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HPLC Determination of Formaldehyde in Flour Samples using 2,4,6-Trichlorophenyl Hydrazine as Derivatization Reagent

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A new simple and sensitive high-performance liquid chromatography (HPLC) method for the determination of formaldehyde in flour samples has been developed. Formaldehyde was quantified after derivatization with a readily available reagent, 2,4,6-trichlorophenyl hydrazine (TCPH) under basic conditions. The formaldehyde-TCPH derivative was eluted with chromatographic mobile phase of 70:30 (v/v) acetonitrile:water at a flow rate of 1.0 mL min^{-1}; wavelength, 222 nm; injection volume, 50 µL, using a C18 ODS Hypersil column (250 mm × 4.5 mm, 5 µm). The calibration curve was linear over the range of 0.001-10 µg mL^{-1} with R^2 = 0.999. Recoveries at three different concentration levels (0.1, 1.0 and 5 µg mL^{-1}) ranged from 92.0-101.7% with RSD less than of 2.2%. The limit of detection (LOD) and limit of quantification (LOQ) were 0.3 and 1.0 ng mL^{-1}, respectively. The developed method was used for the determination of formaldehyde in various flour-based samples.

Keywords: HPLC, Flour, Derivatization, Formaldehyde, 2,4,6-Trichlorophenyl hydrazine.

INTRODUCTION

Carbonyl compounds are widely found in food products. They are originally derived from raw materials, alcoholic fermentation, or even from chemical reactions (Maillard reactions), lipid oxidation, Strecker degradation and aldol condensation [1]. Formaldehyde recently gained a lot of attention due to its extensive use in food products and potential carcinogenic properties [2]. Exposure of formaldehyde causes irritation of the eyes, headache, nausea, allergic skin reactions and drowsiness [3]. Formaldehyde is usually added in controlled amounts to food-products such (*e.g.* meat, mushroom, beverages, bean curd, vermicelli and hydrated foods) as an antiseptic to keep them pleasant [4-6]. The US Environmental Protection Agency (USEPA) has set an acceptable daily intake of 0.2 mg kg^{-1} body weight for formaldehyde. Therefore, at exposures greater than the daily dose, the potential for the adverse health effects increases [7]. Due to its widespread use, an effective method for the determi-nation of formaldehyde in flour is needed.

Several analytical methods have been reported for the determination of formaldehyde, such as spectrophotometry [8-12], thin layer chromatography [13,14], gas chromatography [15,16], fluorometry [17,18], biosensor [19], capillary electrophoresis [20], high-performance liquid chromatography [7,21-26], gas chromatography [15,27,28] and isotope dilution mass spectrometry [29]. Most of the spectrophotometric methods have been developed based on formaldehyde reactions with reagents (*e.g.* purpald) to form coloured derivatives [1]. Currently, HPLC-fluorescence detection employing Tagging reagent 2-[2-(7*H*-dibenzo[*a*,*g*]carbazol-7-yl)ethoxy]ethyl carbonylhydrazine as derivatization reagent is the most commonly method for the determination of formaldehyde [22]. The derivatization step is necessary to improve the sensitivity of the method. Lately, 2,4-dinitrophenylhydrazine (DNPH) reagent become a closely controlled item but is not commercially available. The main objective of this study is therefore to develop a new and sensitive HPLC method for the determination of formaldehyde by chemical derivatization with TCPH in flour-based samples using a more

readily available derivatization reagent. This reagent was introduced to reduce the problems experienced using DNPH and GC analysis. Moreover, the reaction using DNPH takes place under strongly acidic conditions. Although this reagent has been used with GC analysis, elimination of excess DNPH is required prior to injection to avoid detector and column deterioration. Thus, frequent cleaning of the inlet liner is deemed necessary. Interest in the study stems from the isolated reports on the detection of formaldehyde in flour samples.

EXPERIMENTAL

Chemicals and reagents were used as received without additional purification. Formaldehyde (37%) was purchased from Merck (Darmstadt, Germany). Acetonitrile (HPLC grade) was purchased from Fisher-Scientific (UK). TCPH 99% was supplied from Merck (Switzerland). Sodium hydroxide and 4-hydrazineobenzenesulfonic acid were purchased from Acros Organics (USA). Chromotropic acid disodium salt and pararosaniline were purchased from Merck (USA), while 2,4-dinitrophenylhydrazine (DNPH) was supplied from Ajax Chemicals (Australia). 4-(Trifluoromethoxy)phenylhydrazine hydrochloride and 1,1-diphenylhydrazine hydrochloride were purchased from Fisher-Scientific (USA). Ultrapure water (18 Ω cm^{-1}, Nanopure Diamond, Barnstead, USA) was used throughout.

Instrumentation and chromatographic conditions: Chromatographic analysis was performed using a Waters Alliance HPLC system (model 2695) (Milford, USA) equipped with Waters photodiode array detector (PDA) (model 2998) Chromatographic separation in the isocratic mode was carried out using a C18 ODS Hypersil column (250 mm × 4.5 mm, 5 µm) (Thermo Fisher Scientific, USA) at ambient temperature. The mobile phase was 70:30 (v/v) acetonitrile: water at a flow rate of 1.0 mL min^{-1}; wavelength, 222 nm; injection volume, 50 µL. Unless otherwise stated, preparations and determinations were done in triplicates.

Preparation of standard solution: Stock solution of 0.1% (m/v) of formaldehyde was prepared in water (27 mL of 37% formaldehyde solution was diluted to 100 mL with water to obtain a 10% intermediate stock solution, further 1 mL was diluted to 100 mL with water). Since formaldehyde is volatile, the exact concentration of formaldehyde 37% standard solution was determined prior to analysis using the AOAC method [30]. The concentration of formaldehyde solution used was found to be 37.10%. The stock solution was diluted with water to yield the appropriate working standard solutions. To obtain the calibration curve, the working solution (1 mL) was derivatized and injected into the HPLC unit. Standard solutions were kept in refrigerator after using.

Preparation of flour samples: Various types of flour samples were obtained from local markets of five countries. Flour sample (1 g) was accurately weighed and transferred to a 25 mL volumetric flask with the aid of 20 mL acetonitrile and sonicated for 10 min and then was topped-up to the mark with acetonitrile. The mixture was centrifuged (4000 rpm) for 10 min. The supernatant was used for derivatization as prescribed under derivatization procedure and then injected into the HPLC unit.

Derivatization procedure: In a sample vial, standard or sample extract (1 mL) was mixed with 1 mL of the derivatization solution (75 mg of TCPH 99% in 50 mL acetonitrile, daily prepared) and 0.1 mL of 0.5 M NaOH solution was added. The mixture was heated (70 ºC) on a water bath for 20 min. Then the solution was left to remain at room temperature for 10 min. The mixture was then injected into the HPLC unit. The proposed reaction occurred between formaldehyde and TCPH is shown in Fig. 1.

RESULTS AND DISCUSSION

As mentioned earlier, the non-commercial availability of the common derivatization reagent 2,4-dinitrophenylhydrazine (DNPH) necessitates the exploration of alternative substitutes in the HPLC determination of formaldehyde. Due to time constraints, the use of similar reagents becomes our strategy. Thus, several hydrazones compounds such as 4-hydrazineobenzene sulfonic acid, 1,1-diphenylhydrazine hydrochloride, 4-(trifluoromethoxy)phenylhydrazine hydrochloride, chromotropic acid disodium salt, pararosaniline, DNPH and TCPH were investigated.

Among the reagents investigated, only TCPH was able to achieve the best sensitivity compared with the other reagents studied and moreover, it was selected as a replacement for the common reagent DNPH that have been used previously. In the proposed method, the derivatization procedure took place in simple steps as prescribed under section derivatization procedure. In the work of Wang *et al*. [3], one step involves the addition of a surfactant. Moreover, in the work of Xu *et al*. [1], the derivatization procedure involves extra steps such as using

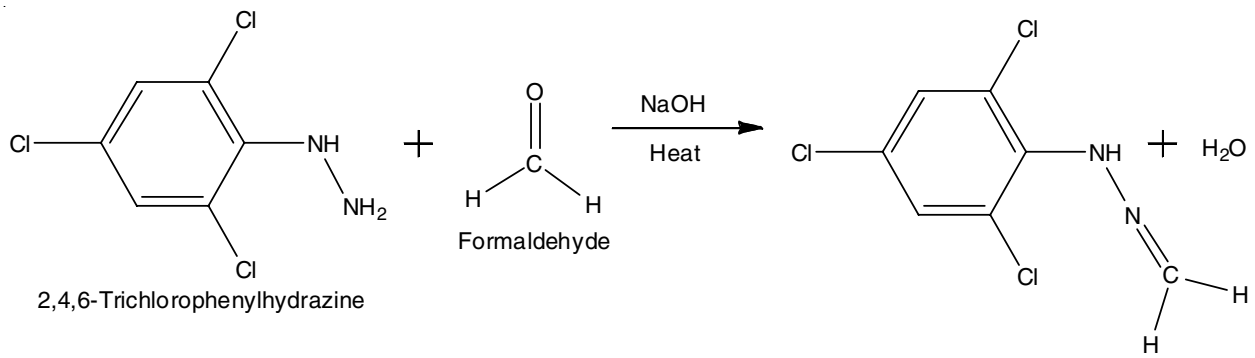

Fig. 1. Derivatization reaction scheme

microwave-assisted derivatization and dispersive liquid-liquid microextraction, which make the two procedures longer and time consuming when compared with the proposed one.

Optimization of derivatization conditions: Several parameters affecting the derivatization conditions were studied and optimized (*e.g.* temperature, heating time, derivatization agent concentration and sodium hydroxide concentration).

Effect of heating temperature: The effect of temperature (50-90 ºC) on reaction yield was studied. It can be shown that the peak area rises from 50 to 70 ºC with increase in temperature and then gradually decreases (Fig. 2). Further heating may cause decomposition of the derivative and thus 70 ºC was selected for the following experiments.

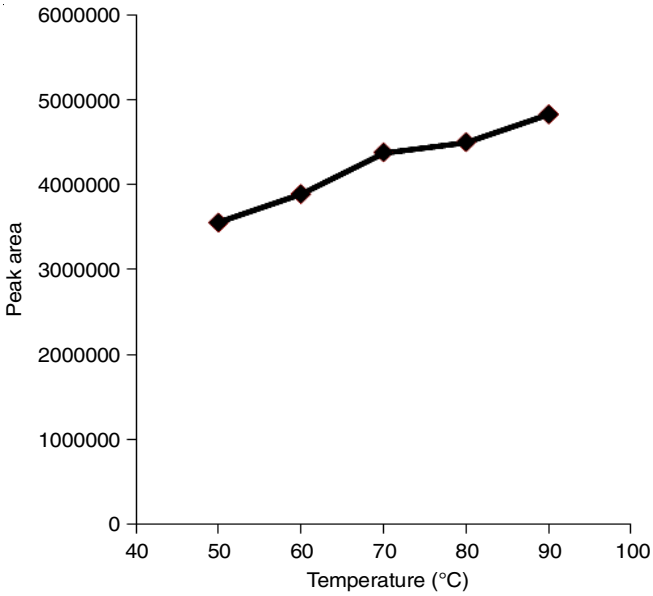

Fig. 2. Effect of heating temperature on peak area of formaldehyde derivative. Experimental conditions: heating time: 15 min; NaOH solution (1M) and TCPH: 1500 µg mL^{-1}

Effect of heating time: The effect of heating time was studied from 5-25 min. The highest peak area was observed when heated for 20 min (Fig. 3), after that the area decreased sharply due to the completion of the reaction. Therefore heating for 20 min was optimized for the further studies.

Effect of 2,4,6-trichlorophenyl hydrazine (TCPH) concentration: Generally, the derivatization efficiency improved as the derivatization reagent concentration increases, whereas overloaded of the derivatization reagents may affect the determination of the target analyte [31]. The effect of concentration of TCPH (500-2500 µg mL^{-1}) was studied (Fig. 4). The peak area increased sharply with increasing TCPH concentration until 1500 µg mL^{-1}, followed by an abrupt decrease (Fig. 4). Thus, 1500 µg mL^{-1} was chosen for the next experiments.

Effect of NaOH concentration: The Schiff base reaction between formaldehyde and TCPH that leads to the formation of the derivative is known to favour alkaline medium. The effect of different sodium hydroxide solution concentrations (0.1-1.0 M) were studied. Sodium hydroxide solution (0.5 M) was selected as the highest peak area was obtained (Fig. 5). A sharp decrease in signals when NaOH concentration greater

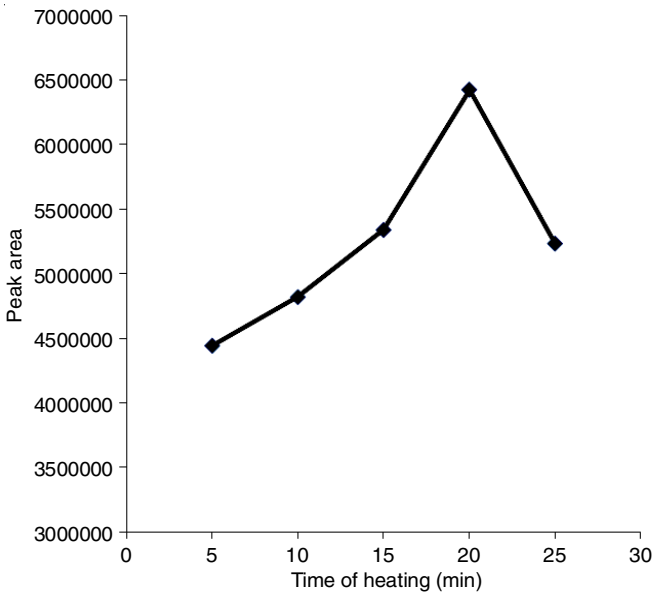

Fig. 3. Effect of time of heating on peak area of formaldehyde derivative. Experimental conditions: heating temperature: 70 ºC; NaOH (1M) and TCPH: 1500 µg mL^{-1}

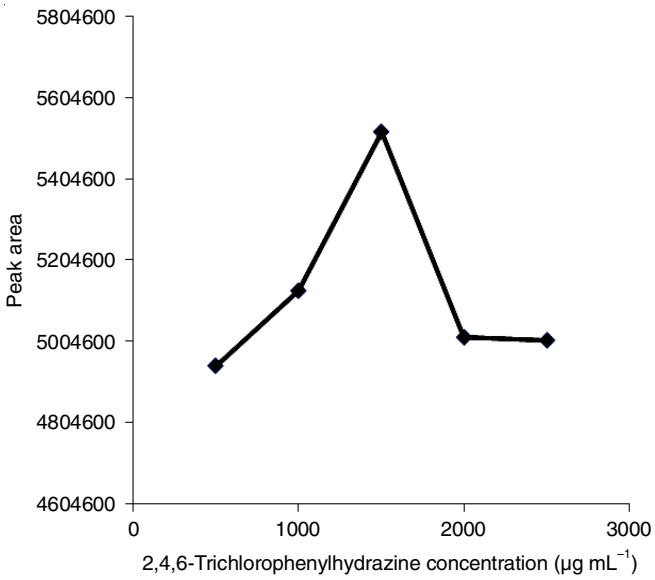

Fig. 4. Effect of derivatization agent concentration on peak area of formaldehyde derivative. Experimental conditions: heating temperature: 70 ºC; heating time, 20 min and NaOH solution (1 M)

than 0.5 M used, which might be due to the derivative product decomposition.

Adopted derivatization conditions: Based on the above experiments, the adopted conditions were derivatization temperture 70 ºC, duration of heating, 20 min; TCPH concentration, 1500 µg mL^{-1}; and NaOH concentration, 0.5 M.

Stability of derivative: The stability of the derivative was investigated by analyzing the formaldehyde derivative at a concentration of 0.6 µg mL^{-1}. When the derivatization reaction was prepared, then the derivative solution formed stood for 0, 15, 30, 45, 60, 75, 90, 105 and 120 min and then analyzed. The derivative was found to be stable under room temperature for the whole range tested (Fig. 6).

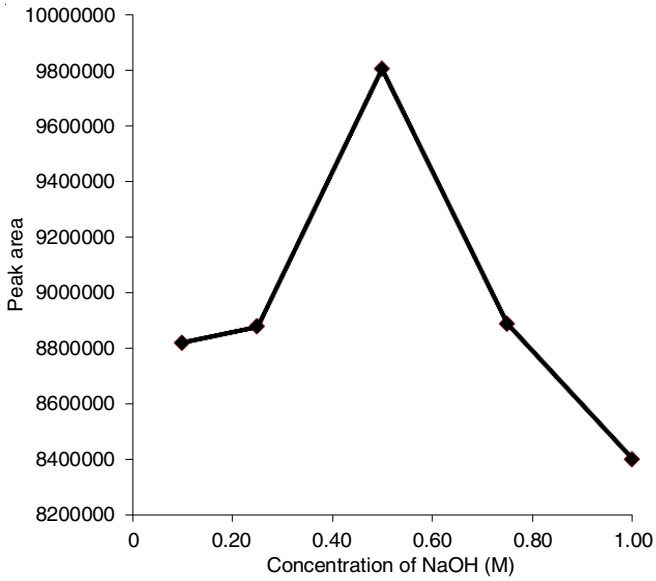

Fig. 5. Effect of NaOH concentration on peak area of formaldehyde derivative. Experimental conditions: heating temperature: 70 ºC; heating time: 20 min and TCPH: 1500 µg mL^{-1}

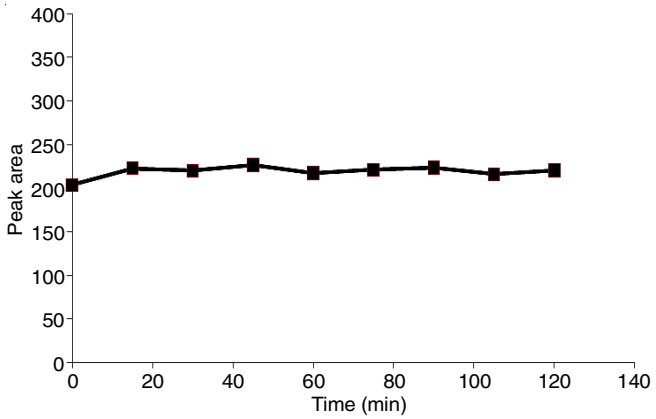

Fig. 6. Stability of formaldehyde derivative (0.6 µg mL^{-1}). Experimental conditions: heating temperature: 70 ºC; heating time: 20 min; TCPH: 1500 µg mL^{-1} and NaOH solution: 0.5M

Method validation

Calibration curve, limits of detection and quantification: The calibration curve was obtained by using eight different concentrations (0.001-10 µg mL^{-1}) of the standard and was performed in triplicate. Calibration plot with the equation y = 938024*x* + 102095 (R^2 = 0.999) was obtained by plotting the peak area (y) as a function of analyte concentration (*x*) in µg mL^{-1}. The limit of detection (LOD) was 0.3 ng mL^{-1}, while the limit of quantification (LOQ) was 1.0 ng mL^{-1}. LOD was calculated as the amount of the injected sample to yield a signal-to-noise ratio of 3 and the LOQ was taken as the amount of the injected sample to give a signal-to-noise ratio of 10. However, the proposed method is sensitive when compared with the reported HPLC-UV method, when DNPH is used as the standard and the common derivatization agent [3], (LOD was 0.30 and 0.70 ng mL^{-1}, respectively). Moreover, the LOQ (1.0 ng mL^{-1}) obtained by the proposed method is lower than the values obtained by the standard method (2.0 ng mL^{-1}) [3].

In addition, the current method was sufficiently sensitive and quick compared to the reported work [32]. For instance, in the present study, the sample preparation time consumed till injecting it in the HPLC unit was about 50 min, while the time used by Lawrence & Iyengar [32] was more than 1 h in the step of steam distillation only excluding the extraction time used in the separator funnel and the dryness steps conducted again using the rotary vacuum evaporator.

Precision: Intra- day and inter-day variations of peak area and retention time were performed to determine the accuracy of the method produced by testing three concentrations (0.1, 1 and 5 µg mL^{-1}) of standard solutions. The intra-day precision was performed by evaluating the nine replicates on the same day. In the same way, inter-day precision were performed over three consecutive days. Intra-day precision for both retention times and peak areas, expressed as a percent relative standard deviation, (%RSD) ranged from 0.04 to 0.28 and 1.0 to 1.8%, respectively (Table-1), while inter-day precision ranged from 0.32 to 0.44 and 1.5 to 2.7% for both retention times and peak areas, respectively, suggesting the good precision of the method being developed.

TABLE-1
INTRA- AND INTER-DAY PRECISION FOR THE DETERMINATION OF FORMALDEHYDE

Analyte (µg mL^{-1})	RSD (%)	
	Retention time	Peak area
Intra-day precision (n = 9)		
0.1	0.15	1.7
1.0	0.04	1.0
5.0	0.28	1.8
Inter-day precision (n = 27)		
0.1	0.32	2.7
1.0	0.34	1.5
5.0	0.44	2.0

n = no. of introductions, (three preparations for each concentration)

Accuracy: Accuracy study was performed by weighing 1 g of flour sample and spiked with three different concentration levels (0.1, 1.0 and 5 µg mL^{-1}) of standard formaldehyde. Each concentration was prepared in triplicate. The obtained results were 101.7, 93.8 and 92.0%, respectively (Table-2).

TABLE-2
ACCURACY RESULTS FOR THE DETERMINATION OF FORMALDEHYDE IN SPIKED SAMPLES

C_{added} (µg mL^{-1})	C_{found} (µg mL^{-1})	Recovery (%)	RSD*
0.1	0.102	101.70	2.20
1.0	0.938	93.80	1.60
5.0	4.600	92.00	1.80

*n = 3

Analysis of flour samples: The developed method was successfully applied for the determination of formaldehyde in several different flour samples (Table-3). Fig. 7 shows typical chromatograms for standard and samples. The percentage of formaldehyde was calculated using the following equation:

$$\text{Formaldehyde (\%)} = \frac{C \times V}{W}$$

TABLE-3
RESULTS FOR THE ANALYSIS OF FLOUR SAMPLES

S. No.	Producing area	Matrix	Concentration (mg kg^{-1} ± SD)	S. No.	Producing area	Matrix	Concentration (mg kg^{-1} ± SD)
1	Selangor, Malaysia	Custard	19.72 ± 0.88	62	Selangor, Malaysia	NA	29.55 ± 1.27
2	Selangor, Malaysia	Custard	12.82 ± 0.53	63	Butterworth, Malaysia	NA	19.42 ± 0.23
3	Selangor, Malaysia	Wheat	8.11 ± 0.34	64	Butterworth, Malaysia	NA	35.30 ± 0.59
4	Selangor, Malaysia	Wheat	15.00 ± 1.70	65	Kuala Lumpur, Malaysia	NA	23.96 ± 0.85
5	Bangkok, Thailand	Starch	10.48 ± 0.27	66	Selangor, Malaysia	NA	24.92 ± 0.79
6	Kuala Lumpur, Malaysia	Corn flour	6.03 ± 0.28	67	Kuala Lumpur, Malaysia	NA	11.83 ± 0.51
7	Kuala Lumpur, Malaysia	Custard	8.72 ± 0.15	68	Penang, Malaysia	NA	8.69 ± 0.60
8	Bukit Mertajam, Malaysia	Custard	40.76 ± 2.08	69	Bukit Mertajam, Malaysia	NA	11.75 ± 0.35
9	Penang, Malaysia	Potato	5.72 ± 0.23	70	Selangor, Malaysia	NA	8.51 ± 0.02
10	Manila, Philippines	Custard	10.81 ± 0.64	71	Kedah, Malaysia	NA	9.48 ± 0.77
11	Penang, Malaysia	NA	5.33 ± 0.06	72	Kedah, Malaysia	NA	11.18 ± 0.50
12	Penang, Malaysia	Rice flour	7.40 ± 0.31	73	Kedah, Malaysia	NA	43.59 ± 3.92
13	Hague, The Netherlands	Potato starch	7.76 ± 0.22	74	Kedah, Malaysia	NA	BLD
14	Bangkok, Thailand	Rice flour	6.37 ± 0.09	75	Kedah, Malaysia	NA	BLD
15	Alor Star, Malaysia	NA	6.17 ± 0.03	76	Kedah, Malaysia	NA	19.50 ± 1.48
16	Alor Star, Malaysia	Peanut flour	5.13 ± 0.28	77	Kedah, Malaysia	NA	37.84 ± 3.03
17	Alor Star, Malaysia	NA	5.87 ± 0.38	78	Kedah, Malaysia	NA	0.21 ± 0.01
18	Alor Star, Malaysia	Custard	5.63 ± 0.14	79	Penang Malaysia	NA	BLD
19	Alor Star, Malaysia	Rose flour	12.05 ± 0.60	80	Seoul, Korea	NA	0.05 ± 0.06
20	Alor Star, Malaysia	NA	5.61 ± 0.32	81	Bangkok, Thailand	NA	2.79 ± 0.03
21	Alor Star, Malaysia	NA	4.97 ± 0.25	82	Penang, Malaysia	NA	4.69 ± 0.29
22	Alor Star, Malaysia	NA	24.13 ± 1.26	83	Kedah, Malaysia	NA	3.17 ± 0.18
23	Alor Star, Malaysia	NA	4.60 ± 0.18	84	Johor Bahru, Malaysia	NA	ND
24	Selangor, Malaysia	Red millet flour	ND	85	Kedah, Malaysia	NA	6.03 ± 0.12
25	Selangor, Malaysia	Corn starch	5.37 ± 0.17	86	Bukit Mertajam, Malaysia	NA	47.67 ± 1.40
26	Selangor, Malaysia	NA	ND	87	Selangor, Malaysia	NA	49.70 ± 1.62
27	Selangor, Malaysia	Rice flour	9.66 ± 0.21	88	Bukit Mertajam, Malaysia	NA	29.35 ± 1.53
28	Selangor, Malaysia	NA	ND	89	Kuala Lumpur, Malaysia	NA	8.21 ± 0.69
29	Seoul, Korea	Corn starch	ND	90	Bukit Mertajam, Malaysia	NA	3.54 ± 0.20
30	Penang, Malaysia	NA	ND	91	Bukit Mertajam, Malaysia	NA	5.01 ± 0.15
31	Bukit Mertajam, Malaysia	Rice flour	2.99 ± 0.12	92	Bukit Mertajam, Malaysia	NA	7.52 ± 0.12
32	Bukit Mertajam, Malaysia	NA	25.33 ± 0.57	93	Bukit Mertajam, Malaysia	NA	11.22 ± 0.44
33	Bukit Mertajam, Malaysia	NA	23.23 ± 1.12	94	Bukit Mertajam, Malaysia	Cake flour	2.00 ± 0.12
34	Bukit Mertajam, Malaysia	NA	11.03 ± 0.35	95	Kedah, Malaysia	Corn flour	5.53 ± 0.26
35	Kuala Lumpur, Malaysia	NA	8.13 ± 0.47	96	Bukit Mertajam, Malaysia	NA	6.49 ± 0.28
36	Kuala Lumpur, Malaysia	NA	ND	97	Kedah, Malaysia	NA	23.77 ± 0.23
37	Kuala Lumpur, Malaysia	NA	3.97 ± 0.08	98	Bukit Mertajam, Malaysia	NA	18.96 ± 0.44
38	Manila, Phillippines	NA	3.85 ± 0.06	99	Bukit Mertajam, Malaysia	NA	34.38 ± 2.11
39	Bukit Mertajam, Malaysia	NA	21.67 ± 1.21	100	Bukit Mertajam, Malaysia	NA	15.44 ± 0.51
40	Kuala Lumpur, Malaysia	NA	10.84 ± 0.90	101	Kuala Lumpur, Malaysia	NA	16.49 ± 1.43
41	Bukit Mertajam, Malaysia	NA	17.41 ± 1.17	102	Kuala Lumpur, Malaysia	NA	14.12 ± 0.97
42	Bukit Mertajam, Malaysia	NA	11.74 ± 0.20	103	Bukit Mertajam, Malaysia	Cake vanilla	56.97 ± 2.91
43	Bukit Mertajam, Malaysia	NA	14.18 ± 1.29	104	Bukit Mertajam, Malaysia	Dark chocolate	32.76 ± 1.99
44	Bukit Mertajam, Malaysia	NA	25.32 ± 1.40	105	Bukit Mertajam, Malaysia	Pineapple	98.70 ± 5.71
45	Bukit Mertajam, Malaysia	NA	18.17 ± 0.64	106	Bukit Mertajam, Malaysia	NA	14.75 ± 0.64
46	Selangor, Malaysia	NA	38.48 ± 0.89	107	Bukit Mertajam, Malaysia	NA	17.14 ± 0.13
47	Selangor, Malaysia	NA	17.18 ± 1.10	108	Bukit Mertajam, Malaysia	NA	40.37 ± 2.72
48	Selangor, Malaysia	NA	23.27 ± 1.14	109	Bukit Mertajam, Malaysia	Butter cake chocolate	14.40 ± 0.03
49	Selangor, Malaysia	NA	267.30 ± 24.13	110	Bukit Mertajam, Malaysia	NA	47.27 ± 1.32
50	Selangor, Malaysia	NA	26.07 ± 1.32	111	Bukit Mertajam, Malaysia	NA	43.87 ± 1.13
51	Bukit Mertajam, Malaysia	NA	33.39 ± 1.47	112	Bukit Mertajam, Malaysia	NA	16.02 ± 0.72
52	Butterworth, Malaysia	NA	18.73 ± 0.22	113	Bukit Mertajam, Malaysia	NA	22.49 ± 0.79
53	Butterworth, Malaysia	NA	50.84 ± 0.54	114	Bukit Mertajam, Malaysia	Pancake	82.76 ± 4.36
54	Butterworth, Malaysia	NA	ND	115	Kedah, Malaysia	NA	58.74 ± 2.80
55	Bukit Mertajam, Malaysia	NA	23.18 ± 0.40	116	Bukit Mertajam, Malaysia	Cake	72.78 ± 2.29
56	Butterworth, Malaysia	NA	19.26 ± 1.72	117	Kedah, Malaysia	Orange cake	14.39 ± 0.80
57	Bukit Mertajam, Malaysia	NA	15.37 ± 0.66	118	Kedah, Malaysia	Strawberry cake	60.29 ± 1.56
58	Butterworth, Malaysia	NA	2.31 ± 0.06	119	Kedah, Malaysia	Chocolate Cake	20.30 ± 1.50
59	Butterworth, Malaysia	NA	13.54 ± 0.75	120	Kedah, Malaysia	Vanilla cake	46.51 ± 2.66
60	Butterworth, Malaysia	NA	25.31 ± 1.29	121	Bukit Mertajam, Malaysia	Cake	57.94 ± 2.79
61	Kuala Lumpur, Malaysia	NA	19.71 ± 1.73	122	Bangkok, Thailand	Rice flour	10.71 ± 0.35

ND = Not detected; NA = Not available; BLD = Below limit of detection

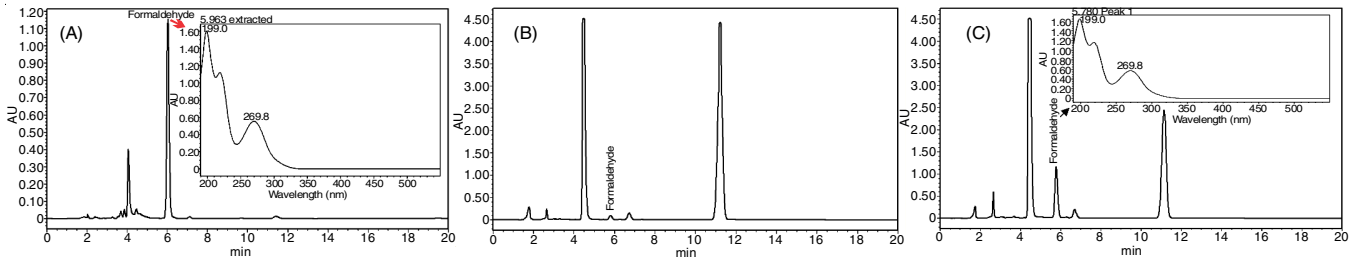

Fig. 7. Typical chromatograms obtained upon running under the validated conditions. (A) Standard injection, (B) sample # 1, (C) sample # 49

TABLE-4
COMPARISON OF THE DEVELOPED METHOD WITH OTHER REPORTED METHODS FOR THE DETERMINATION OF FORMALDEHYDE

Method	Matrix	Extraction time (min)	Linearity (ng mL^{-1})	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Recovery (%)	Ref.
Current method	Flour samples	–	1-10000	0.3	1.0	92.0-101.7	–
Spectrophotometric	Food samples	3	10000-100000	80	–	98.3-101.1	[10]
FIA-SPD[a]	Alcohol fuels	40	50-1500	30	–	–	[9]
FIA-FD[b]	Alcoholic beverages	–	up to 30300000	3.1	–	–	[17]
Mini-CE-ED[c]	Food	60	50-50000	9.1	–	96.1-106.0	[20]
HPLC-DAD[d]	Spirits	20	250-2000	24	80	93.4-100.4	[7]
IL-based DLLME[e]	Beverages	1.5	0.50-50	0.12	–	84.9-95.1	[1]
HPLC-UV[f]	Beer	20	2-1000	0.6	–	94.1-102.8	[3]

[a]Flow injection analysis coupled with spectrophotometric detection; [b]Flow injection analysis coupled with fluorimetric detection; [c]Miniaturized capillary electrophoresis coupled with electrochemical detection; [d]High performance liquid chromatography coupled with diode array detector; [e]Ionic liquid-based dispersive liquid-liquid microextraction; [f]High performance liquid chromatography coupled with ultraviolet detector.

where C is the concentration of sample (μg mL^{-1}) obtained from the calibration curve, V is the final volume (mL) and W is the sample weight (g).

Comparison with the reported methods: The analytical characteristics of the newly developed method were compared to the other methods mentioned (Table-4). When the newly method was applied, the LOD was lower compared with the other reported methods and comparable with the work of Xu *et al*. [1]. This is mainly due to the good enhancement achieved due to the derivatization step using TCPH. To the best of our knowledge this is the first analytical technique which describes the derivatization of formaldehyde using TCPH reagent. Moreover, comparable recoveries (92.0-101.7%) were obtained using simple sample preparation compared with other methods that employ different and sometimes tedious sample preparation techniques. Moreover, few studies [21,33] demostrated that the use of DNPH as a derivatizing agent has led to a small formaldehyde peak behind the large reagent peak in blank chromatography reagent, although several techniques have been implemented to address this problem but without success.

Conclusion

A simple and sensitive method for the determination of formaldehyde in flour samples proceeded by a derivatization step using TCPH, which is comparatively low-cost reagents compared with other reagents, has been developed and validated. The developed method gives low detection limit, good linearity, precision and recovery over the studied concentration range. Moreover, the method exhibits sufficient sensitivity to be used for the determination of formaldehyde in flour stuffs samples. The contents of formaldehyde in these samples were in the range of 0.05-267.3 mg kg^{-1}. The good sensitivity achieved, as indicated by the results obtained, enables the method to be comparable in terms of sensitivity to the reported IL-based DLLME [1] and Mini-CE-ED [20] or FIA-SPD methods [9]. Based on the results obtained, the newly developed method may be a useful method for the determination of other carbonyl compounds.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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