



Research Article



Effect of pH and Storage Temperature on 5-(Hydroxymethyl) Furfural (5HMF) Formation in USP Syrup Preparation

Faranak Ghaderi^{1,2}, Mohammad Reza Siah Shadbad^{1*}, Molood Hoseinzadeh¹

¹Department of Drug and Food Control, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

²Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran.

ARTICLE INFO

Article Type:
Original Research

Article History:
Received: 12 August 2014
Accepted: 29 October 2014

Keywords:
5HMF
USP syrup
temperature

ABSTRACT

Background: 5-(Hydroxymethyl) furfural (5HMF) is a compound that is formed by during thermal processing or heat sterilization of glucose, fructose and sucrose solution formulations, dialysis fluids and medical syrups. Examinations show that the storage quality dramatically reduces owing to the formation of toxic compound 5-HMF. 5HMF is considered as an irritating substance and is irritating to eyes, upper respiratory tract, skin and mucous membranes. The aim of this study is to evaluate the effect of pH and storage temperature on 5-HMF production in USP syrup. **Methods:** The USP syrups prepared with mixing method without heating at pH 3,4,5,6 and 8 and stored in oven at 50, 60, 70°C. The sampling has been done in several days and the amounts of 5HMF have been immediately determined by simple difference spectrophotometric method. **Results:** The results elucidated that low pH for preparing USP syrups leads to invert sucrose to sugars and increasing the rate of fructose dehydration, therefore an increase in 5HMF formation observed. Eventually, the high pH of syrup preparation delays the formation of 5HMF in different storage temperatures. Although, temperature influences on 5HMF formation, but the role of pH is more remarkable. **Conclusion:** The obtained results of this research indicated that optimal temperature and pH selection in preparation and storage of syrup cause increasing stability and reducing 5HMF formation.

Introduction

5-(Hydroxymethyl)furfural (5HMF), is an organic compound derived from dehydration of certain sugars.¹ The molecule consists of a furan ring, containing both aldehyde and alcohol functional groups.² Its IUPAC name is 5-(hydroxymethyl)-2-furaldehyde and its structure is shown in Figure 1.

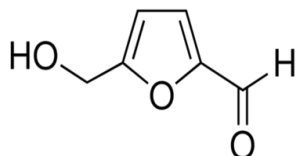


Figure 1. Structure of 5HMF

5-HMF is readily produced by the thermal decomposition of aqueous dextrose solutions during heat sterilization and upon long time storage condition³. Several reports confirm the toxic effect of 5-HMF for human health. Temperature and pH are both vital factors directly affecting on quality of syrup, especially in the long term storage. Therefore, it is an indicator of syrup quality. The thermal degradation of aqueous

dextrose solutions has been extensively studied and 5HMF firmly established as the principal decomposition product in acidic solution.³

5HMF has been determined in parenteral solutions. Ulbricht *et al.* reported the results of several studies that have determined the concentrations of 5HMF in these products.⁴ In sterile glucose solutions, 5HMF concentrations of about 1 to 90 mg/liter have been reported. Parenteral solutions containing invertose or glucose have been reported to have 5HMF concentrations ranging from 3 to 56 and 1 to 4 mg/liter, respectively.

5HMF concentration strongly depends on pH, sterilization temperature, (>110°C) and sterilization time. 5HMF is considered as an irritant and is irritating to eyes, upper respiratory tract, skin and mucous membranes. Human exposures can occur by inhalation, ingestion or skin absorption. Initiation/promotion researches showed that 5HMF may act as an initiator and promoter of colon cancer in rats. No positive or negative epidemiological studies or case reports associating 5HMF with a cancer risk in humans were identified in the available literature.⁵ Also according to

*Corresponding Author: Mohammad Reza Siah Shadbad. Tel: (+98) 41 33341315, Fax: (+98) 41 33344798. E-mail: siahimr@tbzmed.ac.ir

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infusion studies with rats and dogs, 5HMF does not seem to be acutely toxic at normal concentrations in parenteral infusion solutions (e.g., 10 mg/liter). Dosages of parenterally administered 5HMF exceeding 75 mg/kg body weight causes some toxic effects, including increased activity of hepatic enzymes, altered serum-protein fractions, increased relative spleen weight, and hepatic fatty degeneration. Nearly 50% of 5HMF in parenteral solutions is oxidized and eliminated by the kidneys.⁴ Owing to formation of 5HMF, as the prevalent major degradation product of dextrose, a pharmacopeial limit test has been set up for "5HMF and related substances". This limit test was appointed with the publication of the Fourth Supplement to USP XIX on 1 May 1978, including the direct measurement of the absorbance of a portion of appropriately diluted sample solution at 284 nm.⁶

Upper limit for the test is 0.25 absorbance units, which is equivalent to 2 ppm of 5HMF. The test is also essential for a number of infusion fluids based on dextrose mixtures⁷. Many studies have confirmed the presence of 5HMF in food and pharmaceutical products.

Nilsson *et al.* have reported the heat sterilization of commercial peritoneal dialysis fluids give rise to 5HMF, 2-furaldehyde and several aldehydes such as acetaldehyde which may contribute to adverse effects of these fluids on patients⁸. Henderson *et al.* also showed that long-term storage of these fluids increased the level of 5HMF.⁹ Suzan Zein *et al.* showed high levels of 5HMF were mainly found in commercial honey and jams. The presence of 5HMF in these kind of products, generally, was related to the hot climate in Sudan and storage conditions.¹⁰

There are several methods for determination of 5HMF concentration such as difference UV spectrophotometric assay and high performance liquid chromatography (HPLC) methods. Arefhosseiny *et al.* in difference UV spectrophotometric assay involved measurement of the difference absorbance of a solution of 5HMF relative to that an equimolar solution in which absorption had been destroyed by sodium borohydride.¹¹

The difficulty of such study encourages the investigators to use simple methods in order to get fast results. Thus, the main objective of this study is to evaluate the effect of pH and storage temperature on the 5HMF level which produced in USP syrup (85% sucrose in water) with simple and fast spectrophotometric method.

Materials and Methods

Chemicals

5HMF (98%) was purchased from Janssen Pharmaceutica (Beerse, Belgium), sodium borohydride, sodium hydroxide, citric acid monohydrate and ethanol were provided by Merck (Darmstadt, Germany). Sucrose and distilled water were prepared in the pharmaceuticals laboratory (Faculty of Pharmacy,

Tabriz, Iran) and double distilled water was prepared from Zahravi (Tabriz, Iran).

Apparatus

The equipment were used including analytical balance from Sartorius (Gottingen, Germany), magnetic stirrer from Nuova (Illinois, USA), pH meter from Corning (New York, USA), spectrophotometer UV160 from Shimadzu (Kyoto, Japan), oven (50, 60 and 70°C) and ultrasound apparatus from Transonic (Singen, Germany).

Solutions preparation and procedure

Buffer solutions with determined pH (3, 4, 5, 6, and 8) were prepared with combination an appropriate amount of citric acid (0.1 M) and sodium hydroxide (0.1 M). For producing the calibration curve 5HMF several standard solutions accurately were prepared with determined concentration (8, 4, 2, 1 and 0.4 µg/ml).

For each reaction, 5 ml of standard solution (from different concentrations) was transferred into each balloon and in addition to standard solution 2 ml of sodium borohydride solution (0.5%) was added to blank. Then the blank absorbance changes versus the standard solution were obtained at 284 nm using UV-Vis spectrophotometer and the absorbance values were plotted against the related concentrations (Conc. = 8.88 abs- 0.043). USP syrup (85% w/v) simply prepared by mixing method without heating. 85 g of sucrose was dissolved in 42.5 ml of distilled water while stirring, and then bring the total volume to 100 ml with buffer solutions with mentioned pH ranges (3, 4, 5, 6 and 8) to give syrup 85% w/v. To determine the effect of heat treating on the 5-HMF formation, the syrup samples were stored in oven at 50, 60, 70°C during 63 days and sampling was performed 2, 4, 8, 18, 30 and 63 days after storage. After dilution, 5 ml of each sample was poured into the test tubes and blank tubes and then 5 ml of sodium borohydride solution (0.5%) was added to blank tubes. Samples were placed in the ultrasound machine for 5 minutes. Then the absorbance changes in the samples were recorded at 284 nm and the concentrations of 5HMF in diluted samples were determined from the calibration curve. Excel software was used for data analysis.

Results and Discussion

As it is presented in Figure 1, 5 HMF levels in syrups plotted against various pHs (3, 4, 5, 6 and 8) at 50 ° C. Comparing the different values of 5HMF for different sampling days of the same pH range, reveals that 18 days after sample storage a remarkable increasing in the amounts of 5HMF was exerted.

Figures 2 and 3 indicate the concentrations of 5 HMF in oven at 60 ° C and 70 ° C, respectively while the pH range (3, 4, 5, 6 and 8) is same for both. (Since there was no significant difference in the amounts of 5HMF, 30 and 63 day after storage, at 60°C, sampling was not performed at 70 ° C on 63th day.)

There are great increases in 5HMF values 8 and 4 days after storage at 60°C and 70°C, respectively.

In order to feasibility of data comparison, full details are tabulated in table 1.

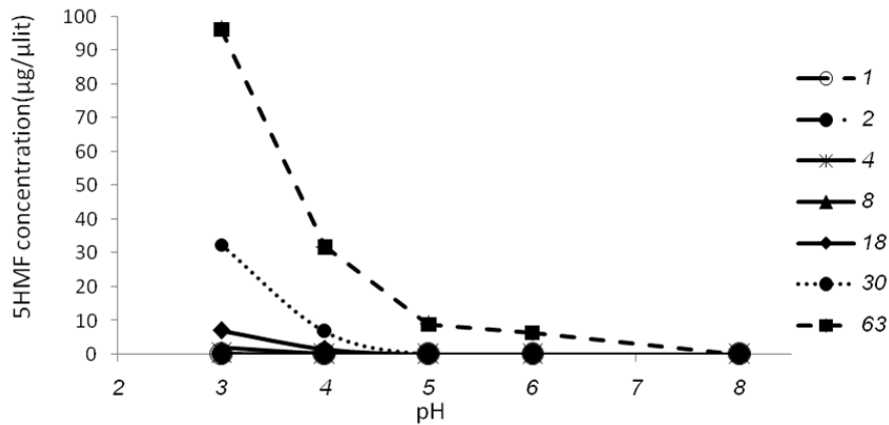


Figure 2. Values of 5HMF ($\mu\text{g}/\text{ml}$) in prepared syrups in pH 3, 4, 5, 6 and 8 and storage temperature 50°C. (Sampling was performed 2, 4, 8, 18, 30, 63 days after storage).

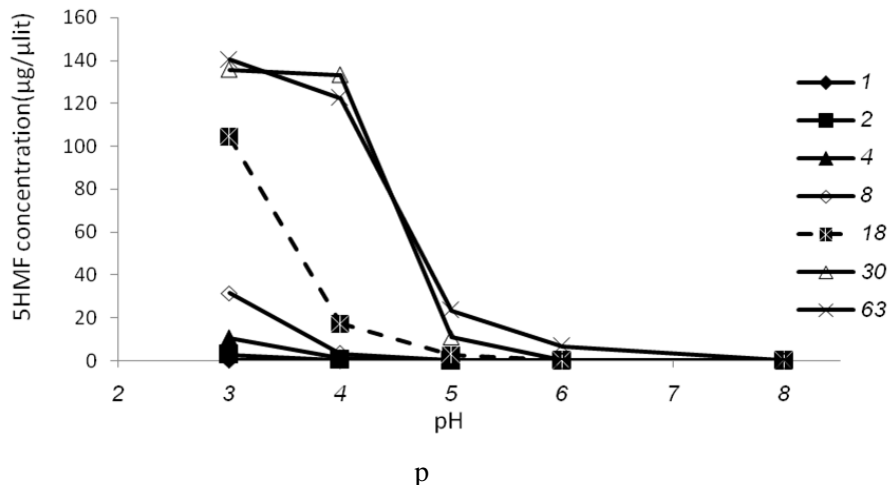


Figure 3. Values of 5HMF ($\mu\text{g}/\text{ml}$) in prepared syrups in pH 3, 4, 5, 6 and 8 and storage temperature 60°C. (Sampling was performed 2, 4, 8, 18, 30, 63 days after storage).

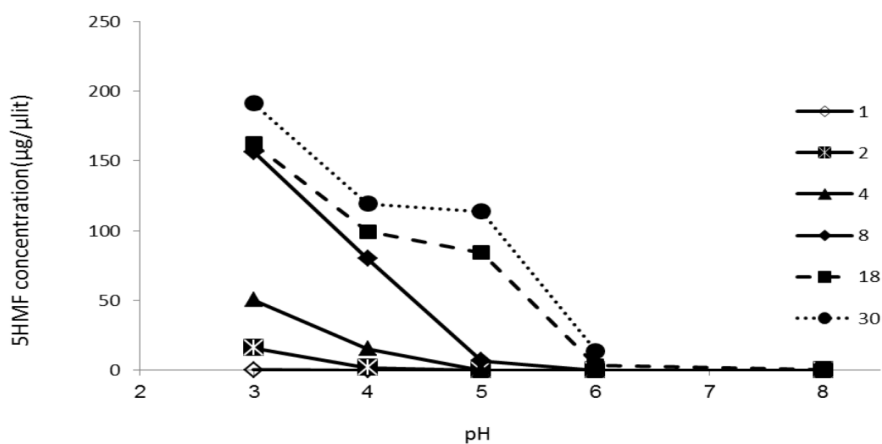


Figure 4. Values of 5HMF ($\mu\text{g}/\text{ml}$) in prepared syrups in pH 3, 4, 5, 6 and 8 and storage temperature 70°C. (Sampling was performed 2, 4, 8, 18, 30, days after storage).

Table 1. Values of 5HMF ($\mu\text{g/ml}$) in prepared syrups at 50, 60, 70°C and pH3,4,5,6 and 8 on 8th, 18th, 30th and 63th days of storage.

Storage days	pH 3			pH 4			pH 5			pH 6			pH 8		
	T (°C)			T (°C)			T (°C)			T (°C)			T (°C)		
	50	60	70	50	60	70	50	60	70	50	60	70	50	60	70
8	156.45	31.49	1.99	80.01	3.41	0.39	6.79	0	0	0.03	0	0	0	0	0
18	162.40	104.37	7.14	99.30	17.27	1.62	84.28	2.70	0	3.41	0	0	0.74	0	0
30	191.30	135.65	32.56	119.20	133.3	6.79	113.8	10.87	0	13.90	0	0	0.92	0	0
63	ND	140.20	96.02	N.D	122.5	31.85	N.D	23.30	8.92	ND	6.76	6.43	ND	0.21	0

ND: No data

Overall, observations showed that the amounts of 5HMF are decreased while increasing in pH value for all three temperatures. It is worth to mention that, on 30th and 63th days, at 60 °C 5HMF values are not so different.

Eventually, with increasing storage temperature in prepared syrups, the amounts of 5HMF increased and the level of 5HMF also decreases with increasing the pH of syrups. These lead to conclusion that, apart from storage time and temperature, 5HMF formation is exacerbated in acidic media. In this account, we discuss at pH 8 there is no production of 5HMF is observed after 8 days of sample storage.

The impact of basic media with reduced glucose degradation is might be ascribed to less formation of fructose from glucose therefore, this will cause less 5HMF formation. It is known that acids have a potent influence on the dehydration rate of fructose. This may result to an increase in the direct generation of 5HMF.¹² In another study Daniel *et al.* showed that fructose has a protective effect on 5HMF decomposition in solution. As a result, at low pH high concentration of 5HMF is produced.¹³ As this study showed the increasing pH could be delayed the production of 5HMF. As aforementioned, when the pH of syrups was high consequently 5HMF formation was low.

In addition, the 5HMF concentrations were observed to increase with heat ascending in all sampling days.

Conclusion

Taken together, it was shown that there is a significant dependence of the formation of 5HMF on pH and temperature of storage. Thus, it can be concluded from this study, the best way to avoid 5HMF formation is to use high pH in preparation of syrup as well as low storage temperature. However there have been no report on dependency of pH and temperature on 5-HMF concentration in syrup, these kinds of investigations lead to recommend the best storage condition.

Acknowledgement

This article was written based on a dataset of Pharm. D Thesis (No.2861), registered in Tabriz University of Medical Sciences. There are no financial interests related to the material in the manuscript.

Conflict of Interest

The authors report no conflicts of interest.

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