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Assessment of 5-hydroxymethylfurfural levels in carbonated soft drinks and milk products on the markets in the Kumasi Metropolis of Ghana

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ABSTRACT

Processing of food products makes them palatable, edible, provides options to consumers and prolong their life spans. Irrespective of these benefits, some of these processes have also shown to increase the build-up of potential toxic metabolites, for example, 5-hydroxymethylfurfural (5-HMF). 5-HMF production results from caramelization and/or Maillard reactions involving carbohydrates and some proteins. Its levels are thus used as an indicator of thermal treatment and suitability of storage conditions. At significantly, higher levels in products, they have been shown to elicit deleterious effects on consumers. The current study was carried out to monitor the levels of 5-HMF in carbonated soft drinks (CSDs) and milk products (MPs) on the market as their consumptions have increased in recent years. Using a validated spectrophotometric method, it was shown that 5-HMF levels in MPs ranged between 1.165 ± 0.007 g/L and 2.638 ± 0.010 g/L and that in the CSDs was between 0.0294 ± 0.00018 g/l and 0.0845 ± 0.00049 g/L. However, the estimated 5-HMF exposure levels from the consumption of these products were shown to be within internationally acceptable limits of 0.005 g/L – 0.15 g/L. The 5-HMF levels observed however demonstrated differences in the two types of products analysed, differences in different brands of similar products, batch-to-batch variations for same brands, and differences in products from different origins. These observations may be indicative of inconsistencies in applicable industrial processing systems. The outcome of this study calls for the need to appraise existing processing systems, to assure reproducible quality products.

Introduction

Food processing has become an inevitable practice in the way man handles food products. It is thought that processing makes food palatable, edible and safe [1]. Additionally, the act tends to offer varieties in foods, gives consumers choices

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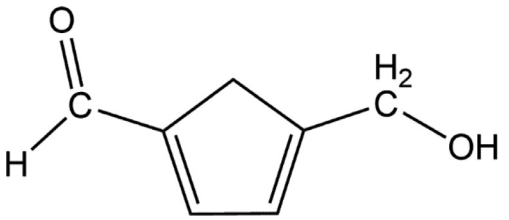

Fig. 1. Chemical structure of 5-HMF.

Table 1
Sampled Carbonated Soft drinks.

Sample	Ingredients	Batch (Lot) Number	Expiry date	Product Origin
CL1A	Carbonated water, sugar, caramel colorant, phosphoric acid,	BS/1/14:52	16/06/2019	Local
CL1B	flavourings, caffeine.	BS/1/14:47	16/06/2019	
CL2A	Carbonated water, sugar, caramel colour E15S0D, acidifier phosphoric acid E338, natural cola flavour, caffeine flavour, preservative	1401192116	14/01/2020	Local
CL2B	(potassium sorbate E202 and sodium benzoate E211)	2401191950	24/01/2020	
CL3A	Carbonated water, caramel, phosphoric acid, potassium sorbate,	20:57	20/08/2019	Local
CL3B	sodium benzoate, aspartame, caffeine.	20:56	20/08/2019	
CF1A	Carbonated water, sugar, colour (caramel), acidulant (phosphoric	G182D17B	01/08/2019	Foreign
CF1B	acid), cola flavour, caffeine	G182C06A	01/08/2019	
CF2A	Carbonated water, sugar, colourant (E150D), phosphoric acid, cola	08/08/2018(2B)	08/05/2019	Foreign
CF2B	flavourings, preservative (sodium benzoate), caffeine.	08/08/2018(2A)	08/05/2019	
CF3A	Water, sugar, carbon dioxide, colourant, acid regulator, vegetable	05/07/18(1B)	04/07/19	Foreign
CF3B	extract, caffeine	13/05/18(6C)	12/05/19	

[2,3] and make products available all year round. Most importantly, processes such as heating, drying, roasting, frying, pasteurization and canning [4] have resulted in extending food shelf lives by eliminating microorganisms responsible for their spoilage, and thus, maintaining their expected physical and nutritional properties [5]. In some cases, over-processed foods have seen some form of deterioration resulting in the reduction of their nutritional values [6–8]. Several indicators exist for monitoring the quality of products and one of the most important ones is 5-hydroxymethylfurfural (5-HMF) [9,10]. In recent times, 5-HMF (Fig. 1) has become a recognized indicator of Maillard reaction (non-enzymatic browning) and caramelization [11]; often serving as an index of deteriorative changes that occur during excessive heating or prolonged storage of carbohydrate-containing foods to a large extent [6,12,13]. Despite benefits observed with Maillard reaction, including adding colour, flavour and some nutritional qualities to products [4,6,12], it has also been shown to yield a number of oxidized metabolites including other furans in addition to 5-HMF, anoidins and a variety of heterocyclic compounds, which are considered mutagenic and genotoxic [14,15].

5HMF has been reported in various foodstuffs including honey [16], milk [6,14], fruit juices [10,13,17], bread [18], jams, tomato juices and cereal products [12,19] among others.

5-HMF is not known to pose any health risk at normal dietary exposure levels [12] but is considered to be cytotoxic, an irritant to eyes, upper respiratory tract, skin and mucous membranes and damages striated muscles and viscera by causing the accumulation of poisons in the body at high levels [10,12]. It has thus become needful to regularly monitor the levels of the compound in food products, as a quality control and assurance requirement. This study was thus premised on this need and forms part of ongoing studies to regularly sample food products from various sales outlets in the country and evaluate their quality by monitoring their 5-HMF levels and make the necessary recommendations to national regulatory authorities, like the Food and Drugs Authority (FDA) and Ghana Standards Authority (GSA), as well as industry stakeholders. The current study focused on milk products (MPs) and carbonated soft drinks (CSDs) sampled from the markets in the Kumasi metropolis, using a simple spectroscopic method adopted from literature [17] and validated for the analyses.

Materials and methods

Samples

24 commercial samples of CSDs (Table 1) and MPs (Table 2) were randomly sampled from different retail outlets in the Kumasi Metropolis. The samples were either analysed immediately after purchase or were stored at −17°C for later analysis. The sampled products included 2 batches each of 6 brands of both MPs and the CSDs.

Table 2
Sampled milk products.

Sample	Ingredients	Date of Manufacture	Expiry date	Product Origin
MF1A	Cow milk, stabilizer E339, Vitamin B_{12}, Folic acid, Vitamins A,	05/2018	11/2019	Foreign
MF1B	D_3, B_1, B_6	09/2018	09/2019	
MF2A	Solid milk non-fat (20%), Vegetable fat (7.6%), Milk fat (0.4%)	10/2018	10/2019	Foreign
MF2B	and Stabilizer (E339 & E407)	10/2018	10/2019	
MF3A	Milk solids, acidity regulator (E339), Emulsifier (soya	01/2019	10/2019	Foreign
MF3B	lecithin), Vitamin A, Thickener (carrageenan)	10/2018	06/2019	
MF4A	Milk solids, Vegetable fat, Milk fat and Stabilizer (E339 &	18/03/20119	17/03/2020	Foreign
MF4B	E407)	27/02/2019	26/02/2020	
ML1A	Milk solids non-fat, Palm Olein, acidity regulator (E339,	01/2019	09/2019	Local
ML1B	E340), Emulsifier (soya lecithin), Thickener (carrageenan)	10/2018	07/2019	
ML2A	Milk solids, acidity regulator (E339), Emulsifier (soya	01/2019	01/2020	Local
ML2B	lecithin), Vitamin A, Vitamin D, Thickener (carrageenan)	02/2019	02/2020	

Table 3
Results of specificity test for 100% concentration of 5-HMF in matrices.

Parameter	Absorbance @ 284nm (N = 5)
Placebo (solvent) – Acetonitrile: Water (1:1)	$0.002 \pm 0.001^{****}$
Matrix (combination of ingredients present in products)	$0.096 \pm 0.006^{****}$
Placebo + 5-HMF	0.890 ± 0.018
Combination (Placebo + Matrix + 5-HMF)	0.904 ± 0.007^{ns}
ANOVA analysis	$F_{(3,16)} = 12034$; $p < 0.0001$

Dunnett *post-hoc* test was performed comparing the absorbance of 5-HMF + placebo with the other parameters: ^{ns} $p > 0.05$; $^{*}0.05 < p < 0.01$; $^{**}0.01 < p < 0.001$; *** $0.001 < p < 0.0001$; **** $p < 0.0001$

Chemicals, reagents and glassware

Chemicals and reagents used included acetonitrile (HPLC grade, Fisher Scientific, UK), distilled water (in-house produced) and a working standard of 5-hydroxymethylfurfural (98 %, Fluorochem, UK). Glassware used included borosilicate glass volumetric flasks (10 ml, Grade A), pipettes (1 mL, 2 mL and 10 mL, Grade A), and scintillation vials (20 ml).

Instrumentation

Spectrophotometric analysis was carried out using a single beam UV spectrophotometer (UVmini-1240, Shimadzu Corporation, Kyoto, Japan) fitted with a 10 × 10 mm cuvette holder, and scanned within a wavelength range of 200 nm – 800 nm, with a resolution of 5 nm and light source produced from 20 W halogen lamp (long-life 2000 hour), and Deuterium lamp (socket type). The results were recorded on a windows computer system, using the UVProbe software. Analytical balance (Kern, Germany, WD140050809), and a refrigerator (Model WRT348FMEZ, Whirlpool, USA) were also used.

Method development and validation

The method was a simple spectrophotometric method adopted from literature [17], with minimum modifications, and validated for the identification and quantitation of 5-HMF in the sampled products as per the International Council of Harmonization (ICH), Q2(R) Guidelines [20]. The validation parameters investigated included linearity and its range, limits of detection and quantitation, specificity, stability of test solution, robustness, accuracy and precision.

Preparation of working standard solution

A 2 mg/mL stock solution of 5-HMF was freshly prepared with acetonitrile: water (50: 50 v/v), in a volumetric flask, and transferred into a labelled scintillation vial. The solution was diluted with the same solvent system, to produce a 0.2 mg/mL working standard solution. This was kept under refrigeration until use in determining the validation parameters.

Specificity and selectivity

The method specficity was as described in literature [17]. The absorbances of the solvent system, placebo, placebo with 5-HMF and their combinations were recorded and analyzed using Analysis of Variance (ANOVA). Specificty and selectivity were demonstrated when the wavelength of detection (λ_{max}) of 5-HMF was prominent and its corresponding absorbance was significantly higher than that from the matrix and the solvent system (Table 3).

Table 4
Results for accuracy studies.

Expected concentration (mg/ml)	Absorbance	Actual concentration (mg/ml)	% Recovery	Mean ± SD
0.01	1.709	0.009997	99.97	100.1
	1.712	0.01002	100.15	±
	1.713	0.01002	100.21	0.125
0.005	0.889	0.005024	100.48	100.7
	0.890	0.005030	100.61	±
	0.893	0.005048	100.97	0.254
0.0025	0.479	0.002538	101.51	101.8
	0.480	0.002544	101.76	±
	0.482	0.002556	102.24	0.371
Acceptance criteria 98 – 102%				

Table 5
Results from regression analysis in the linearity test.

Parameter	Value
Slope	164.9 ± 0.764
y-intercept	0.06051 ± 0.004
x-intercept	-0.0004
1/slope	0.006
R square	0.9997
Sy.x	0.011

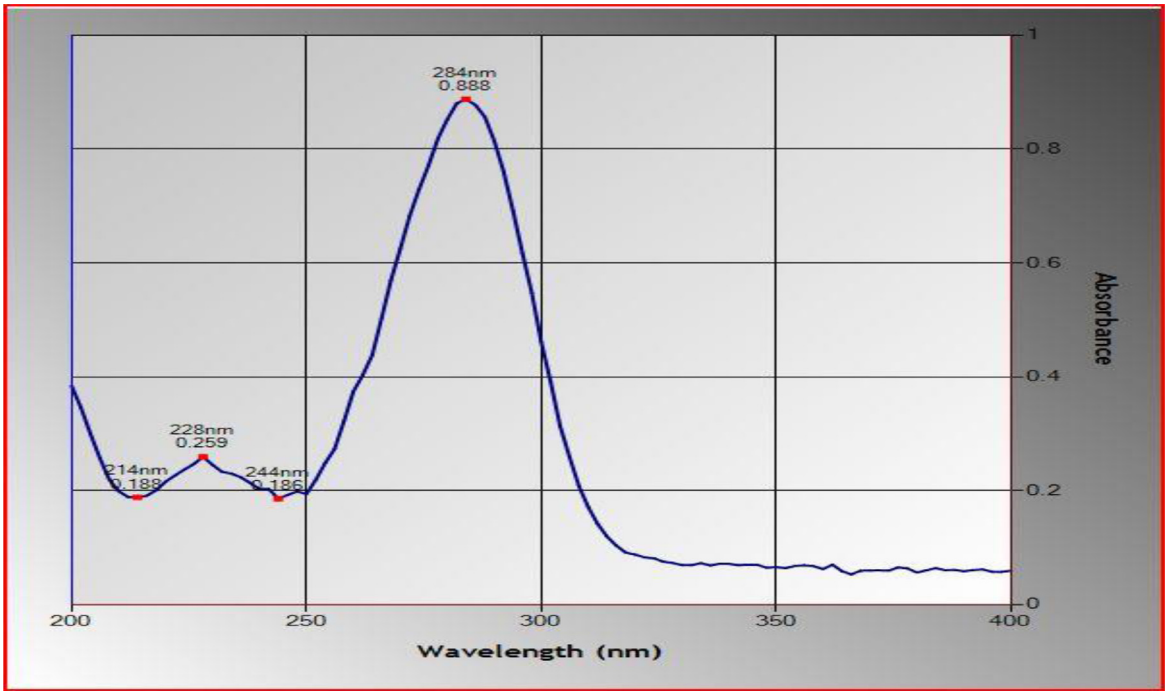

Fig. 2. UV Spectrum 5-HMF.

Accuracy

Method accuracy was determined by calculating the percentage recoveries [20] of 5-HMF over a concentration range (0.0025 mg/mL – 0.01 mg/mL) (Table 4).

Linearity and range

The linearity of the method was demonstrated from triplicate absorbance determinations of varying concentrations of 5-HMF in the range, 0.00025 mg/mL - 0.01 mg/mL, prepared from the working standard solution. Linearity was then predicted from the coefficient of regression, y-intercept and *F*-vaue of the generated regression model (Table 5), as well as from the residual plot [21] (Fig. 2).

Table 6
Results showing proving precision of results from the developed method.

Precision Parameters			Mean absorbance ± SD	RSD	Comparing groups
Intra-Assay Precision	*6 replicate determinations* (0.005 mg/ml)		0.9012 ± 0.002	0.26%	-
Inter-Assay Precision	*Same Day*	Analyst 1	0.9012 ± 0.002	0.26%	Student *t*-test:
		Analyst 2	0.8988 ± 0.004	0.44%	$t = 1.243$; $p = 0.2421$
	Same Analyst	Day 1	0.9012 ± 0.002	0.26%	ANOVA: $F_{(2,15)} = 2.605$;
		Day 2	0.9000 ± 0.006	0.24%	$p = 0.1069$
		Day 3	0.8957 ± 0.004	0.14%	
	Acceptance Criteria			< 2%	$p > 0.05$

Table 7
Robustness of developed method at three different concentrations.

Test	280 nm	284 nm	288 nm	Comment
1	0.886	0.890	0.889	$F_{(2, 6)} = 1.000$; p >
2	0.889	0.887	0.886	0.05
3	0.885	0.883	0.885	Absorbances from
	Mean = 0.887 ±	Mean = 0.887 ±	Mean = 0.887 ±	different wavelengths
	0.002	0.004	0.002	do not differ
	RSD = 0.23%	RSD = 0.40%	RSD = 0.46%	significantly.

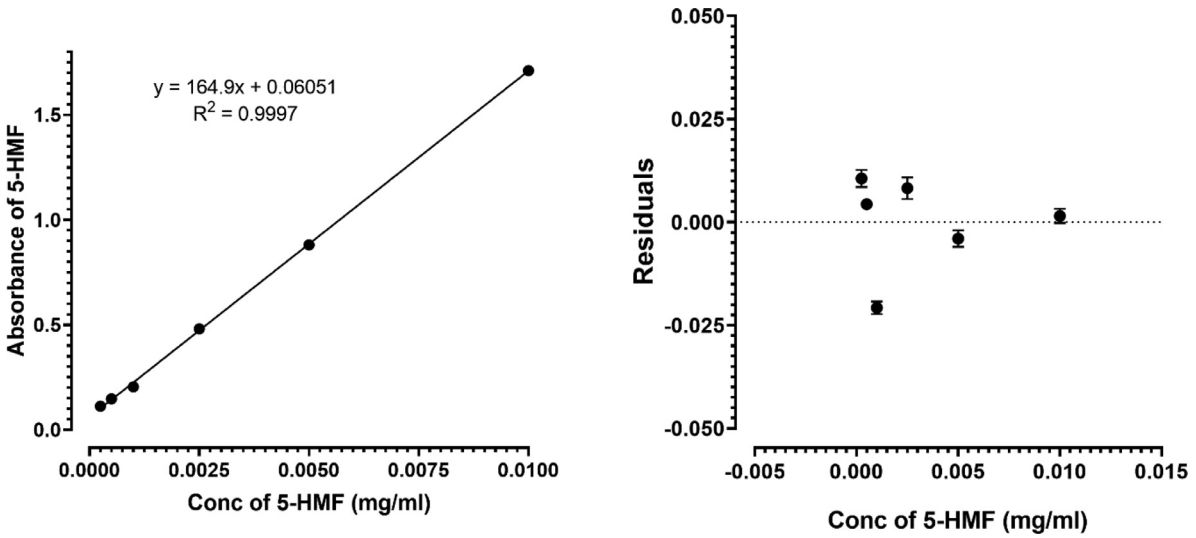

Fig. 3. Linearity Curve and Residual plot for 5-HMF.

Precision

The precision of the method was demonstrated by determining both repeatability and intermediate precision. Repeatability was evaluated from replicate determinations (n = 6) of absorbance of 0.005 mg/mL 5-HMF solution prepared from the working standard solution. The intermediate precision on the other hand, was evaluated by determining the absorbance of the same solution from two different analysts in three consecutive days. In each case, the mean, standard deviation and relative standard deviation were calculated (Table 6).

Robustness

The robustness of the method was evaluated by accessing the effects of delibrate changes in the wavelength of detection (± 4 nm) [21]. The results were analysed using ANOVA (Table 7).

Stability of solution

The stock and the working standard solutions were accessed for their stability by monitoring their concentrations over a 3-week period. A linear regression analysis was conducted on the 5-HMF concentrations to estimate the shelf-life of the respective solutions [21] (Fig. 3).

Analysis of samples

The validated method was then used to evaluate the content of 5-HMF in selected MPs and CSDs. In preparing the samples for analysis, 1 mL aliquots of MPs and 5 mL of CSDs were accurately pipetted and quantitatively transferred into labelled calibrated scintillation bottles respectively, and independently dissolved with acetonitrile: water (50:50 v/v), to produce 20 mL stock sample solutions. From the stock solutions, serial dilutions were carried out, with mixing, to obtain sample solutions with dilution factors of 4000 and 20 for the MPs and CSDs respectively. The resultant sample solutions were labelled accordingly and analysed with the UV spectrophotometer at 284 nm. Replicate determinations were carried out for each sample (n = 3). A linear regression model generated for the working standards in the validation, was used to estimate the content of 5-HMF in the test samples.

Data analysis

The results obtained from the validation and sample analysis were analysed using GraphPad Prism 8 for Windows (Version 8.0.2, GraphPad Software, 2019). Test results were expressed as means ± SD, and relative standard deviations (RSD). Validation data were analysed inferentially using regression analysis, and Student's *t*-test and ANOVA (at 95% confidence level) to determine statistical differences in the 5-HMF contents.

Results and discussion

This section details the results obtained from the study and a discussion on the method development and validation. Further details on the batch-to-batch variations, comparism of values to recommended limits, differences in the local and foreign brands of products as well as any possible health implications are also discussed.

Method development and validation

In a previous study, it was demonstrated that UV-spectrophotometry could be used to estimate 5-HMF contents in beverages [17]. The current method was similarly developed, to be used to control the contents of 5-HMF in CSDs anf MPs. The method was showed to be selective and specific towards the detection of 5-HMF in the presence of product matrix. Fig. 2 shows the spectrum obtained from UV analysis of the 5-HMF. Although the products are thought to contain several components, the detection of 5-HMF at 284 nm was shown to be significantly higher than that from the solvent and product matrices (Table 3). Quantitatively, the method was also demonstrated to be accurate, with percentage recoveries complying with the acceptance criteria of 98% - 102% (Table 4). These response were further shown to be linear within the concentration range, 0.00025 mg/mL - 0.01 mg/m (Fig.e 3; Table 5). Consequently, the absorbance produced by the method were also precise; as shown from both the repeatability and intermediate precision investigations (Table 6). Furthermore, the results produced from different analysts on the same day of analysis were comparable ($t = 1.243$; $p > 0.05$); just as the results from different days of analysis by the same analyst ($F_{2,15} = 2.605$; $p > 0.05$). The method was also shown to be robust, especially with minor changes in the wavelength of detection. Additionally, no significant difference in the absorbance was observed with the changes made ($F_{2,\ 6} = 1.000$; $p > 0.05$) (Table 7). The stock 5-HMF solution prepared was shown to be stable for use within 9 days of preparation whiles its corresponding working standard solution was only stable for use within 3 days of preparation (Fig. 4).

How current results compare to recommended limits

The details of the samples analysed are shown in Table 1 & 2. The results from the analysis showed that 5-HMF levels in the MPs ranged between 1.165 ± 0.007 g/L and 2.638 ± 0.010 g/L; with an average of 1.874 ± 0.379 g/L (Table 8). These levels were thought to be higher than previously reported 5-HMF levels in MPs from Spain, where a range of 0.00014 g/L – 0.00012 g/L was established [6]. In the case of the CSDs, the 5-HMF levels determined ranged between 0.0294 ± 0.0002 g/L and 0.0845 ± 0.0005 g/L, with an average of 0.0517 ± 0.017 g/L (Table 8). Similarly, the levels were in excess of levels reported in CSDs sampled in China by Zhang et al. The 5-HMF levels were reported to be in the range of 0.00017 – 0.00826 g/L [22].

As earlier explained, 5-HMF is the end product of caramelization and/or Maillard reactions involving carbohydrates [6,23]. Thus, 5-HMF levels may be mostly accounted for by the sugar contents [6] in the sampled products (Table 1 & 2). The observed differences with those earlier reported could partly be attributed to storage conditions of the sampled products. In the climatic conditions in Sub-Saharan Africa, it may be expected that the average high temperatures experienced by some products under storage may contribute to their relative high 5-HMF levels than similar products under storage in temperate regions of the world. Also, the differences could be as a result of the differences in industrial processing systems adopted by manufacturers of these products. 5-HMF levels may be indicative of the amount of heat applied during processing of the products [24]. Thus, the high 5-HMF levels observed might also possibly be as a result of high amounts of heat applied in the production.

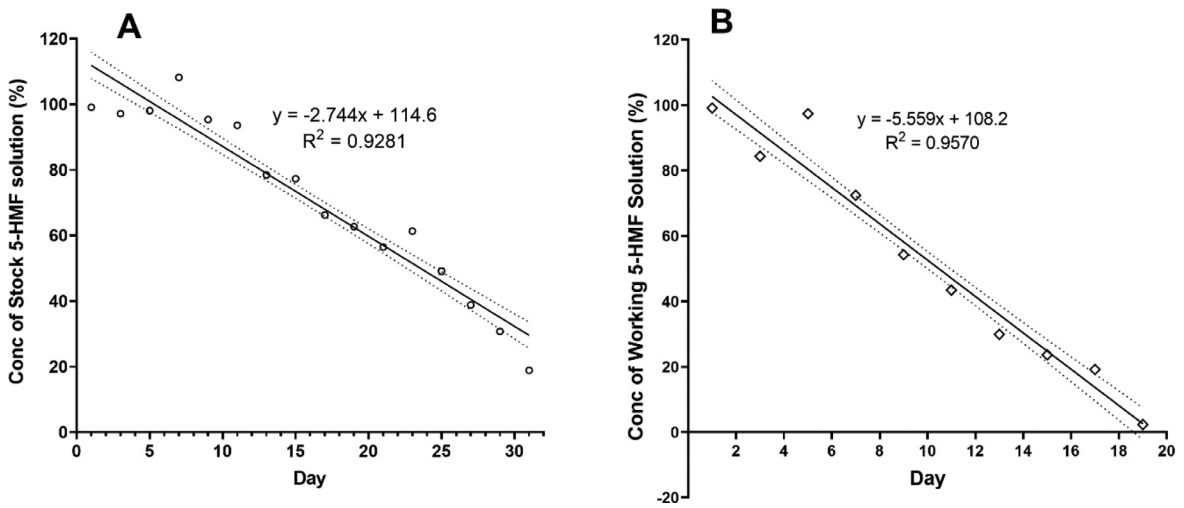

Fig. 4. Stability of 5-HMF Solutions [A] – Investigatiing the stability of the stock solution prepared for all the validation investigations. The regression analysis showed that the solution had a shelf-life of 9 days. [B] – Stability studies involving the working standard solution. The solution had a shelf-life of 3 days.

Table 8
Assayed 5-HMF levels in the sampled products.

Sample	Sample ID	Mean ± SD of 5-HMF levels (g/L)	
		1st batch	2nd batch
Milk Products	MF1	1.852 ± 0.007	1.649 ± 0.007****
	MF2	2.169 ± 0.016	2.077 ± 0.011****
	MF3	2.011 ± 0.017	1.370 ± 0.013****
	MF4	1.165 ± 0.007	1.888 ± 0.007****
	ML1	2.638 ± 0.010	2.179 ± 0.003****
	ML2	1.671 ± 0.015	1.823 ± 0.006****
Carbonated Soft Drinks	CL1	0.049 ± 0.0002	0.052 ± 0.0002****
	CL2	0.053 ± 0.0002	0.053 ± 0.0003ns
	CL3	0.085 ± 0.0005	0.083 ± 0.0002****
	CF1	0.048 ± 0.0001	0.041 ± 0.0003****
	CF2	0.029 ± 0.0002	0.029 ± 0.0002 ns
	CF3	0.048 ± 0.0001	0.048 ± 0.0001 ns

Student *t*-test conducted to compare the 5-HMF levels from the different batches of the products. Significance level were indicated as ****p < 0.0001, ***p < 0.001, **p < 0.01 and *p < 0.05.

Table 9
Estimated 5-HMF exposure levels from sampled products.

Product	Brands	Absorbance (Mean ± SD)	5-HMF levels (g/L)(Mean ± SD)
MPs	MF1	0.421 ± 0.023	0.03676 ± 0.002
	MF2	0.498 ± 0.011	0.04459 ± 0.001
	MF3	0.490 ± 0.072	0.03550 ± 0.007
	MF4	0.375 ± 0.082	0.03206 ± 0.008
	ML1	0.557 ± 0.052	0.05058 ± 0.005
	ML2	0.421 ± 0.017	0.03669 ± 0.001761
CSDs	CL1	0.478 ± 0.017	0.02531 ± 0.001012
	CL2	0.499 ± 0.002	0.02661 ± 0.000120
	CL3	0.752 ± 0.007	0.04191 ± 0.000413
	CF1	0.429 ± 0.033	0.02234 ± 0.002028
	CF2	0.303 ± 0.001	0.01469 ± $8.857e^{-005}$
	CF3	0.459 ± 0.001	0.02416 ± $4.548e^{-005}$

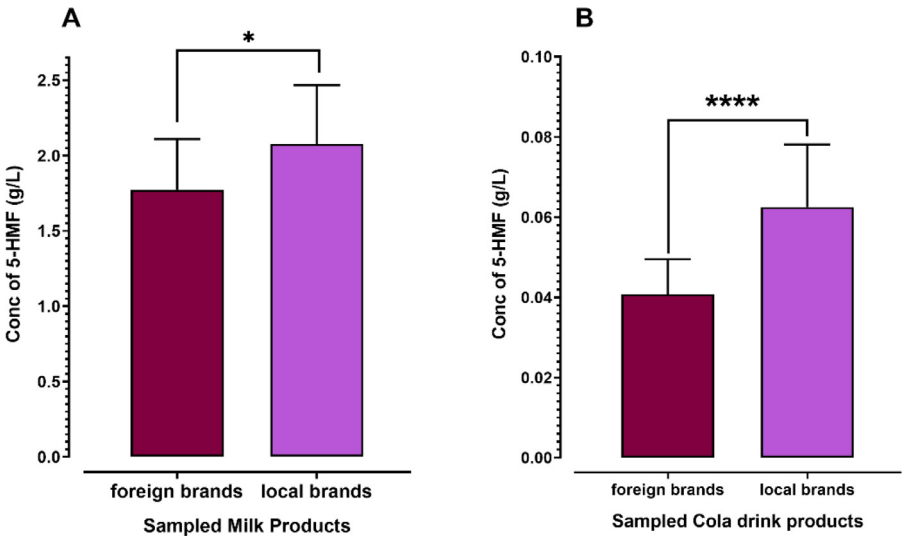

Fig. 5. Comparing 5-HMF levels in MPs and CSDs from different origins. Data expressed as Mean ± SD. Data was analysed using unpaired Student t-test to determine significance of differences in their contents at 95% confidence level. Significant levels were illustrated as ****p < 0.0001, ***p < 0.001, **p < 0.01 and *p < 0.05.

However, upon estimating the 5-HMF exposure levels (Table 9), with ranges of 0.03206 ± 0.008 g/L – 0.05058 ± 0.005 g/L for MPs and 0.01469 ± 8.857e-005 g/L – 0.04191 ± 0.0004 g/L for CSDs, these products were shown to be consistent with internationally recommended limits of 0.005 g/L – 0.15 g/L [12].

5-HMF levels of local versus foreign products

When the products were compared in terms of their origin, it was observed that there were some differences. 5-HMF levels in local brands of the MPs were significantly higher than that of foreign origin ($t = 2.432$, $df = 34$, $p < 0.05$; Fig. 5a). Similar observation was made with the CSDs as well ($t = 5.177$, $df = 34$, $p < 0.0001$; Fig. 5b). Assuming that similar storage conditions were maintained for the products (since products were sampled from the same geographical area), it may be argued that the differences could be attributed to differences in the industrial processes. It is also possible that during processing of such products, the relatively higher environmental temperature and relative humidity could contribute to such high-level of occurrence.

Batch-to-batch variation in 5HMF content

In confirmation to the earlier proposal that differences in the industrial processes possibly contributed to the difference in 5-HMF levels, the results demonstrated batch-to-batch differences. Generally, it was observed that 5-HMF levels in both MPs ($F_{(5,24)} = 2987$, $p < 0.0001$) and CSDs ($F_{(5,24)} = 345.2$, $p < 0.0001$) were all significantly different from each other, irrespective of the batch, brand and origin. For example, there were differences in 5-HMF levels in MF1A and MF1B ($t = 23.01$, $df = 24$, $p < 0.0001$), ML2A and ML2B ($t = 17.16$, $df = 24$, $p < 0.0001$), CL1A and CL1B ($t = 18.88$, $df = 24$, $p < 0.0001$), and CF1 ($t = 37.95$, $df = 24$, $p < 0.0001$). These observations call for a need to review industrial processes, which would ensure stricter adherence to control measures; with the aim of producing products intended for public consumption with reproducible quality attributes.

Implications of the findings from the study

Although the 5-HMF exposure levels of the products considered were deemed acceptable, it is worth noting that the concentrations were relatively higher than estimates from similar products in different places in previous studies. Thus an accumulation of the compound is likely to occur upon consumption of high quantities of these products. For example, MPs and CSDs in the current study had averages of 1.874 ± 0.379 g/L and 0.0517 ± 0.017 g/L respectively. Thus, a consumption of 1L of the MPs means the individual is exposed to 1874 mg of 5-HMF and this is considered higher than the advised tolerable daily intake of 132 mg per person per day [12]. Similarly, a 1L consumption of CSD means such an individual would be exposed to 51.7 mg of 5-HMF which could lead to exceeding the acceptable tolerable daily intake. Although serious health risks have not yet been associated with the dietary intake of high concentrations of 5-HMF [25], these compounds could be metabolized by sulfotransferases to produce an inherently mutagenic metabolite known as 5-sulfoxymethylfurfural [26].

Again as the compound is an indicator of the stability of the product, it value provide some form of information on the quality of storage conditions applicable to such products. The relatively high 5-HMF levels may therefore indicate that the products considered might not have been stored properly. As a quality assurance measure, these concentrations call for a closer look at the storage systems available for these products and make the necessary recommendations of Good Storage Practices, to ensure the integrity of the products. A product may not necessarily produce a foul odour or change colour before its integrity is compromised. The 5-HMF levels may therefore serve as early warning signs to call for the review of existing storage and distribution conditions so as to safeguard the products' quality.

Conclusion

MPs and CSDs sampled recorded relatively high 5-HMF levels as compared to previous studies elsewhere but their exposure levels were within acceptable limits.5-HMF levels were higher in MPs than CSDs; with observations of batch-to-batch variations. In addition, products of local origin recorded higher 5-HMF levels than those of foreign origin. These observations may be the result of differences and/or inconsistencies in processing systems applicable. The current findings call for the need to appraise existing processing systems, to assure reproducible quality products.

Data availability

Processed data used to support the findings of this study are included within the article. The source data are available at the Department of Pharmaceutical Chemistry and can be obtained upon request.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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