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Identification and Quantification of 5-Hydroxymethyl Furfural HMF in Some Sugar-Containing Food Products by HPLC

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Abstract: The study was conducted on some commonly available sugar-containing beverages and foods to evaluate their quality characteristics through the determination of HMF in them. The study covered eighty samples of different beverages and foods (natural honey, commercial honey, carbonated soft drinks, juices, jams, fructose sachets and Sudanese dates). Variable amounts (0-900 mg/k) of HMF were found in these products. High levels of HMF were mainly found in commercial honey and jams. The presence of HMF in these products in general was attributed mainly to the hot climate in Sudan coupled with storage conditions in addition to other possible causes discussed in this study. Due to absence in Sudan of imposed maximum levels for HMF in sugar containing products (low levels reflects food healthy products) this pioneer work is expected to be an initiator for authorities to give such studies weight and concern.

Key words: HMF, HPLC, honey, fructose, Sudan

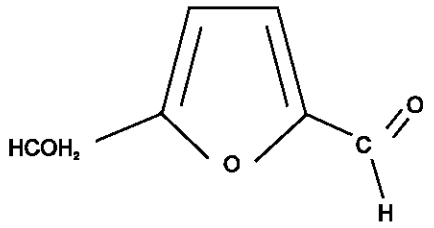
INTRODUCTION

5-hydroxy methyl furfural is an aldehyde and a furan compound. Its chemical abstracts name is 5-(hydroxyl methyl)2-furan carboxaldehyde (Technical Resources International, inc, 1994). Its structure is shown in Fig. 1. HMF is used in the synthesis of some organic compounds (Budavari, 1989), novolak type resins (Brode *et al*., 1982). It is an intermediate in the synthesis of several crown ethers (Larousse *et al*., 1992). It is also utilized to produce polymers, surfactants, solvents, pharmaceuticals and plant protection agents (Kunz, 1993). It should be kept protected from light and air (Budavari, 1989). It is normally formed during thermal decommission of sugars and carbohydrates.

HMF therefore has been reported in many food products including, honey, citrus juices, tomatoes paste, syrups, jams and bread. Its presence may be an indication of the spoilage, adulteration or heat stress of the sugar containing materials.

Humans are potentially exposed to HMF through pharmaceutical preparation, cigarette smoke and consumption of a number of commonly available beverages and foods.

Human exposures can occur by inhalation ingestion or skin absorption (Aldrich Chemical Co, 1994). Although HMF is not yet considered a harmful substance, the National Institute of Environmental Heath Science nominated HMF for testing based on the widespread of HMF in daily consumed foods, evidence for carcinogenic potential of other members of this class and the fact that little is known about HMF toxicity. The reported toxicity tests are mostly confined to mice and rats (Ulbricht *et al*., 1984).



5-HydroxyMethylFurfural

Fig. 1: 5-Hydroxy methyl furfural

The international Honey Commission recommends three methods for the determination of HMF in honey: two spectrophotometric methods (after white and after winkler) and an HPLC method (FPA, 2006).

In Sudan there is a wide spread and great consumption of sugar-containing foods and beverages; s such products are expected to contain HMF due to the strong heat and the prevailing sunlight.

Ambient temperature above 35°C is normal during the summer season which extends for about 6 months. The objective of this study, therefore is to investigate the presence and the content of HMF in sugar-containing foods and beverages which are exposed to high temperature during storage and distribution. The study is meant also to be a guide line for authorities to take measures that will ensure distribution of good quality foods and drinks

MATERIALS AND METHODS

Samples: The samples were collected from different local markers in Khartoum under different conditions of storage. The samples namely: natural honey (28

samples), commercial honey (10 samples), jam (11 samples), water based beverages (carbonated) 14 samples and juice (14 samples), Fructose (1 sample) and Dates (2 samples)

Apparatus:

- Membrane filters.
- Syringe membrane filters (0.45 µm).
- Analytical balance-from (Kern/Germany).
- Magnetic stirrer-from (Labtech).
- Micro filtration assembly-from (Janting).
- Micro syringe for HPLC-from (Waters/U.S.A).

Instrument:

- Isocratic HPLC pump with Inline degasser-Waters (USA)
- UV-Vis Detector-Waters (USA)
- C18 column-Waters (USA)
- Guard column-Waters(USA)

Methods: Five grams of (honey samples or jam) were dissolved in aprox 25 ml deionized water and transferred quantitatively to a 50 ml volumetric flask, diluted to 50 ml with deionized water, filtered through a 0.45 um membrane filter before injection into the HPLC column. Juices and carbonated soft drinks were injected directly in HPLC column without dilution.

HPLC condition:

Mobile phase:	5% acetic acid w/v in water, methanol (80:20)
Column:	C18 (5 µm)
Flow rate:	1 ml/min
Injection volume:	20 µL
Detection:	285 nm

RESULTS AND DISCUSSION

The stability studies and storage guidelines for photo labile or thermo labile compounds which are used by humans (drugs, foods, drinks), usually refere to temperate climate (with less sun shine and therefore with cold conditions). These studies may not be relevant to actual extreme climate conditions. Thus the stability of such compounds distributed and used in hot, humid and sunny climates can pose serious problems in African countries where strong heat (day and light) and sun shine prevail. Investigation of stability of such compounds should receive major concern. This is important to establish types of possible degradats, their toxicity, set limits for their LD_{50} doses as well as means for their stabilization or proper storage.

The Hydroxyl Methyl Furfural (HMF) is an aldhyde and a furan compound that is formed during the thermal decomposition of sugars and carbohydrates.

The objective of this work was therefore to investigate the possible presence and determine levels of hydroxylmethylfurfuran that can be formed in sugar containing foods and drinks due to the hot climate in Sudan, Khartoum state.

The presence of HMF is an indicator for spoiled, adulterated or products that were exposed to heat stress or bad storage conditions. On the other hand, HMF is considered an irritant and is irritating to eyes, upper respiratory tract, skin and mucous membrane. No positive or negative definite reports associating HMF with a cancer risk in humans were identified in available literature (FPA, 2006). However the National Institute of Environmental Health Sciences nominated HMF for testing based on the extensive human exposure, lack of a adequate data characterizing its toxicity and carcinogenicity. Miller (1994) in studies based on mice and rats, proposed that sulphonation of HMF may lead to multagenicity and carcinogenicity. Janzowski *et al.* (2002), studied the DNA damaging potential and reactivity of HMF towards cellular glutathione as an assessment of multagenicity of HMF. It is also reported that HMF damages striated muscles and viscera by combining to protein and thus causing the accumulation of poisons in the body (Pamplona *et al.*, 1995; Chi *et al.*, 1998). These ongoing reports about HMF toxicity whether in humans or animals makes the determination of HMF in foods and drinks an important concern at present time before solid toxicity reports can be documented.

There are three main methods in current use for measuring HMF: a colorimetric method (winkler method), a direct measurement using HMF absorbance at 284 nm (White method) (Stefan Bogdanov, 2002) and an HPLC method with detector set at 284 nm. The HPLC method was used in this study as it is a separation tool that can resolve the HMF from the complex matrix of the analysed products. In this present work, we studied the content of HMF in: 28 claimed natural honey samples coded as H_1-H_{28} (Table 1), 10 commercial honey samples coded as HP_1-HP_{10} (Table 2), 14 soft drink coded D_1-D_{14} (Table 3), 14 juices coded Ju $_1$-Ju $_{14}$(Table 4), 11 jam samples coded JA_1-JA_{11} (Table 5) and one sample of fructose sachets for diabetics coded FR_1. The samples were collected from the local market. The collection was varied so as to have samples from places of poor to good storage condition (those with or without cooling system or efficient ventilation or over crowded small places with high room temperature).

The concentration of HMF in these samples was calculated reference to a linear regression analysis data or from a direct sample/standard comparison method. The standard HMF area–concentration plot was rectilinear over the range 1-80 µg/ml with correlation coefficient (r) value 0.9998, slope (B) 252642 and intercept (A) 81098. The obtained results in ug/ml were converted to mg/kg for honey and jam samples and mg/L for the beverages.

Table 1: Results obtained for the HMF content in natural honey sample

Sample name	Level of HMF (mg/kg)	Production date
H_1	8.8	1/008
H_2	ND	10/007
H_3	15.2	8/005
H_4	ND	2/008
H_5	2.3	8/005
H_6	ND	12/007
H7	ND	9/007
H_8	ND	2/006
H_9	ND	2005
H_{10}	ND	1/007
H_{11}	9.5	2007
H_{12}	15.2	2007
H_{13}	19.8	2006
H_{14}	13	2007
H_{15}	13.1	2007
H_{16}	ND	2007
H_{17}	798.1	2006
H_{18}	232	2007
H_{19}	ND	3006
H_{20}	6.9	2007
H_{21}	922	2003
H_{22}	280	2007
H_{23}	274	2007
H_{24}	192	2008
H_{25}	4.9	2007
H_{26}	118.3	2007
H_{27}	48.6	2007
H_{28}	ND	2007

ND: Not Detected

Natural honey samples: Table 1 shows the results obtained for the different samples collected with different production dates (2003-2008). The HMF content of these samples showed great variation. Some samples were either free from or having negligible amount of HMF; amounts ranging from 5 mg/kg to 922 mg/kg were also obtained. Sample H_{21} (production date 2003) was having the highest amount of HMF. It is interesting to quote here that the owner of this sample claimed that he intentionally keeps such sample for long periods to use them in a formulation to treat certain eye diseases. Other samples with HMF content above 100 mg/kg could be due to bad storage, ambient temperature over 35°C in Sudan or possible adulteration with invert sugars (White, 1980). It is reported that it is common for honey sold in hot climates to have HMF content well over 100 mg/kg due to high temperature that honey is exposed to in the distribution channel (Dvid A-Cushman, 2007). Figure 2 and 3 shows typical chromatogram of injected standard HMF and bee honey sample with high HMF content.

Although HMF is not yet been ascertained as a harmful substance however, limits for its content in honey are imposed in many countries. This is to guard against distribution of honey of low quality. HMF is an indication of over heating, storage at elevated temperature or an aged sample or adulterated with invert sugars (FPA, 2006; White, 1980). A limit of 40 mg/kg was imposed by

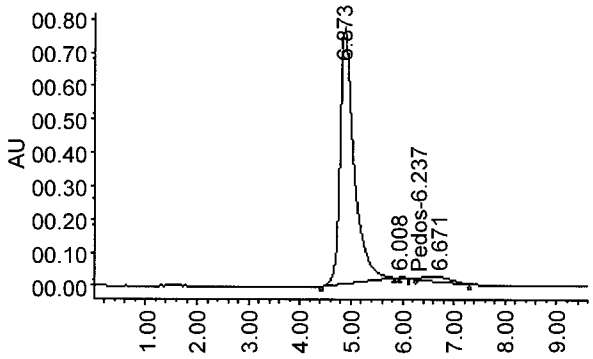


Fig. 2: Chromatogram of injected standard HMF

Table 2: The results obtained for the content of HMF in Commercial honey sample

Sample name	Level of HMF (mg/kg)	Production date
HP_1	614	11/007
HP_2	535	12/007
HP_3	303	2/008
HP_4(syrup)	341	7/007
HP_5	344	2006
HP_6	285	12/006
HP_7	67.3	6/007
HP_8	11	9/007
HP_9	121	11/006
HP_{10}	170	2/008

Czech legislation for HMF in honey (FPA, 2006). The international honey commission sets a limit of 80 mg/kg of HMF in honey. In Sudan there are no limits set for HMF content in foods and drinks.

Commercial honey samples: Table 2 shows results obtained for the commercial locally manufactured or imported honey samples. Most of these samples showed HMF content above 100 mg/kg. This could be due to excessive heating during manufacture or bad storage at elevated temperature. It is reported that the use of high fructose corn syrup as sweetener can lead to high HMF content reaching 100-1000 mg/kg. The breakdown of fructose to HMF is an acid-catalyzed process. It is also reported that many sugars type products e.g (golden syrup, molasses, etc), have levels of HMF that are 10-100 times that of honey (Dvid A-Cushman, 2007). In this study, one sample HP_4, is a golden syrup which showed an HMF content of about 341 mg/kg. If the accepted limits of honey are between 30-100 mg/kg, then this sample could be of a good quality. Figure 4 shows a chromatogram of HMF in a commercial honey sample.

Water based beverages

Carbonated soft drinks: Table 3 shows the results obtained for soft drinks manufactured in Sudan. The production dates for all were during the year 2008. Results of HMF varied between 0-3 mg/L. These drinks

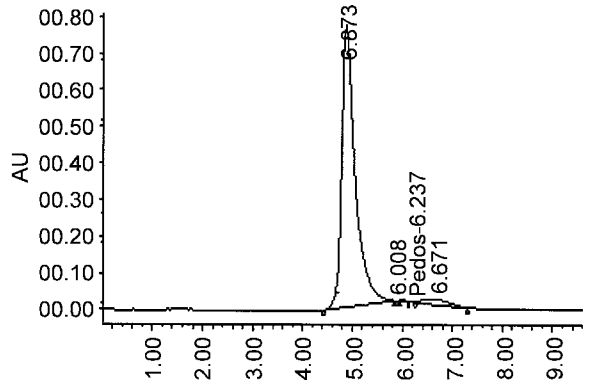


Fig. 3: Chromatogram of HMF in a natural honey sample

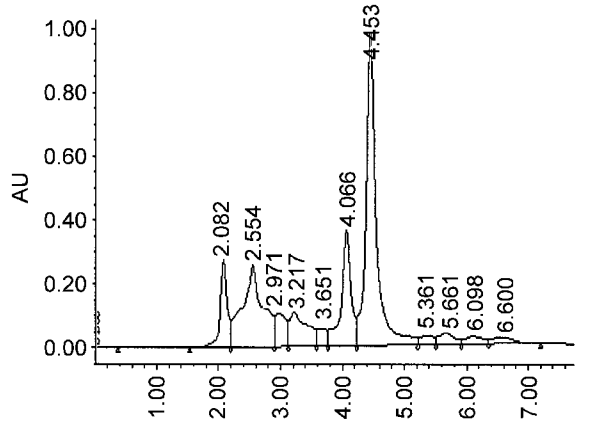


Fig. 4: Chromatogram of HMF in a commercial honey sample

Table 3: Results obtained for the content of HMF in Carbonated soft drink sample

Sample name	Level of HMF (mg/L)	Production date
D_1	ND	4/008
D_2	ND	5/008
D_3	ND	4/008
D_4	ND	3/008
D_5	ND	4/008
D_6	ND	4/008
D_7	0.19	4/008
D_8	0.28	4/008
D_9	3	4/008
D_{10}	ND	4/008
D_{11}	2.9	4/008
D_{12}	0.58	4/008
D_{13}	3.3	4/008
D_{14}	1.9	1/008

ND: Not Detected

have low sugars content i.e unlike honey samples whose sugar contents normally exceeds 80% w/w (Stefan Bogdanov, 2002). As mentioned before food items sweetened with high fructose corn syrups like carbonated soft drinks can have levels of HMF between 100-1000 mg/L (Dvid A-Cushman, 2007). These studied carbonated soft drinks seems not to be sweetened with fructose corn syrup (Fig. 5).

Table 4: Results obtained for the content of HMF in Juice sample

Sample name	Level of HMF (mg/L)	Production date
JU_1	1.3	4/008
JU_2	0.6	4/008
JU_3	5.4	4/008
JU_4	7.9	6/008
JU_5	1.2	4/008
JU_6	5.8	4/008
JU_7	2.0	4/008
JU_8	16.5	4/008
JU_9	ND	5/007
JU_{10}	4.8	4/008
JU_{11}	ND	3/008
JU_{12}	0.76	2/008
JU_{13}	ND	2/008
JU_{14}	75.5	1/008

ND: Not Detected

Table 5: Results obtained for the content of HMF in Jam sample

Sample name	Level of HMF (mg/kg)	Production date
JA_1	139.4	7/007
JA_2	117.3	3/008
JA_3	807	4/008
JA_4	205.9	3/008
JA_5	231.6	6/007
JA_6	394	1/007
JA_7	161.8	9/008
JA_8	291.2	8/008
JA_9	26.1	3/008
JA_{10}	31.8	1/008
JA_{11}	139.3	12/007

Juices: Table 4 shows results obtained for juices locally manufactured or imported samples. Results varied between 0-75 mg/l.

The indicators for good manufacture and proper storage of juices is the low levels of HMF and furosine which is used as a common quality marker. High furosine levels and low HMF levels are considered indicators for prolonged storage, while high HMF values and low furosine values may indicate severe heat treatment during manufacture (FPA, 2006). The temperature under which this prolonged storage rook place was not stated. Therefore in hot climates prolonged storage is expected to increase both furosine and HMF. In this study only HMF was determined in juices. The European Union (EU) set a limit of 20 mg/kg HMF for juices made for children (FPA, 2006). The low sugar content of the investigated juices could be the cause of low HMF values generally observed in these samples. Figure 6 shows a chromatogram of HMF in a sample of juices.

Jams: Table 5 shows the results obtained for the jam samples studied. HMF values ranged between 26-807 mg/kg. As for juices, HMF and furosine are criteria used to assess the quality of samples (FPA, 2006). Most of these samples showed HMF levels above 100 mg/kg.

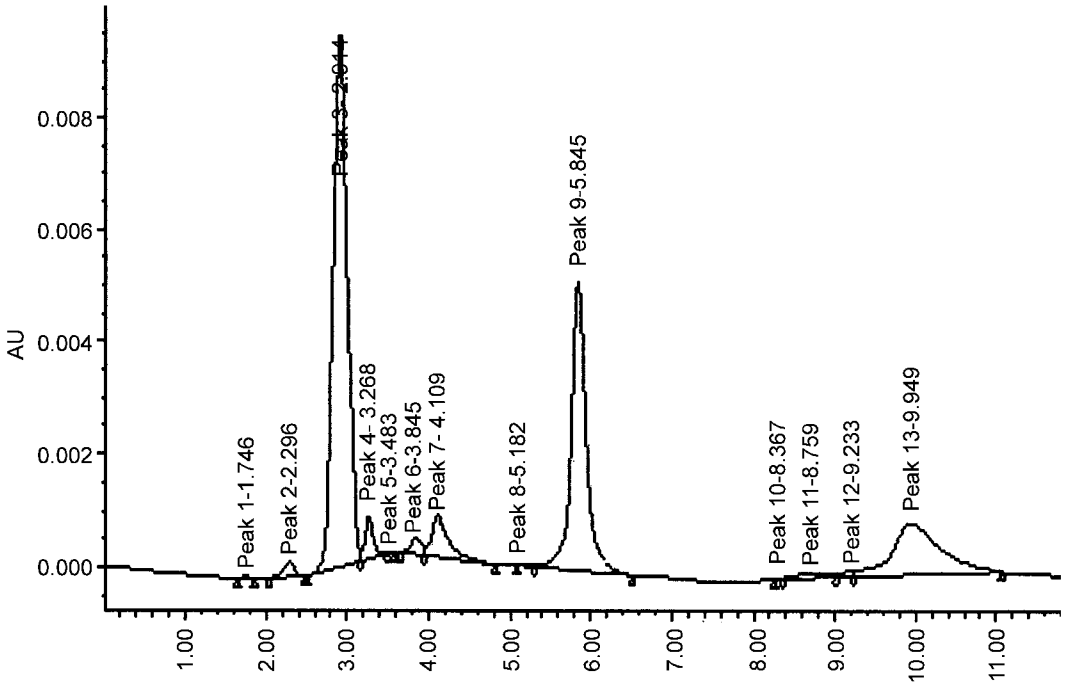


Fig. 5: Chromatogram of HMF in a carbonated soft drinks sample

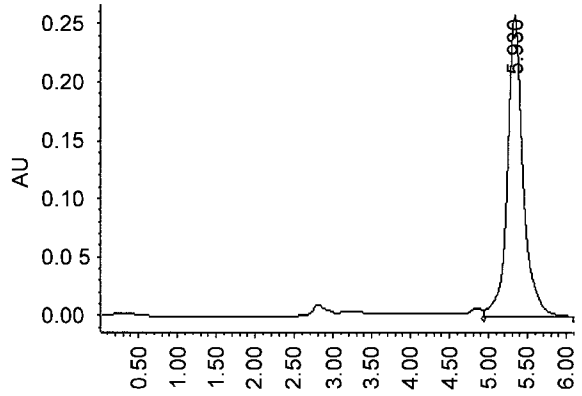


Fig. 6: Chromatogram of HMF in a juice sample

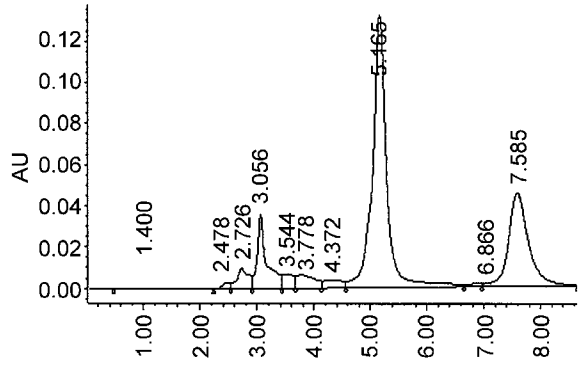


Fig. 7: Chromatogram for HMF in a jam sample

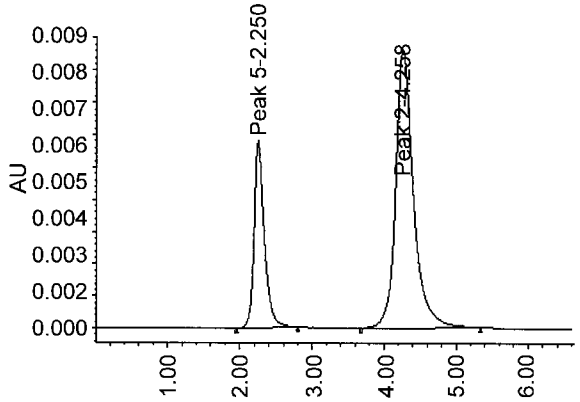


Fig. 8: Chromatogram for HMF in a fructose sample

The high sugar content of these samples, coupled with the hot condition to which these samples are exposed to in the distribution channel could be the major cause of such high values.

No limits are set for HMF content in jams to our knowledge. Figure 7 shows a chromatogram for HMF in a jam sample.

Besides the above mentioned samples, a sample of fructose provided in sachets for diabetics and two old samples of Sudanese dates were also investigated for their HMF content. The results were 4.2 mg/kg for the fructose sample (Fig. 8) and 147 mg/kg and 310 mg/kg for the date samples. The aged date samples which were exposed to sun light and high temperature were

very dark in color. The high HMF content was therefore expected.

This study was carried during a three month period. An increase of the retention time of the eluting peaks was observed during this period, despite the use of guard column or column-washing from time to time with aqueous acid solvent. This increase could be due to some sort of column poisoning with the complex matrix of the injected products, which also have different pH values. This necessitates the daily injection of standard HMF before and after samples injectionss. Sometimes, samples spiking with HMF was necessary to ensure the retention time of HMF in the sample for accurate HMF content determination.

Conclusion: As many countries impose maximum levels of HMF for food products, it is important to impose limits for HMF content in hot climate countries for many food items. This will ensure distribution of food products of good quality and less possible health hazards. In Sudan, many food products are manufactured locally but there is no proper control on the actual good manufacturing practice for these products. The follow up of these products is mainly concerned with physical changes rather than possible chemical decomposition or tests for adulterants. This pioneer work was carried in khartoum city only where better conditions for storage are available compared to areas outside khartoum province. No doubt extension of this work to include investigation from different parts in Sudan is essential to accumulate data for such products so as to be the quality guide line for setting maximum levels of HMF in commonly available beverages and foods.

Mean while and before future studies can be done we recommend that all sugar-containing products should be stored at temperature below 30°C This is based on the study carried by White and Subers (1964) who proved that the time for 30 mg/kg HMF to accumulate in honey samples at 30°C is 100-300 days.

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