

Research Article

Development and Validation of UV-Visible Spectrophotometric Method for the Determination of 5-Hydroxymethyl Furfural Content in Canned Malt Drinks and Fruit Juices in Ghana

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A simple, rapid, accurate, and less expensive spectrophotometric method has been developed for the quantitation of 5-hydroxymethyl furfural (5-HMF) levels in canned malt drinks and fruit juice drinks sampled in the Kumasi Metropolis, Ghana. The quantitation is based on the selective maximum absorption of ultraviolet radiation by 5-HMF at the wavelength (λ_{\max}) of 284 nm using acetonitrile: water (50 : 50 v/v) as the solvent system. The method was established to be specific, precise, and accurate over a concentration range of 0.001 mg/ml–0.02 mg/ml. 5-HMF levels in fruit juice samples (A1–A10) were between 0.132 mg/ml and 0.438 mg/ml, and these levels were shown to be comparable ($t = 2.200$; $p = 0.0553$) to the contents in the canned malt samples (M1–M10) which were between 0.3140 mg/ml and 0.7170 mg/ml. The study failed to show any dependence of 5-HMF levels on the composition of the product as well as the manufacturing process adopted. The length of storage did also not significantly affect the 5-HMF levels in the products.

1. Introduction

Processed foods including fruit juices and malt-based drinks have become a common stay on the Ghanaian market. Usually, these products are subjected to processing stages such as pasteurization, baking, roasting, and sterilization, in order to obtain desirable sensory properties or texture features, assure microbiological safety, and eliminate enzymatic activities, hence the name, processed foods. These treatments are however shown to result in the production of some undesired by-products, such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, acrylamide, chloropropanols, furan, transfatty acids, nitrosamines, and biogenic amines [1, 2], which may show up in the finished products. It is thus, necessary to check these products for these undesired products, as they may pose serious health risk [2–4].

The influx and increased consumption of processed products, as a result of lifestyle modifications, trade liberalization, and urbanization [5], brings into question issues of safety with the use of these products.

5-hydroxymethylfurfural (5-HMF) is one of the known compounds formed during the thermal treatments of carbohydrate-containing foods (for example, fruit juices and malt-based drinks) because of Maillard reaction, a non-enzymatic browning reaction [6, 7]. 5-HMF is generally known as a quality indicator during storage for a wide range of food products, especially carbohydrates [1, 8] or an indicator of deterioration, resulting from excessive heating [9]. It also serves as an indicator of stress to food processing [1]. The content of 5-HMF in food varies considerably depending on the production technology as well as storage conditions. Although its toxicological significance remains uncertain [10], it still remains relevant to assess its levels in

processed food products in attempts to safeguard food safety [3]. For instance, some studies in rats and dogs have showed that 5-HMF can be toxic when given at doses in excess of 75 mg kg^{-1} body weight [11, 12].

Several analytical methods have been developed and used to identify and quantify 5-HMF content in various food products, including spectrophotometry and colourimetry [13–15], and reverse-phase liquid chromatography [3, 6, 16], together with its hyphenated systems [17]. The chromatographic methods are currently the most commonly used ones, because of the challenges that were associated with the use of the spectrophotometric methods. Some of these included lack of specificity, process being cumbersome, and use of toxic or hazardous chemicals [18]. That notwithstanding, spectrophotometry may provide relatively cheaper options compared to the capital-intensive chromatographic methods. Hence, the purpose of this study was to develop and validate a simple, fast, and specific UV-Vis spectrophotometric method, void of the challenges raised by previous authors, for assessing the levels of 5-HMF in selected fruit juices and malt-based beverages on the Ghanaian market.

2. Materials and Methods

2.1. Samples. A total of 20 samples of liquid food products were randomly collected in commercially available quantities, from different retail outlets in the Kumasi Metropolis during the period from February to March 2018. The collected samples included canned malt drinks (M1–M10, $N = 10$) and fruit juices (A1–A10, $N = 10$). Basic information on the products, including, manufacturing and expiry dates, name of manufacturer, and origin were recorded. The samples were analyzed immediately after being purchased; otherwise, they were stored at -17°C for later analysis.

2.2. Chemicals and Reagents. Fisher Scientific (United Kingdom) borosilicate glass volumetric flasks (10 ml, 250 ml, and 500 ml, Grade A), pipettes (2 ml and 10 ml, Grade A), measuring cylinders (100 ml, Grade A), conical flasks (250 ml, Grade B), and scintillation vials were used for the study. Solvents employed included acetonitrile (HPLC grade, Fisher Scientific, UK) and distilled water (in-house produced).

Working standard of 5-hydroxymethylfurfural (98%) was purchased from Fluorochem (United Kingdom).

2.3. Instrumentation. Spectrophotometric analysis was performed using a single beam 7315 UV-visible spectrophotometer (Jenway Scientific Equipment, United Kingdom), fitted with a 10×10 mm cuvette holder, and scanned within a wavelength range of 198 nm–1000 nm produced from a Xenon lamp. The results of the analysis were recorded on a windows computer system, using the Jenway 73 series. Analytical balance (Kern, Germany/WD140050809), centrifuge (Model C257-120, Wagtech, UK) and a refrigerator

(Model WRT348FMEZ, Whirlpool, USA) were among equipment employed for the study.

2.4. Preparation of Solution. A stock solution of the working standard, 5-HMF (0.1 mg/ml), was prepared with an acetonitrile:water (50:50 v/v) solvent system, in a volumetric flask and transferred into a labelled scintillation vial. The solution was then kept under refrigeration (2°C – 8°C) away from sunlight for later use. Sample solutions for method development and validation were prepared by pipetting determined quantities of the stock solution with the same solvent system.

Determined aliquots of each of the canned malt samples were pipetted to prepare sample solutions (M1–M10) with distilled water in volumetric flasks. For fruit juice samples (A1–A10), aliquots pipetted were centrifuged at 5000 rpm for 5 minutes, after which the supernatants were collected and diluted with distilled water.

2.5. Method Development. The spectrophotometric methods developed for content assay of 5-HMF were based on the principle that the compound was able to absorb ultraviolet radiation at a wavelength (λ_{max}) of 284 nm, with little interference from the constituents of the malt drinks and the fruit juices. The direct method of assay therefore involved dilution of the initially prepared malt sample, while the fruit juice was centrifuged before dilution. Both types of samples were further diluted to specified concentrations and absorbances taken at 284 nm, without further sample preparations.

2.6. Analytical Method Validation. The developed methods were then validated in accordance with recommendations from the ICH guidelines, for specificity, precision, linearity and range, robustness, and stability of the sample solutions.

2.6.1. Specificity. The specificity of the direct spectrophotometric method was assessed by comparing the absorbances obtained from the solvent system alone (placebo), placebo together with constituents of the matrix, placebo with 5-HMF, and a combination of all the above. The results were then analyzed using ANOVA (Figures 1 and 2; Table 1).

2.6.2. Linearity and Range. In testing for linearity, determined aliquots were pipetted from the stock solution (0.1 mg/ml) to prepare solutions of concentrations ranging from 0.001–0.02 mg/ml using acetonitrile:water (50:50 v/v). The absorbances of these solutions were recorded in replicates, using the same solvent system as the blank. Linearity was demonstrated from regression analysis (Table 2; Figure 3). The residuals of the absorbance were also plotted against concentration to further prove linearity (Figure 3).

2.6.3. Precision. The precision of the method was demonstrated by intra-assay and interassay studies. In the intraday studies, six replicate absorbance readings of 100% concentration (0.005 mg/ml) of 5-HMF were taken. In addition,

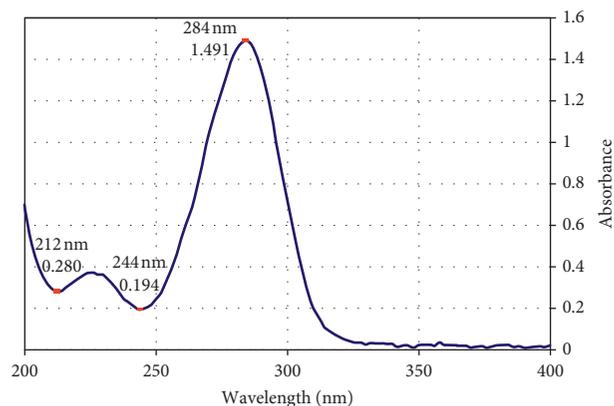


FIGURE 1: UV spectrum of 5-HMF in a fruit juice. Maximum absorption of the compound is observed to occur at 284 nm, which may be indicative of the presence of 5-HMF.

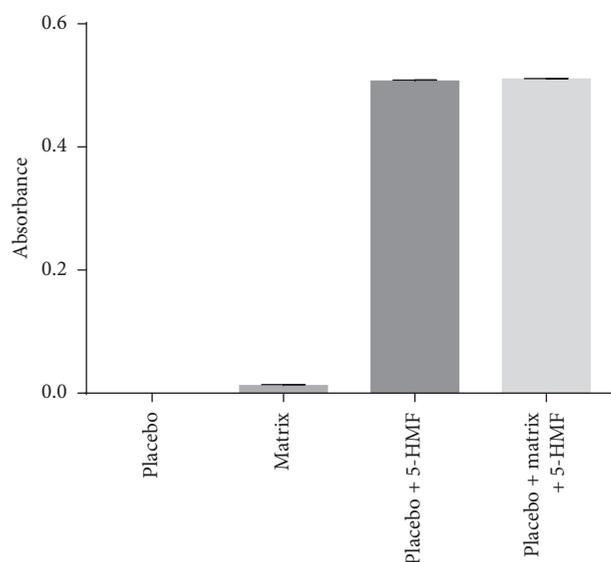


FIGURE 2: Mean absorbances \pm SD ($N = 5$) recorded under different test conditions, to prove specificity of the developed method.

TABLE 1: Results of specificity test for 100% concentration of 5-HMF in matrices ($N = 5$).

Parameter	Mean absorbance \pm SD (at 284 nm)
Placebo (solvent system)	0.00 \pm 0.00
Matrix (combination of ingredients present in products)	0.0122 \pm 0.0007348
Placebo + HMF	0.5070 \pm 0.0007071
Combination (placebo + matrix + HMF)	0.5102 \pm 0.0003
One-way ANOVA	$p < 0.0001$; $F_{(3,16)} = 285407$

absorbances of three different concentrations of 5-HMF, representing 80%, 100%, and 120% of the test solution (that is, 0.0025 mg/ml, 0.005 mg/ml, and 0.02 mg/ml), were also taken. In the interday variation studies, the solutions of same concentration (0.005 mg/ml) were prepared and analyzed in triplicates by 2 independent analysts, for three consecutive

TABLE 2: Results from regression analysis in the linearity test.

Best-fit values	
Slope	142.1 \pm 0.6579
Y-intercept when $X = 0.0$	-0.01806 \pm 0.006219
X-intercept when $Y = 0.0$	0.0001271
1/Slope	0.007036
R^2	0.9997
Sy.x	0.01847

days, and the absorbances were recorded. Results were analyzed by determining the mean absorbances and RSD from the replicate determinations (Table 3).

2.6.4. Accuracy. The accuracy of the developed method was established by determining percentage recoveries of prepared solutions of 5-HMF over a concentration range (0.001–0.005 mg/ml) in triplicates (Table 4).

2.6.5. Robustness. The robustness of the method was determined by investigating the change of wavelength on the absorbance and concentration of the sample. The results were analyzed using one-way ANOVA (Table 5).

2.6.6. Stability of Solution. The stability of the solutions employed in the method was assessed on two fronts: the stability of the working standard solution (0.005 mg/ml) prepared from the stock solution (0.1 mg/ml) over a 30-day period and the stability of the 100% concentration calibration solution (0.005 mg/ml) over an 11-day period. The results obtained were analyzed by plotting relative responses on each day and determining the confidence interval within which period, the prepared solutions may be used for analysis (Figure 4).

2.7. Analysis of Sampled Products. In order to accurately assay the sampled products, several concentrations of 5-HMF were prepared from the working standard solution (0.02 mg/ml, 0.01 mg/ml, 0.005 mg/ml, 0.002 mg/ml, 0.0025 mg/ml, and 0.001 mg/ml) to obtain the calibration curve. 1 ml of the malt samples was pipetted into 10 ml volumetric flasks and diluted with distilled water. From the resulting solution, 1 ml was pipetted and further diluted to 10 ml in another volumetric flask, and absorbance readings at 284 nm. For the fruit juice samples, 5 ml was pipetted after shaking and centrifuged in 20 ml tubes at 5000 rpm for 5 minutes. The supernatants were collected and diluted (1 in 50 dilutions) with distilled water. The absorbances of the resulting solution were recorded at 284 nm.

2.8. Statistical Evaluation of the Results. The results obtained from the method development and validation were analyzed using GraphPad Prism 6 for Windows (Version 6.01, GraphPad Software, 2012). Test results were expressed as means \pm SD and relative standard deviations (RSD), and also analyzed inferentially, using Student's t -test and one-way ANOVA (at 95% confidence level) to determine statistical

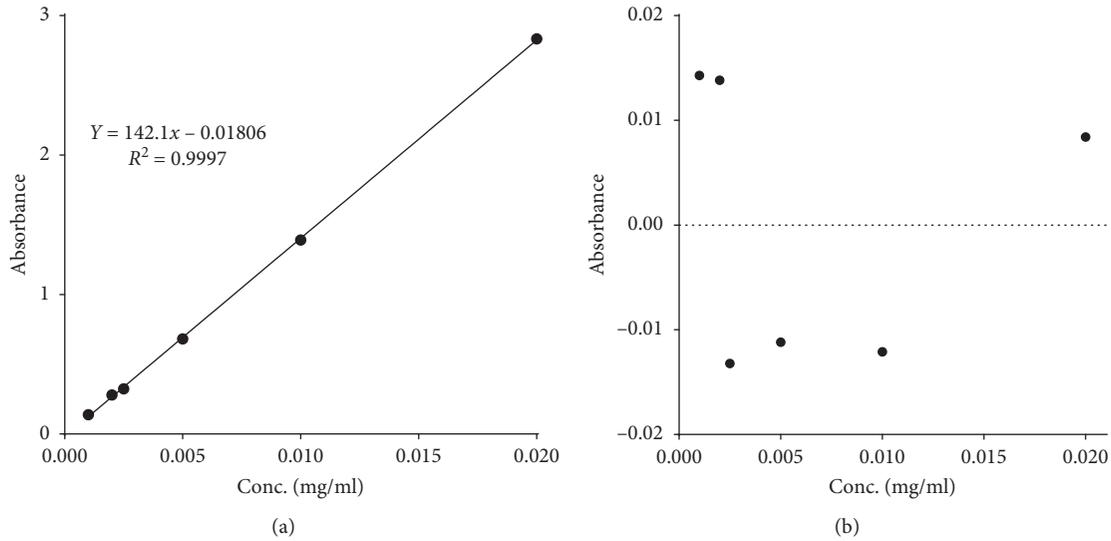


FIGURE 3: Proof of linearity and its corresponding concentration range for achieving the linearity. (a) Linearity plot involving the concentrations used (abscissa) and their corresponding mean absorbances \pm SEM (ordinate). (b) Residual plot showing the randomness of the plotted figures, which further proves linearity.

TABLE 3: Results showing precision of results from the developed method.

Precision parameters			Mean absorbance \pm SD	RSD (%)
Outcome from 6 replicate determinations			0.5040 \pm 0.00698	1.37
Intra-assay precision	Triplicate determinations from three different concentrations	0.0025 mg/ml	0.3240 \pm 0.001	0.31
		0.005 mg/ml	0.6813 \pm 0.010	1.50
		0.02 mg/ml	2.833 \pm 0.031	1.09
Interassay precision	Day 1	Analyst 1	0.5037 \pm 0.005	0.90
		Analyst 2	0.5003 \pm 0.002	0.46
	Day 2	Analyst 1	0.5043 \pm 0.004	0.70
		Analyst 2	0.4990 \pm 0.003	0.69
	Day 3	Analyst 1	0.4753 \pm 0.005	1.04
		Analyst 2	0.4767 \pm 0.006	1.37
<i>Acceptance criteria</i>				<2

TABLE 4: Results showing accuracy of results from the developed method.

Expected concentration (mg/ml)	Mean absorbance \pm SD	Mean% recovery \pm SD
0.001	0.1253 \pm 0.001	100.91 \pm 0.8126
0.0025	0.3357 \pm 0.002	99.57 \pm 0.5860
0.005	0.6930 \pm 0.005	100.08 \pm 0.7037
<i>Acceptance criteria</i>		[98–102%]

TABLE 5: Robustness of developed method at three different concentrations.

	280 nm	284 nm	288 nm
Mean absorbance \pm SD	0.496 \pm 0.002	0.498 \pm 0.004	0.500 \pm 0.002
Mean concentration \pm SD	0.003618 \pm 1.407e-5	0.003634 \pm 2.664e-5	0.003648 \pm 1.625e-5
RSD	0.40%	0.76%	0.45%
One-way ANOVA of concentration results		$F_{(2,6)} = 1.789$; $p = 0.2459$	

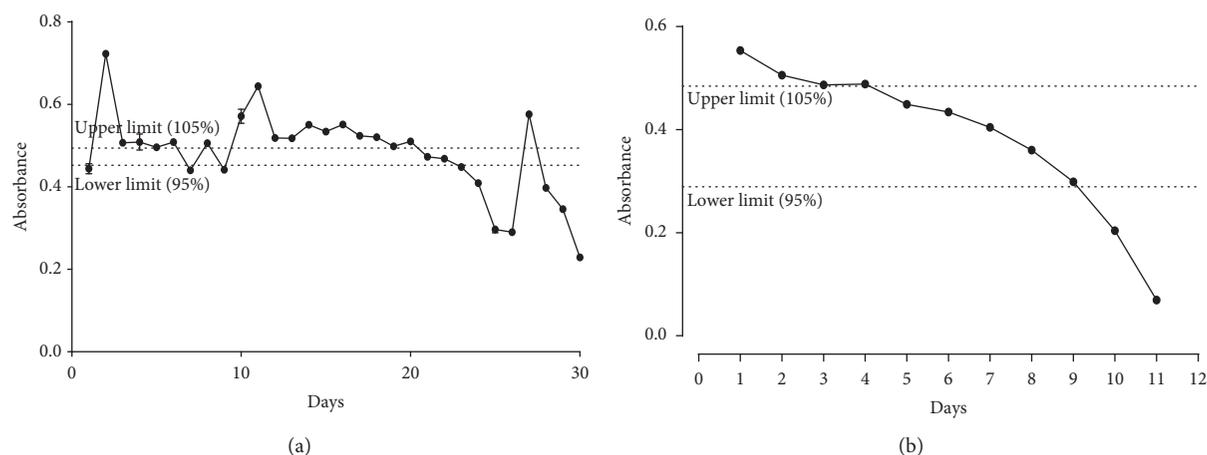


FIGURE 4: Results showing the stability of the solutions used in the method. (a) The stability of stock solution prepared and kept under refrigeration. The results show that the solution was stable for about 23 days, after which the absorbances recorded were significantly different from the initially recorded values. (b) The stability of the 100% concentration calibration solution prepared during the samples analyses. The result showed that the prepared solution was stable for 9 days, beyond which it may not be advisable to use such solution.

differences in the results generated. Results from the 5-HMF assay were also expressed as percentages (in pie charts) and means \pm SD and test for statistical differences using Student's *t*-test (at 95% confidence level) from SPSS Statistics (IBM Corporation, version 20, 2011).

3. Results and Discussion

3.1. Method Development and Validation. Contrary to previously reported studies, that spectrophotometric methods were not ideal to analysing the contents of 5-HMF in products because of matrix effects, this study has showed that, there may be exceptions to that axiom. The developed method was showed to be selective towards the compound, 5-HMF in the presence of the other components of the drink (Figure 1). This specificity of the method was further proven with replicate spectrophotometric determinations ($N = 5$) showing that absorption of placebo and matrix components were significantly lower than that of 5-HMF ($p < 0.0001$; Table 1 and Figure 2). The method was also showed to be precise (Table 3), accurate (Table 4), and linear within the concentration range, 0.001 mg/ml–0.020 mg/ml (Figure 3 and Table 2), as well as robust, with respect to the change in the wavelength of detection ($p = 0.2459$; Table 5). The test solutions were observed to be stable within 9 days of preparation while the stock solution could last up to 23 days (Figure 4). These outcomes indicate how simple and convenient the method is, to be used for analysis of large samples of similar products.

3.2. 5-HMF Levels in the Sampled Products. Analysis of the sampled products showed 70% of the fruit samples were of foreign origin while 30% were of local origin ($N = 10$; Figure 5). On the other hand, 90% of sampled canned malt products were found to be of foreign origin while 10% were of local origin ($N = 10$). It was established that the concentration of 5-HMF in canned malt samples (M1–M10) ranged between

0.3140 mg/ml and 0.7170 mg/ml, while its concentration in the juice samples (A1–A10) was between 0.132 mg/ml and 0.438 mg/ml (Table 6). The 5-HMF levels detected in both local and foreign juice samples were found to be comparable ($F_{(6,2)} = 12.05$, $t = 1.121$, $p = 0.1572$). In addition, the content of 5-HMF in the canned malt samples was found to be relatively higher than that in the fruit juices, although the difference was not significant ($t = 2.200$; $p = 0.0553$; Figure 6). This could partly be attributed to the differences in manufacturing processes employed for the two types of food products. It is widely reported that increasing temperature in the manufacturing process for some products result in relatively higher 5-HMF levels due to Maillard reactions and caramelization [19, 20]. It has been shown that 5-HMF levels in fresh foods are usually close to zero and higher in processed foods [18]. It could thus be inferred that the manufacturing processes for the canned malts may contribute to the relatively higher 5-HMF levels. It is also reported that 5-HMF levels in a product may be indicative of the quality and stability of a product [18–21]. Storage conditions (for example, temperatures) deemed unfavourable and may affect the quality of processed products through nonenzymatic browning reactions. For this reason, processed foods may be observed to undergo flavour, taste, colour, and nutritional changes when stored at warm temperatures and/or for prolonged periods of time [18], and the 5-HMF levels are used as indicators to monitor the quality of such products during storage [18, 19].

It was deduced from the labels of the sampled products that the fruit juice samples had 1–15 months remaining of their shelf life as at the time of sampling, while the canned malt samples had 1–12 months remaining (Figure 5). Contrary to the above supposition, the current study failed to show any significant correlation between the remaining months on the shelf life and the concentration of the 5-HMF levels determined for both fruits juice samples ($F = 0.2907$, $p = 0.6044$, $R^2 = 0.03504$) and canned malt samples ($F = 0.03645$, $p = 0.8533$, $R^2 = 0.00454$) (Figure 7). In effect, the concentrations of 5-HMF were not dependent on

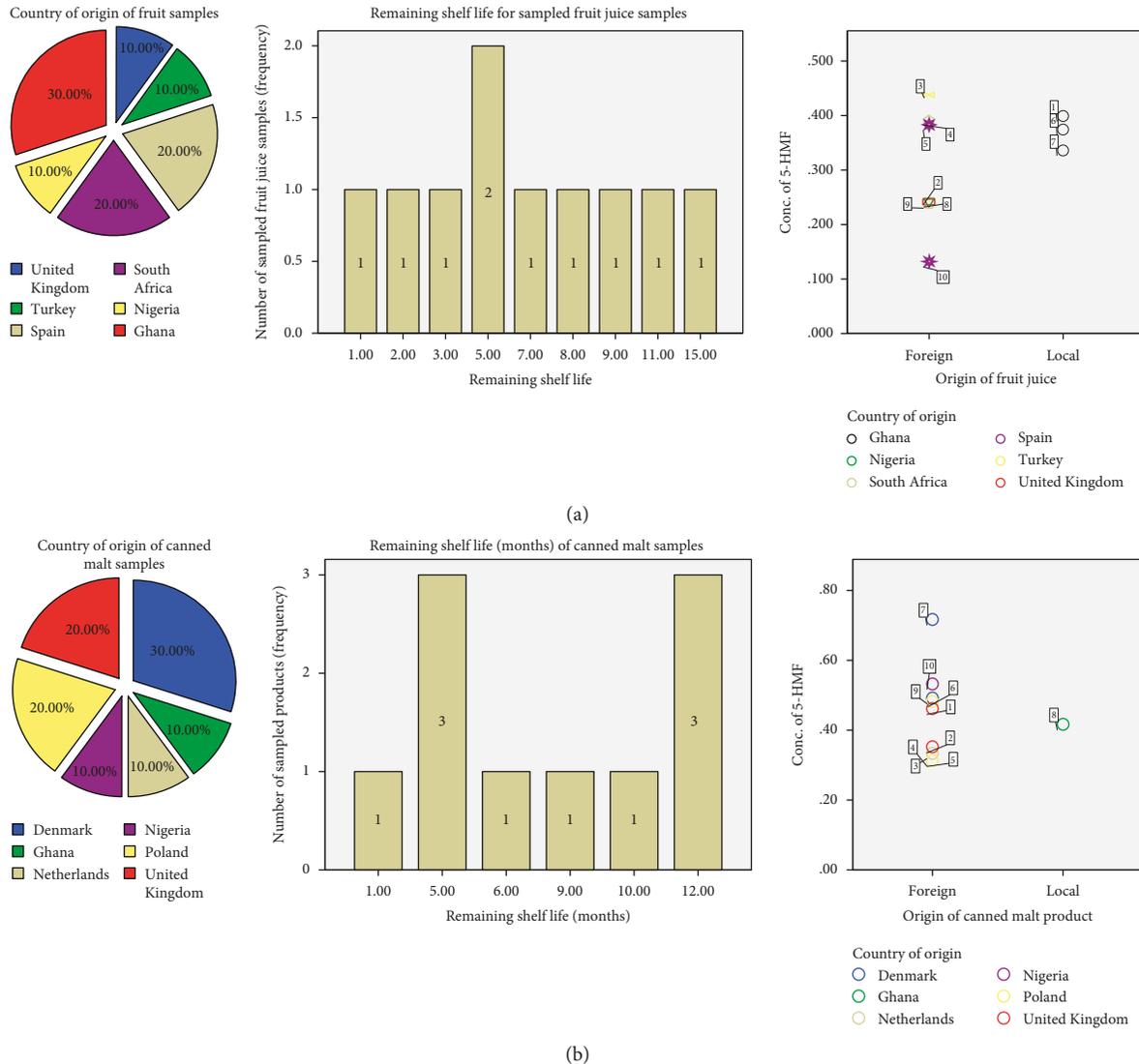


FIGURE 5: (a) Data generated from fruit juice products sampled from the market ($N = 10$). The data indicate that 30% ($N = 3$) of fruit juice samples taken were of the Ghanaian origin. Sampled products had 1–15 months remaining of their shelf lives at the time of collection. (b) Data generated from canned malt products sampled from the market ($N = 10$). The data indicate that 10% of canned malt samples ($N = 1$) taken were of the Ghanaian origin. Sampled products had 1–12 months remaining of their shelf lives at the time of collection.

how long the products had stayed on the shelf after manufacturing.

3.2.1. Specificity. The results of specificity are given in Table 1.

3.2.2. Linearity and Range. Figure 3 shows the proof of linearity and its corresponding concentration range.

3.2.3. Precision. Table 3 shows the results proving precision of results from the developed method.

3.2.4. Accuracy. Table 4 shows the results of accuracy of results from the developed method.

3.2.5. Robustness. Table 5 shows the robustness of developed method at three different concentrations.

3.2.6. Solution Stability. Figure 4 shows the results of the stability of the solutions used in the method.

4. Conclusions

A simple, convenient, and cheaper spectrophotometric method has been developed and validated to quantify 5-HMF levels in

TABLE 6: Results from the analysis of the samples.

Sample	Mean absorbance \pm SD (N = 3)	Content of 5-HMF (mg/ml)
<i>Canned malt drinks</i>		
M1	0.654 \pm 0.004	0.462
M2	0.483 \pm 0.003	0.353
M3	0.458 \pm 0.005	0.335
M4	0.428 \pm 0.003	0.314
M5	0.428 \pm 0.003	0.314
M6	0.668 \pm 0.003	0.483
M7	1.001 \pm 0.002	0.717
M8	0.575 \pm 0.004	0.417
M9	0.682 \pm 0.001	0.493
M10	0.739 \pm 0.003	0.533
<i>Fruit juice</i>		
A1	0.549 \pm 0.002	0.399
A2	0.324 \pm 0.011	0.241
A3	0.605 \pm 0.005	0.438
A4	0.536 \pm 0.004	0.390
A5	0.526 \pm 0.001	0.383
A6	0.513 \pm 0.004	0.374
A7	0.459 \pm 0.004	0.336
A8	0.326 \pm 0.001	0.242
A9	0.321 \pm 0.005	0.238
A10	0.170 \pm 0.005	0.132

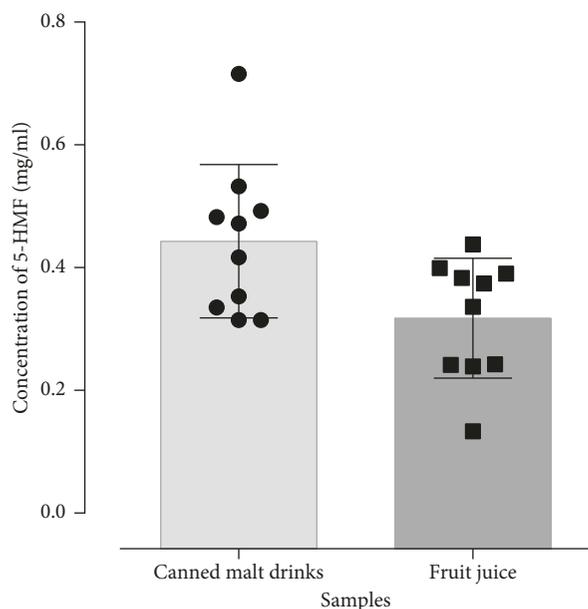


FIGURE 6: Comparison of the concentrations of 5-HMF in the samples analyzed. Results were presented as mean \pm SEM and analyzed using paired Student's *t*-test. Comparison of the concentrations in the two types of products shows that 5-HMF levels were similar ($t = 2.200$; $p = 0.0553$).

canned malt drinks and fruit juices. The study results showed that content of 5-HMF in fruit juice samples was comparable to that in canned malt drinks. The 5-HMF levels were not dependent on the duration of storage of the products. In addition,

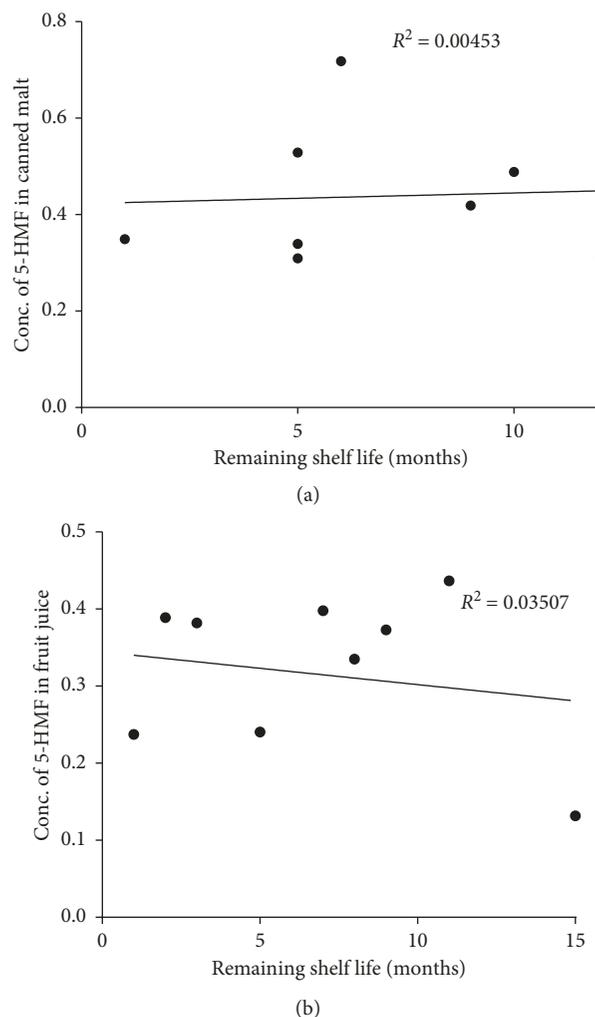


FIGURE 7: Relationship between remaining shelf life of products sampled and 5-HMF levels. Results show poor correlations between concentrations of 5-HMF and remaining shelf life. In effect, the months left of the products' shelf life do not necessarily contribute to 5-HMF levels in the sampled products.

there was no difference in 5-HMF levels for sampled foreign and locally manufactured products.

Data Availability

The data are available from the Laboratory of the Pharmaceutical Chemistry, Faculty of Pharmacy, KNUST, Ghana.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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