

Kinetic Determination of 2-Furfuraldehyde Based in a Modified Winkler's Method

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A kinetic study of the Winkler's reaction for the determination of 2-furfuraldehyde (FUR) has been made on the basis of the absorbance–time curves. Winkler's method has been modified and only one step is necessary to add all reactive components. A kinetic method for the determination of FUR was developed with a range of application between 0.12 and 2 $\mu\text{g mL}^{-1}$. The proposed kinetic method permits one to determine FUR in spirit beverages without the instability problems of the conventional Winkler's method. Only, 40 s per sample is necessary for the analysis.

Keywords: Furfuraldehyde; Winkler's method; kinetic; spirit beverages

INTRODUCTION

2-Furfuraldehyde (FUR) is the principal product of the hydrolysis of pentoses. This compound and 5-(hydroxymethyl)-2-furfuraldehyde (HMF) seem to be related with browning processes of diverse food. Both are used as indicators of temperature abuse or storage time in diverse food (Codex Alimentarius, 1969; Mijares et al., 1986; Beeman, 1987; Sandoval and Hidalgo, 1967; Villalón Mir et al., 1992; Jeuring and Koppers, 1980). The analysis of FUR is very important in the food industry. For example, FUR is formed from the pentoses during wine distillation, and the FUR amount in several spirits (brandy, wine, cognac, etc.) is considered as a quality criterion. Several spectrophotometric methods have been applied to analyze FUR (Dinsmore and Nagy, 1974; Linares et al., 1987; Alonso et al., 1983; Espinosa-Mansilla et al., 1993). However, the most commonly employed spectrophotometric method is that of Winkler (Winkler, 1955; Espinosa-Mansilla et al., 1992) because of the high sensitivity of the method and the larger bathochromic shift exhibited by the derivatized product. However, an important limitation to the Winkler method is the instability of the product generated. Because of this instability, only an interval of 1–4 min is optimum to make the measurement and therefore the recovery values obtained are highly variable.

A continuous flow injection analysis (FIA) system to analyze HMF in honey was proposed to resolve this problem (Salinas et al., 1991). When the semiautomatic system was applied to analyze high-content alcoholic samples, two limitations were observed. First, sparing sensitivity, and second, a light dispersion effect due to the solvent content and because of the lower solubility of the FUR derivative in comparison with that of the HMF derivative.

In this paper, a kinetic study of the derivatization of FUR by Winkler's reaction is described. The influence of physical chemistry parameters were also studied. The aim of this work was to develop a sensitive kinetic method to determine FUR in diverse colored and/or turbid spirit samples without the problem of product instability found with the use of the equilibrium method.

EXPERIMENTAL PROCEDURES

Apparatus. A Milton Roy Spectronic 3000 diode-array spectrophotometer interfaced to a PC 386 microcomputer were used to register the kinetic curves. All measurements were

performed in a 10-mm quartz cell, at 40 °C, with a thermostated cell holder and a Selecta unitronic 320 OR thermostated bath. Milton Roy software (Rapid Scan) was used for all data acquisition, measurement, and analysis of all kinetic data.

Reagents. *Reagent for Derivatization.* A solution was prepared in a 100 mL calibrated flask by adding 7.63 g of *p*-toluidine (Panreac), 0.05 g of barbituric acid (Fluka AG), 3 mL of glacial acetic acid, and 50 mL of ethanol 96%, and diluting to the mark with deionized water.

Furfural Stock Solution. A 0.01% (w/v) solution was prepared by dissolving 0.01 g of reagent (Sigma) in 96% ethanol.

Procedures. *Proposed General Procedure To Determine FUR.* Two milliliters of the reagent for derivatization was placed in a 3-mL quartz cell, a known volume of sample containing 0–2.0 $\mu\text{g}\cdot\text{mL}^{-1}$ of FUR was added, and ethanol was added for dilution to a final volume of 3 mL. The evolution of the absorbance was scanned, with the time at $\lambda = 585$ nm, over 60 s. The temperature was maintained at 40 °C. The measurement time period selected was between 0 and 40 s.

Procedure for the Determination of FUR in Spirit Beverages. Two milliliters of the reagent for derivatization was placed in a 3-mL quartz cell, 100 μL of spirit beverage was added, and ethanol was added for dilution to a final volume of 3 mL. The kinetic curves were registered at 585 nm, and the aforementioned general procedure was followed.

RESULTS AND DISCUSSION

FUR reacts with barbituric acid and *p*-toluidine in acid medium to form a violet product with an absorption maximum located at 585 nm. The derivatized product is sparingly soluble in water, and a high content in ethanol is necessary to maintain it in solution. The derivatized FUR exhibits a high rate of formation but it is unstable. In Figure 1a, the kinetic curve obtained for the derivative product is shown and the absorption spectra of this product is shown in Figure 1b. A linear relation is observed between the variation of the absorbance at 585 nm with the reaction time up to ~50 s and for reaction times >100 s, the absorbance rapidly decreases.

Kinetic Behavior. The influence of the *p*-toluidine concentration was studied in the range 0.15–0.50 M. The initial rate remains practically constant for concentrations >0.310 M and increases (a 1-partial order was found) for smaller values. In Figure 2a, a logarithmic graph of the rate of reaction versus concentration of *p*-toluidine is shown. A 0.56 M *p*-toluidine concentration was selected as optimum.

The influence of barbituric acid was studied in the range 0–4 $\times 10^{-3}$ M. The increase in the rate of the

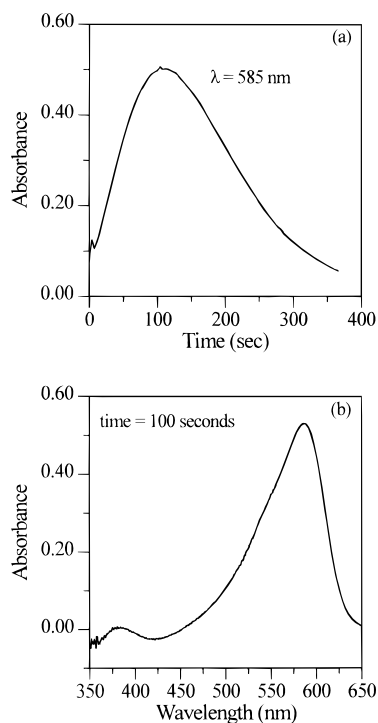


Figure 1. (a) Kinetic curve for the derivatization reaction, registered at 585 nm. (b) Absorption spectrum for 100 s of reaction time. ([FUR] = 1.0 $\mu\text{g}\cdot\text{mL}^{-1}$).

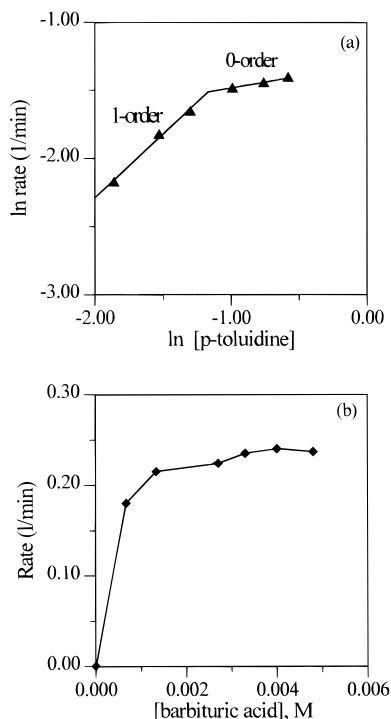


Figure 2. (a) Logarithmic plot rate versus *p*-toluidine concentration. (b) Influence of the barbituric acid concentration in the rate of the reaction.

reaction as the concentration of barbituric acid increases is shown in Figure 2b, where it is evident that the rate increases up to 2×10^{-3} M barbituric acid. For concentrations higher than this, the rate of reaction remains constant (a 0-partial order was found). A barbituric acid concentration of 2.7×10^{-3} M was selected as the optimum for the analytical procedure.

The acidity of the medium affects the development of the derivatized reaction. The rate of reaction was maximal and constant for acetic acid concentrations

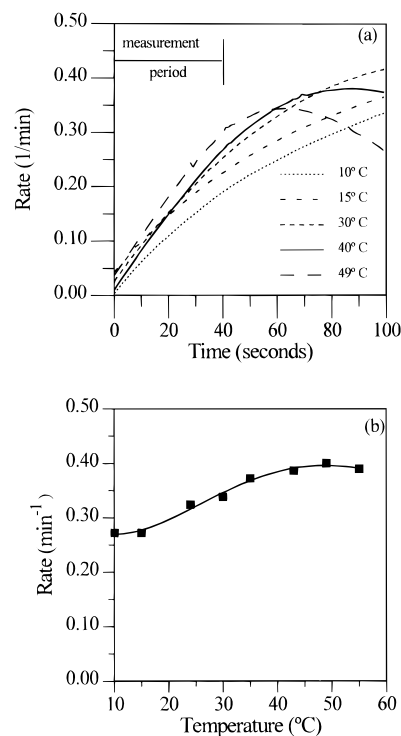


Figure 3. Influence of the temperature on (a) the kinetic curves and (b) the rate of the reaction.

between 0.30 and 0.55 M, and decreased markedly for smaller values. A 0.35 M acetic acid concentration was selected as optimum.

The influence of the proportion of the ethanol in the medium is very important because the derivatized product obtained is unstable in aqueous solution. The proportion of the ethanol in the medium was varied between 40 and 75%. The rate of the reaction is constant for proportion values of ethanol between 50 and 66% and the rate decreases for values lower and higher than this range. A percentage of ethanol of 66% in the medium was selected.

The results obtained with sequential addition of the several reagents necessary for the reaction and with only one addition of a solution containing all the reagents in the optimal established conditions (barbituric acid plus *p*-toluidine plus ethanol) were similar. On the other hand, the stability of the derivatization reagent over time has been proven for >20 h.

Influence of Temperature. The effect of the temperature on the reaction rate was examined between 10 and 60 °C, for samples containing $1.3 \mu\text{g}\cdot\text{mL}^{-1}$ of FUR. In Figure 3a, the different kinetics curves have been represented. A slight increase in the rate of reaction with temperature was observed up to 35 °C, and constant values were obtained at higher temperatures. The stability of the derivatized product decreased for temperatures >40 °C. The variation of the rate of the reaction with the temperature is shown in Figure 3b. A temperature of 40 °C was chosen as optimum.

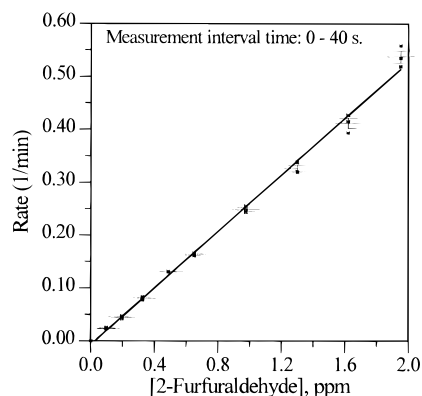
Calibration Curves. Absorbance–time curves were recorded for solutions containing different amounts of FUR at the experimental conditions indicated in Experimental Procedures. The rate of the reaction was found to be first-order with respect to FUR concentration in the range $0.13\text{--}2.00 \mu\text{g}\cdot\text{mL}^{-1}$.

Analytical Parameters. The determination of FUR by the modified Winkler's method involves the construction of a calibration graph by measurement the rate of

Table 1. Determination of 2-Furfuraldehyde (FUR) in Spirit Beverages Using the Proposed Kinetic Method

sample	added ^a	found ^a	recov (%)	slope of calibration curve (mL·μg ⁻¹ ·min ⁻¹) ^b	regression coefficient
cherry brandy	0	20.9		0.345	0.9980
	12.9	34.2	102		
	17.1	39.0	105		
	25.8	49.5	110		
herb brandy (Spain)	0	1.6		0.330	0.9980
	30.0	31.2	99		
	42.9	46.2	104		
herb brandy (Portugal)	0	4.4		0.320	0.9996
	21.6	26.1	101		
	30.1	35.8	104		
oak brandy (Spain)	0	1.6		0.320	0.9981
	21.6	22.3	96		
	30.1	32.29	102		

^a In μg·mL⁻¹ in the original spirit beverage. ^b Standard addition method (slope reference, 0.31 mL·μg⁻¹·min⁻¹).

**Figure 4.** Calibration graph obtained for the derivatization reaction.

the reaction between 0 and 40 s. The calibration curve obtained for different standard samples containing between 0.13 and 2.0 μg·mL⁻¹ is shown in Figure 4. For each standard sample, three replicates were made. The slope of the calibration plots was 0.356 mL·μg⁻¹·min⁻¹, and the residual mean square was 8.8×10^{-5} . The coefficient of determination (R^2) was 0.9975. For a series of 10 measurements of a solution containing 1.0 μg·mL⁻¹ of FUR, the relative standard deviation (RSD) was 7% and the relative error was 5% (confidence level, 95%). A detection limit (Clayton et al., 1987) of 0.12 μg·mL⁻¹ was determined.

Applications. The proposed kinetic method based on the Winkler's reaction has been tested with spirit beverages spiked and unspiked with FUR. The determination is very simple because pretreatment is not necessary. Results obtained by the method of standard additions are summarized in Table 1. The slopes of the different calibration curves are similar in all cases and also similar to the reference slope. This fact shows that sample matrices do not interfere with the kinetic determination. The amount of FUR found in the different beverages assayed are included in Table 2. Similar contents are obtained by external standard and standard addition methods.

Conclusions. The proposed kinetic method based on the Winkler's reaction allows the determination of FUR in spirit beverages without previous separation procedures. The modifications introduced in the Winkler's procedure permit addition of the reagent for derivatization in only one step. Also, the kinetic measurements allow determination of FUR in samples with colored background. The instability problems of the conven-

Table 2. Comparison between External Standard and Standard Addition Calibration Procedures

sample	FUR ^a (μg·mL ⁻¹)	
	external standard	standard addition
cherry brandy	20.9	21.0
herb brandy	1.6	1.9
herb brandy (Portugal)	4.35	4.35
oak brandy (Spain)	1.6	1.3

^a Content in the original spirit beverage.

tional Winkler's method are avoided by using kinetic measurements.

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