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Analysis of 5-hydroxymethyl-2-furfural in Invert Sugar Collected after Clarification in a Sugarcane Ethanol Industry in Brazil

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***Abstract*—5-Hydroxymethyl-2-furfural (HMF), a furan derivative, is one of the major contaminants formed during the chemical treatment step in ethanol production. In addition to being toxic to humans, HMF is a fermentation inhibitor that interferes with the production of sugarcane derivatives. Of the different methods that can be used for identification of HMF, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) is the most used. In this study, we evaluated the presence of HMF in invert sugar collected from fermentation tanks in a Brazilian sugarcane ethanol industry by using a simple and fast sample preparation method. HMF quantification was performed by HPLC-UV at 285 nm. One of the samples was found to contain 118 µg/kg, indicating the need for monitoring of HMF levels for improved process control in the sugarcane ethanol industry.**

***Index Terms*—biofuel, contaminant, fermentation inhibition, liquid chromatography.**

I. Introduction

In ethanol production from renewable resources, such as sugarcane, fermentation inhibitors formed during the pretreatment step can negatively affect production yields and the environment [1]–[3]. During chemical treatment for removal of impurities and recovery of sucrose, it is important to avoid sucrose inversion and decomposition to monosaccharides, such as glucose and fructose, which can further decompose to 5-hydroxymethyl-2-furfural (HMF) [1]. The presence of furfural and HMF, two major inhibitors of sugar fermentation, in sugarcane bagasse strongly influences biomass hydrolysis, decreasing the efficiency of second-generation ethanol production and leading to reduced ethanol yield.

If, on the one hand, HMF is one of the most toxic fermentation inhibitors found in lignocellulosic hydrolysates [4], on the other hand, it is a renewable platform compound that serves as a substitute for petroleum-derived building blocks in the production of chemicals [5]–[7]. This furan derivative is a six-carbon heterocyclic compound containing aldehyde and alcohol functional groups (Fig. 1). It is obtained as a yellowish solid with high water solubility and low melting point [8].

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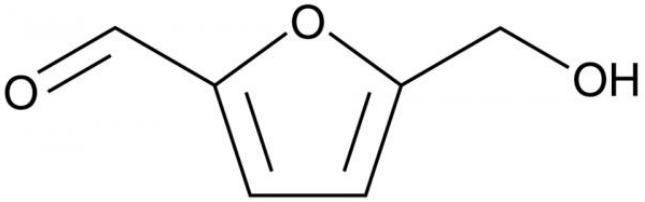

Fig. 1: Chemical structure of 5-hydroxymethyl-2-furfural (Source: ChemSpider).

Several approaches have been developed for detection of HMF in different matrices, such as flame atomic absorption spectrometry and liquid chromatography coupled with mass spectrometry and diode array detection [9]–[11]. High-performance liquid chromatography with detection in the ultraviolet range (HPLC-UV) is a pioneering method for HMF analysis in molasses, honey, and sugarcane juice [12]–[14].

In view of the importance of HMF both for the sugarcane ethanol industry and as a platform molecule for biofuel production, this study aimed to evaluate the presence of HMF in invert sugar collected from industrial fermentation tanks by adapting a previously reported method [12].

II. Material and Methods

Samples

Four samples of invert sugar were collected from fermentation tanks at a sugarcane ethanol industry in São Paulo, Brazil. Invert sugar was produced by an enzymatic route using invertase. Samples were stored under refrigeration (5 ± 2 °C) until analysis.

Sample preparation

Samples were analyzed according to [12]. Briefly, 150 mg of invert sugar was diluted in 10 mL of 5 mmol/L H_2SO_4 (J.T.Baker, USA) under vigorous manual stirring, filtered through a polyvinylidene difluoride membrane filter (0.20 µm; Millipore, USA), and immediately analyzed by HPLC-UV.

HPLC-UV

The analysis was conducted using an ultra-high performance liquid chromatograph connected to an UV detector (Waters, USA) equipped with an analytical column (300 mm × 7.8 mm, 5 µm; Rezex Roa-Organic Acid H^+,

Phenomenex, USA). The column temperature was set at 55 °C. The mobile phase consisted of 5 mmol/L H_2SO_4 at a flow rate of 0.6 mL/min. HMF was detected at 285 nm. The injection volume was 20 µL. A calibration curve was constructed by injecting standard solutions at five concentrations (0.66–4.98 µg/mL). The curve had a correlation coefficient (R) greater than 0.99.

Statistical analysis

Statistical analysis of sample concentration and linearity data was performed using Statistica software version 7.0.

III. Results and Discussion

Jeuring and Kuppers [12] proposed a rapid method for HMF determination in honey and cognac by HPLC-UV. Previous reference methods were considered expensive and toxic to analysts [15], [16]. Given the similarity between invert sugar and honey, in the current study, we adapted the method proposed by [12].

The analytical column used was Rezex Roa-Organic Acid H^+, with 8% coating in the form of ionic hydrogen. The column is commonly used for quantification of organic acids, alcohols, and neutral compounds. Therefore, the use of a compatible mobile phase was necessary. Under the analytical conditions used here (Table 1), the retention time of HMF was 38.4 min (Fig. 2). Similar to honey, invert sugar does not contain many interferents. Therefore, HMF can be quantified by performing simple dilution, followed by immediate injection.

HMF content in invert sugar samples was determined using external standards (0.66 to 4.98 µg/mL) by constructing a linear analytical curve obtained by the least squares method. The model was subjected to analysis of variance, and linearity deviation was not observed ($p < 0.05$). R was greater than 0.99, and residuals, which were randomly distributed, did not exceed 20% of the variance (Fig. 3).

Table I: Analytical conditions.

Parameter	Condition
Instrument	HP 1050 series HPLC, Rheodyne manual injector
Column	Rezex ROA-Organic Acid H^+
Mobile phase	5 mmol/L H_2SO_4
Flow rate	0.6 mL/min
Temperature	55 °C
Detector	Waters 486, 285 nm

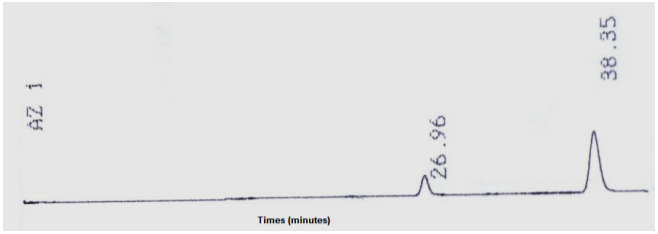

Fig 2: Chromatograph of a standard solution of 5-hydroxymethyl-2-furfural injected at 0.66 µg/mL (retention time of 38.35 min).

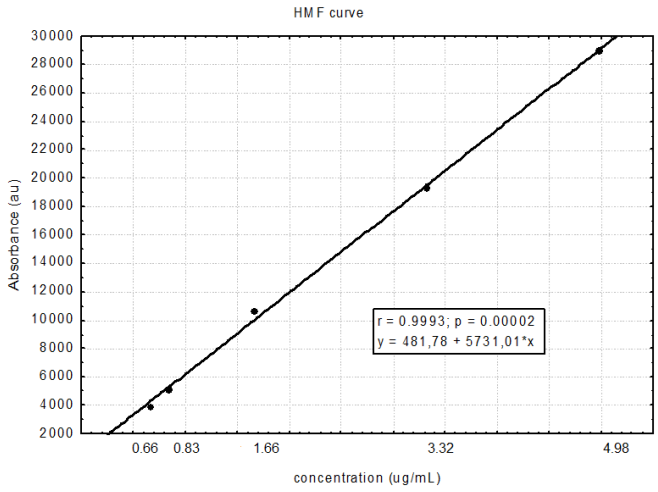

Fig 3: Analytical curve of 5-hydroxymethyl-2-furfural at concentrations ranging from 0.66 to 4.98 µg/mL.

Of the four samples analyzed (in triplicate), only sample A contained quantifiable HMF levels (118 µg/kg) (Fig. 4).

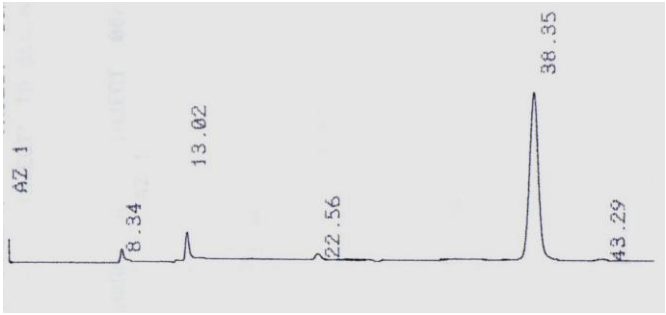

Fig. 4: Chromatogram of invert sugar (sample A) contaminated with 5-hydroxymethyl-2-furfural (retention time of 38.35 min).

The presence of HMF indicates that, during pretreatment, sucrose might have decomposed into sugars such as glucose and fructose. These sugars, when subjected to the high temperatures of fermentation tanks, might have reacted, forming furan derivatives such as HMF. The presence of HMF is common in the sugarcane ethanol industry; thus, various interventions and control strategies are used to monitor the levels of these contaminants in fermentation broths. The method used here is fast and may be easily applied in the industry.

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