as a possible human carcinogen as demonstrated (Smedje et al., 2011). Consumption of' formalin contaminated food is the health hazard. Ministry of Public Health issued Act No.ISI (B.E.2S36) specified the use of formalin in food is prohibited. It is commonly used as a sterilizer in numerous humanoid drugs, makeups, disinfected in veterinary medicines, for the fungus infection, in fabrics and for preserving solutions (P. Feick et al., 2006 and Md. Faruque Miah et al., 2013).

In the human body formaldehyde is an ordinary metabolic creation so, excess amount revelation of formaldehyde upsurges the threat of severe injuring, also it can lead to deadliness like cancer (lARC, 2006 and Xiaojiang Tang et al., 2009). During food processing, it is also used as antiseptic agent. According to Food and Drugs Act at the level of up to 2ppm (2mg/kg) formaldehyde is allowed in maple syrup to prevent the bacterial attack in the holes of maple trees for the use of paraformaldehyde in Canada. In many countries some food producers illicitly used formaldehyde in foods for the preservation purpose. (Aminah, A, S. et al., 2013).

Noodles are one of the most important foods in Asian cuisine. Approximately 40% of the total wheat flour consumed is in the form of noodles in Asia. Unluckily, in outdated market noodles is frequently found to be contaminated with formaldehyde dishonestly (Gani, A.A. et al., 2013). Foods such as noodles, tofu, meatballs, vermicelli, salted dried fish, milk and fruits are commonly used by formalin as a preservative. It was reported by Kaminski et al. (1993) that, the limit of formaldehyde in marketable 2% milk and fresh milk from farm cows fed on a classic North American dairy total mixed food was determined. Beside the price of formalin is cheap, it also beneficial to extend the life of food. It can also improve the external appearance of food and protect them from various microorganism like fungi, bacteria etc. As a result, it is widely used in food products. A recent study by Save The Environment Movement has found that, formalin is used in 90 percent of noodles, 60 percent of aubergines, 82 percent of mangoes, 91 percent of bananas, 59 percent of apples, 95 percent of grapes, 77 percent of dates, 75 percent of tomatoes, 20 percent of cucumbers, 100 percent of vermicelli and oranges. It is assessed that consumption of formaldehyde through food may range from 1.5 and 14mg/person/day, which might surpass the RfD in a poorest-circumstance consequence (Feron et al., 1991).
It is reported by Badan Pengawas Obat dan Makanan (BPOM), The National Agency of Drug and Food Control of Republic of Indonesia, formaldehyde is using widely by a unit of deceitful dealers to preserve food items such as noodles, fish, and tofu. A laboratory test accomplished by BPOM in December (2005), of different food samples as well as oyster, tofu, wet noodles, shrimp, squid and dried salted fish, from six different towns at Lampung, South Sumatra which revealed sixty-four tasters were established with formaldehyde contamination (BIMC Hospital, Indonesia, 2017). Formaldehyde is poisonous and injurious to the human body. Extensive digestion of it might be deadly for health and outcome as memory loss, annexations, changed behavior, and to end with death or unconsciousness for the people (Wantke F. et al., 2000).
A subtle High Performance Liquid Chromatography technique was authenticated for the numerical determination of formaldehyde in foods like fruits, fish, meat. Henceforth, the objectives of this study were to determine the formaldehyde content in commercial brand noodles in order to evaluate the possible risk of noodles consumption and to improve the simple, delicate, correct, and discerning formaldehyde determination technique by using HPLC analysis. The study describes the current scenario on the content, consumption, and exposure to formalin from various commercial noodles. This might help the traders, policymakers to develop public awareness and take proper legitimate action towards formaldehyde addition.

2. MATERIAL AND METHODS

Chemicals

Formaldehyde (37%), Phosphoric acid (H3PO4) purity 85%, Merck (Germany). HPLC solvent Acetonitrile (FW: 41.05) and 2, 4- dinitrophenylhydrazine (DNPH) procured from Daejung, Korea. De-ionized water used for chromatography processing was (Water PRO PS) Labconco.

Materials

Three different brands, as well as different batches of noodles, were purchased from three renowned bazaars in Dhaka city, Bangladesh. A Total of eighty-one noodles samples were collected for the determination of formaldehyde content. Three different samples of three different brands noodles were selected from three different shops each of three bazaars. The expiry dates of all samples were within the study period. All collected samples were preserved at the room temperature until analysis.

HPLC system

The HPLC system equipped with a Shimadzu binary solvent system, a degasser, column, a UV detector, SHIMADZU CLASS-VP chromatographic data system as software were used for data acquirement and experiment.

Sample injected with the volume of twenty microliters (20 µl) into the injector. A Luna 5 µm C18 100 Å column (250×4.60 mm) was used for the chromatographic separation. The formaldehyde analysis was implemented using 50% acetonitrile in water (v/v) binary system. Chromatograms were documented at 355nm with the flow rate of 1.0ml/min.

Preparation of 2,4, DNPH (Dinitrophenylhydrazine) reagent

DNPH was recentralized before using. Recentralization was completed by liquefying with additional of DNPH in 10 ml hot acetonitrile analytical grade to produce a drenched solution. After finalizing the suspension, it was cooled to room temperature in a covered bottle and kept overnight at 4^0C for further crystallization. The crystals were culled by Whatman filter paper. 150 mg of DNPH crystal were accurately weighted ±0.00 and dissolved in 49.5 ml acetonitrile and mixed with 0.5 ml of H_3PO_4 (85%).

Preparation of standard solution

Standard was prepared from CRM (Certified Reference Material) first stock solution which concentration was 4033µg/ml and a second stock solution or working stock solution of 403.3µg/ml was organized by dissolving 1 ml of CRM standard solution into 9 ml H_2O. Standard solutions were prepared by these following concentrations 0.0, 10.0, 20.0 and 40.0 µg/ml.

Preparation of Sample solution

Approximately 5 gm sample was taken and added with 5 ml analytical grade acetonitrile to mix by vortex. The sample was sonicated for 30 min at room temperature (25^0C- 30^0C) and centrifuged for 5 min. with 600rpm at 21^0C. Whatman filter paper (125mm) was used to filtrate the sample and the upper layer of the extract was taken approximately 4 ml in a biker to add 2 ml working DNPH solution. After vortex, the solution was filtered to get the supernatant by syringe micro filter and micro centrifuged at 600 rpm for 5 min. at 21^0C. Again, the solution was filtered to get the final supernatant solution using (pore size 0.22µm) syringe filter and taken into Eppendorf tubes and mixed well with the vortex mixer. Exactly 20 µl of this sample mixture was injected onto the HPLC column.

Experimental analysis

Analysis of formaldehyde: A high performance liquid chromatographic technique was used to determine the formaldehyde concentration by using the modified method described by Tomkins et al. (1989). The final supernatant solution of the sample was transferred into dry Eppendorf tube and centrifuged for 5 min. The clear aqueous solution was filtered through PTFE syringe filter (pore size 0.22µm). Then the solution was transferred to the dry HPLC vials and was injected into the column for quantification and detection.

Optimization: To determine the times of formaldehyde analysis UV-spectrophotometer was used. The λ_{max} for formaldehyde was found to be at 355nm. Therefore, the HPLC analyses were carried out at 355nm.

Quantification: The HPLC equipped with SHIMADZU CLASS-VP chromatographic data system as the software was used for the calibration curve and the quantification of the samples. The quantification of the samples was founded by the comparison of the peak areas of the standard with those of the samples.

Limit of detection and quantification: The limit of detection (LOD) is defined as the lower amount of analyte in a taster which can be identified but not certainly measured as a precise value, whereas the limit of quantification (LOQ) states to the lowest level of analyte which can be determined with an acceptable degree of confidence. In this work, the detection limit (LOD) and quantification limit (LOQ) values were designed based on standard deviation of the response and the slope of the calibration curve (ICH, 1997). The concentration was multiplied by 3 and 10 to obtain the limit of detection and quantification, respectively.

HPLC analysis: The following performance parameters were calculated for HPLC method validation such as linearity, quantification limit, precision, detection limit and the extended uncertainty. The precision of the analytical method was assessed by calculating

the chromatographic peak area of formaldehyde three times for the same sample. Peak areas from HPLC chromatogram were plotted alongside the known concentrations of stock solutions of variable concentrations.

Statistical analysis: A elaborative analyses considering means, median, the coefficient of variation and standard errors were calculated. A single mode analysis of variance (ANOVA) was carried out using Microsoft Excel 13 at an impact level of 5%. The Least Significant Difference (LSD) experiment was used to discover differences in means.

3. RESULTS AND DISCUSSION

HPLC is the most suitable and precise technique used for the analysis of the pharmaceutical industry, food, and beverage industry, research, and development or in analytical chemistry to distinct, classify and measure the composites.

A High Performance Liquid Chromatography technique has been developed for the determination of formaldehyde concentration by using the mobile phase performed with the binary solvent system using deionized water and 50% acetonitrile buffer. The eluent was supervised by UV detector at 355 nm with the flow rate of 1ml/min. The retention time of formaldehyde was 12.01±0.2 min (Figures 1 and 2).

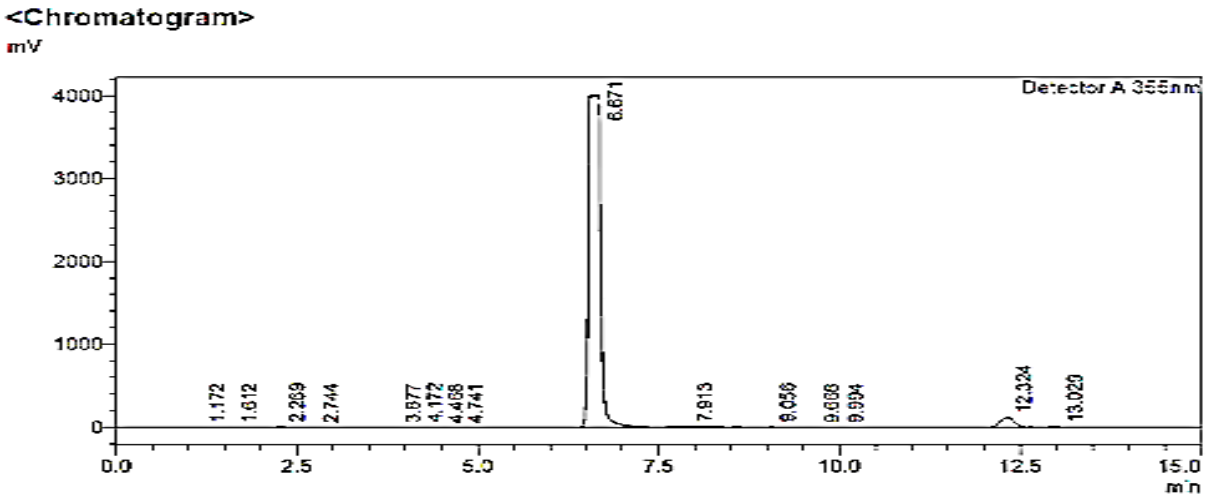

Fig. 1. HPLC chromatograms at 355nm for 20 µg/ml formaldehyde standard solution
The retention time for the peak of formaldehyde in sample solution was 12.32 minutes

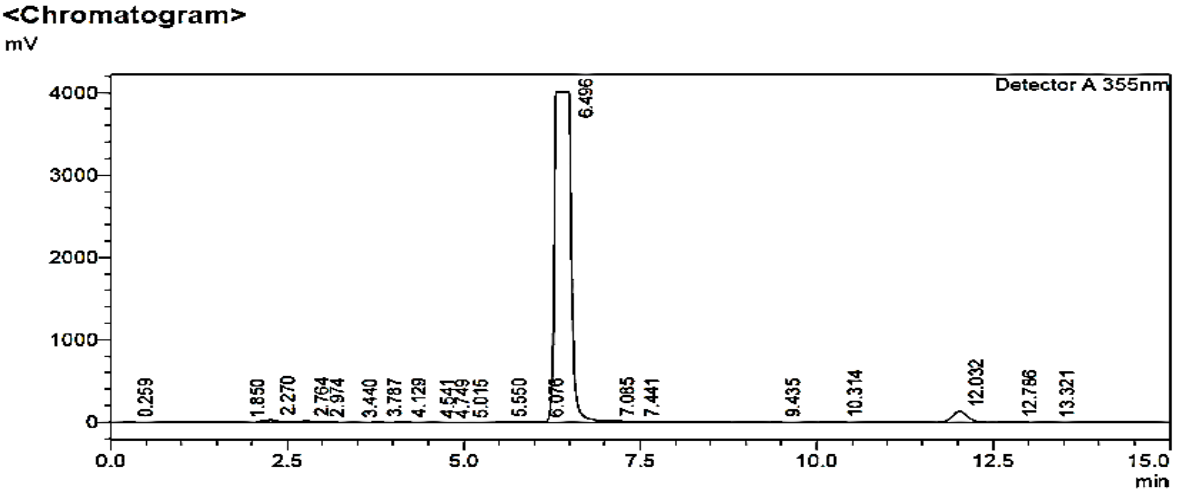

Fig. 2. HPLC chromatograms at 355nm for 20 µg/ml formaldehyde sample solution
The retention time for the peak of formaldehyde in sample solution was 12.03 minutes

Figure 3 shows the calibration curve for formaldehyde was attained by plotting the peak areas of different concentrations of working standard solutions described in Table 1 equipped with the stock solutions. A very good linearity for formaldehyde was obtained as it is presented in Figure 3 with an admirable regression factor (0.9854). A linear regression line was achieved y = 153181x – 121623. Table 2 shows the analytical parameters and their values of HPLC method.

Table 3 showed the mean concentration of formaldehyde of Brand 1 noodles from various shops of selected bazaars which were tested for the determination of formaldehyde content. Each of the tests was carried out with two replicas. As reported in Table 3 the amount of formaldehyde concentration (mg/100gm) of Brand 1 noodles found 2.43±0.11 mg/100g in Malibag Bazaar, 1.94 ±0.78 mg/100g in Mouchak Bazaar and 2.12±0.34 mg/100g in Gulistan Bazaar respectively. The mean concentration of formaldehyde of Brand 2 noodles is presented in Table 4. The concentration of formaldehyde level obtained as 1.74±0.41 mg/100g in Malibag Bazaar, 1.88 ±0.34 mg/100g in Mouchak Bazaar and it was 1.88±0.05mg/100g for Gulistan Bazaar. Table 5 exhibited the concentration of formaldehyde of Brand 3 noodles from various shops of selected bazaars. The mean concentration of formaldehyde was acquired as 2.70±0.36 mg/100g in Malibag Bazaar, 1.86 ±0.23 mg/100g in Mouchak Bazaar and it was 1.72±0.57 mg/100g for Gulistan Bazaar respectively.

Figure 4 showed the comparison of formaldehyde concentration (mg/100g) among different brands of noodles with handmade noodles (control) in three different Bazaars. The range of formaldehyde concentration (mg/100g) of brand 1 noodles varies from 1.94mg/100g to 2.43mg/100g, in brand 2 noodles from 1.74mg/100g to 1.88mg/100g and in brand 3 noodles from 1.72mg/100g to 2.70mg/100g in three different bazaars, whereas the concentration was found as 1.69mg/100g in handmade noodles.

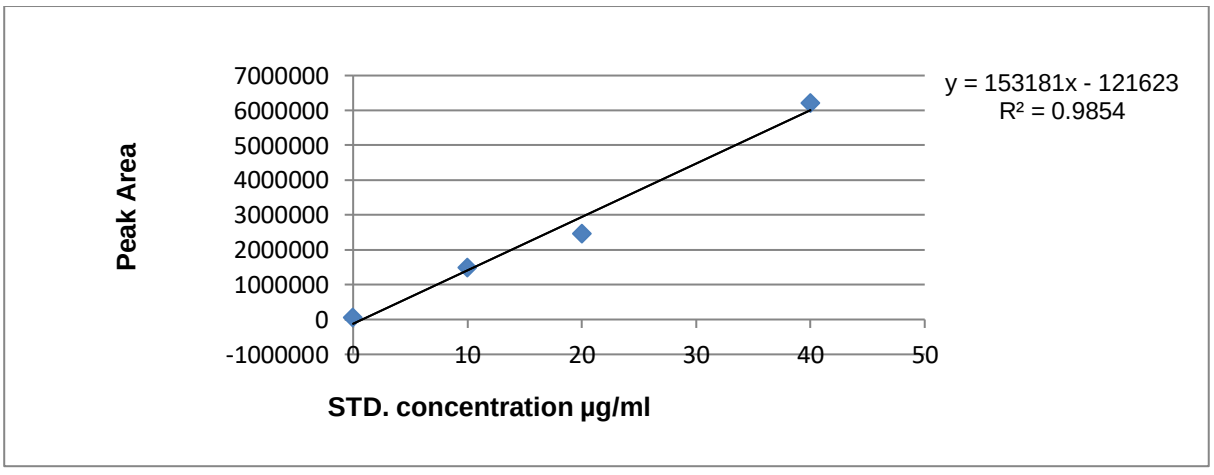

Fig. 3. Calibration curve for the formaldehyde concentration

Table 1. Different concentrations of formaldehyde standard and their peak area

Standard concentration (µg/ml)	Peak area	Found concentration
0	53984	1.146402
10	1489046	10.51483
20	2472483	16.93495
40	6220640	41.40382

Table 2. Analytical Characteristics of HPLC method

Parameter	Value
Slope	153181
Intercept	121623
Linearity range	4.96 µg/ml to 33.13 µg/ml
Linear equation	y = 153181x - 121623
Correlation coefficient	0.9854
Standard error of intercept	298168.9582
Standard deviation of intercept	596337.9164
LOD	1.94mg/100gm
LOQ	5.88mg/100gm

Table 3. Summary of the concentration of formaldehyde (mg/100g) in Brand 1 noodles

Name of bazaar	Concentration of formaldehyde(mg/100gm)			Mean	SD
	Shop 1	Shop 2	Shop 3		
Malibag	2.37	2.37	2.57	2.43	0.11
Mouchak	2.24	1.06	2.54	1.94	0.78
Gulistan	2.45	1.77	2.15	2.12	0.34

Table 4. Summary of the concentration of formaldehyde (mg/100g) in Brand 2 noodles

Name of bazaar	Concentration of formaldehyde(mg/100gm)			Mean	SD
	Shop 1	Shop 2	Shop 3		
Malibag	2.22	1.57	1.44	1.74	0.41
Mouchak	1.57	1.82	2.25	1.88	0.34
Gulistan	1.95	1.85	1.86	1.88	0.05

Table 5. Summary of the concentration of formaldehyde (mg/100g) in Brand 3 noodles

Name of bazaar	Concentration of formaldehyde (mg/100gm)			Mean	SD
	Shop 1	Shop 2	Shop 3		
Malibag	2.48	3.13	2.51	2.70	0.36
Mouchak	1.60	1.91	2.07	1.86	0.23
Gulistan	1.89	1.08	2.19	1.72	0.57

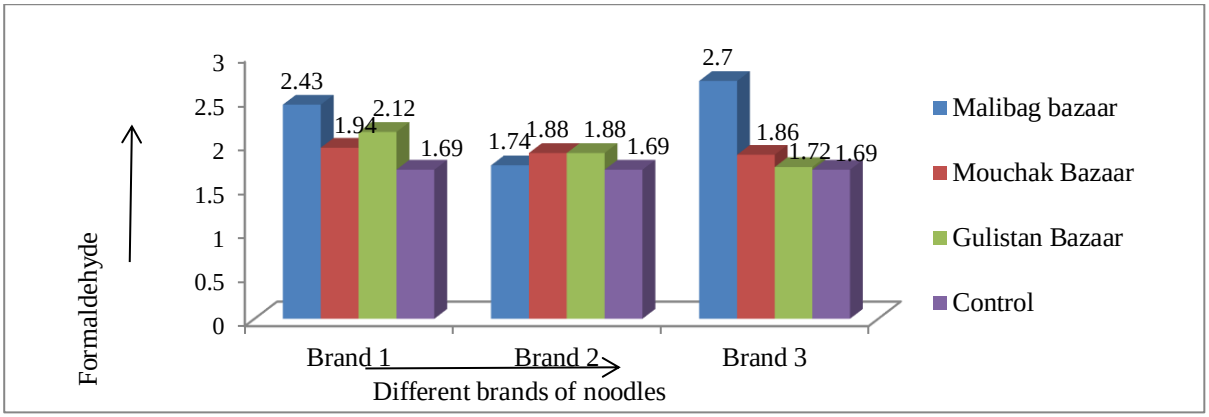

Fig. 4. Comparison of formaldehyde concentration (mg/100g) among different brands of noodles with control in three different Bazaars

From this experiment, the highest amount formaldehyde was found in brand 3 noodles from Malibag bazaars. As reported in Figure 4, the levels of formaldehyde in noodles were ranged from 1.72mg/100g to 2.70mg/100g might have within the permissible limit found by International Programme on Chemical Safety (IPCS 1989). According to IPCS formaldehyde exposure by food may range between 1.5 and 14 mg/person/day. The US Environmental Protection Agency (EPA) has established a maximum daily dose reference (RfD) of 0.2 mg kg-1 body weight per day for formaldehyde.

4. CONCLUSION

The present study was based on a simple, selective, cost-effective, less use of hazardous chemicals and fast binary solvent system for the determination of formaldehyde content in different brand noodles. Formaldehyde is a common preservative used by manufacturers in the industries and deceitful traders to extend the shelf life. The results show that formaldehyde found in different brand noodles varied from 1.72mg/100g to 2.70mg/100g which was within the range of handmade noodles perceived as 1.69±0.04 mg/100g and might have within the permissible limit found by International Program on Chemical Safety (IPCS 1989). From the experiment, it is presumed that formaldehyde is not added in noodles. Moreover, the described analytical method appears to accomplish the principles to reach precise and defined result for the analysis of formaldehyde in the conditions under study.

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