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Determination of 5-(Hydroxymethyl)-2-Furfural (HMF) in Tomato Products: Proposal of a Rapid HPLC Method and its Comparison with the Colorimetric Method

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ABSTRACT

The proposed method includes analysis of the serum obtained after suitable sample dilution and Carrez addition followed by 12 000 rpm centrifuging for 2 h by HPLC, using a reverse-phase C18 column and an ultraviolet detector operating at 285 nm in isocratic elution with 90:10 water–methanol. Juice and paste (double and triple) samples have been analysed by means of two methods (Winkler and HPLC) and statistical analysis, carried out on the results, clearly shows a difference between them. In particular, the colorimetric method which includes no centrifuging, but only filtration, almost always yields results which are lower than the HPLC ones.

INTRODUCTION

Tomatoes, like most other fruit, undergo discoloration during processing and storage.

In research work on the nonenzymatic browning of food systems, due to reaction between reducing sugars and proteins (or amino acids), particular attention has been given to the role of HMF (Weast & Mackinney, 1941; Porretta, in press). HMF does not in itself significantly change the flavour of

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food products; however, the colour changes associated with its formation are such as to make it one of the primary quality parameters (Porretta *et al.*, 1989).

In the case of tomato products, neither the EEC nor the USDA has set limits for the HMF content, thus inducing producers and dealers to set them for their own purposes of commercial exchange, and these limits are often a source of dispute.

Although the different HMF determination methods for tomato products do not include the use of HPLC, this method has proved efficient. The commonly employed colorimetric method is the Winkler (1955) one, which is based on the use of particularly toxic reagents such as barbituric acid and *p*-toluidine, to be prepared daily. Other authors have proposed methods using instruments which are not commonly available in analysis and control laboratories (ultracentrifuge beyond 96 000***g*** and ultrafiltration membranes).

The object of this study was to propose a rapid HPLC method for determining HMF and to compare it with the spectrophotometric method (Winkler, 1955).

MATERIALS AND METHODS

Colorimetric method

Five grams of tomato paste (28° Brix), or 30 g of tomato juice, were placed in a 100 ml calibrated flask; 2 ml each of Carrez I and II was then added and the volume made up to the mark with distilled water. The Carrez clarifying agent consisted of two aqueous solutions, one of 15% (w/v) potassium ferrocyanide (Carrez I), the other of 30% (w/v) zinc sulphate (Carrez II).

The contents of the flask were filtered, two 2-ml aliquots of the filtrate were taken and to each of them was added 5 ml of a solution of *p*-toluidine (Carlo Erba Analyticals, Milan, Italy) prepared by dissolving 10 g of the substance in 90 ml of isopropanol + 10 ml of glacial acetic acid (this solution must be renewed daily).

To one of the aliquots (blank) was added 1 ml of distilled water, to the other (sample to be measured) 1 ml of a 0·5% (w/v) barbituric acid aqueous solution.

After 'several' minutes (the uncertainty resulting from the expression 'several' will be discussed later) the extinction was read at 550 nm on a Jasco Spectroscopic Co. (Tokyo, Japan), Model 7800, spectrophotometer,

attempting to record its maximum value. In the presence of HMF a compound with a red–violet colour is formed whose intensity is proportional to concentration.

The relevant calibration equation is HMF (ppm) = 0·87 + 39·2 A 550 nm ($R = 0{\cdot}99$).

Chromatographic method

The HPLC system used in this research consisted of a Model 712 automatic sample injection module with 20 μl injection loop, a Model 480 LC spectrophotometer, all from Waters Associates (Milford, MA, USA), and a Model RT 250-4 Radial pack C-18 column (250 mm × 4 mm i.d.) with a mean particulate diameter of 10 μm from Merck (Darmstadt, FRG). The flow rate was 1·5 ml/min and injection volume 10 μl. The peaks and areas were calculcated with an integrator (Model C-R3A Chromatopac, Shimadzu, Japan).

HPLC grade organic solvents and water were purchased from Carlo Erba Analyticals (Milan, Italy) and Baker (Deventer, Holland), respectively. Standard solutions were prepared by dissolving, in distilled water, suitable amounts of HMF (Sigma Chemical Co., St Louis, MO) with a purity of 99% checked spectrophotometrically (the molar absorptivity was determined to be 16 660). All solutions were filtered through Waters Associates, Model HVLP, 0·45 μm filters and degassed. Five grams of tomato paste or 30 g of tomato juice was placed in a 100 ml centrifuge tube, 2 ml each of Carrez I and II was added with slow stirring and the volume made up with distilled water. After standing for 30 min, the mixture was centrifuged for 1 h at 12 000 rpm using a Beckman Instruments (Palo Alto, CA), Model J2-21, centrifuge (if this kind of equipment is not available, 2 h at 8000 rpm is sufficient to obtain the same result).

One millilitre of clear supernatant was pipetted into a syringe and filtered, before injection, through a Sartorius (Göttingen, FRG), Model Minisart NML, 0·45 μm disc filter.

Statistical analysis

Replicate HMF determinations by both methods were carried out on 30 samples of tomato juice and paste.

To find out differences, if any, between the two methods the results obtained were subjected to linear regression analysis and the *t*-test paired method using statistics software (NWA STATPAK, 1984) with a 95% confidence limit (Miller & Miller, 1988).

RESULTS AND DISCUSSION

Table 1 lists the results of HMF determination by the two methods and Fig. 1 shows that the results obtained by HPLC were nearly always higher than the corresponding colorimetric values. With the HPLC method, recoveries were 100% from samples of tomato paste up to 28° Brix spiked at 1, 5, 10 and 100 ppm HMF; for samples with higher Brix, an additional determination on the centrifuged serum-free pulp was required to obtain 100% recoveries.

TABLE 1
Results of HMF Determination (ppm) by the Two Methods carried out on Samples of Tomato Products of Different Concentrations

Sample no.	*Conc. (°Brix)*	*Winkler*	*HPLC*
1	7·0	0	1·3
2	7·4	0	2·5
3	7·6	5·6	7·0
4	7·8	4·3	13·2
5	8·4	12·5	15·0
6	8·5	12·0	16·0
7	12·1	17·2	16·3
8	12·6	14·0	17·0
9	14·2	16·5	20·1
10	17·8	19·5	20·0
11	18·0	11·6	20·9
12	18·1	19·1	22·0
13	21·0	15·2	22·2
14	22·3	6·3	22·8
15	26·0	16·7	22·8
16	28·1	25·1	24·7
17	28·1	7·1	26·6
18	28·3	25·8	27·9
19	28·4	22·3	30·3
20	28·8	27·3	30·6
21	28·8	28·3	31·1
22	29·0	21·5	31·4
23	29·0	30·7	32·1
24	30·1	31·1	34·3
25	30·3	43·5	35·5
26	30·3	27·2	36·2
27	30·8	35·8	37·7
28	38·1	178·0	180·0
29	39·2	174·0	185·0
30	39·6	169·0	186·0

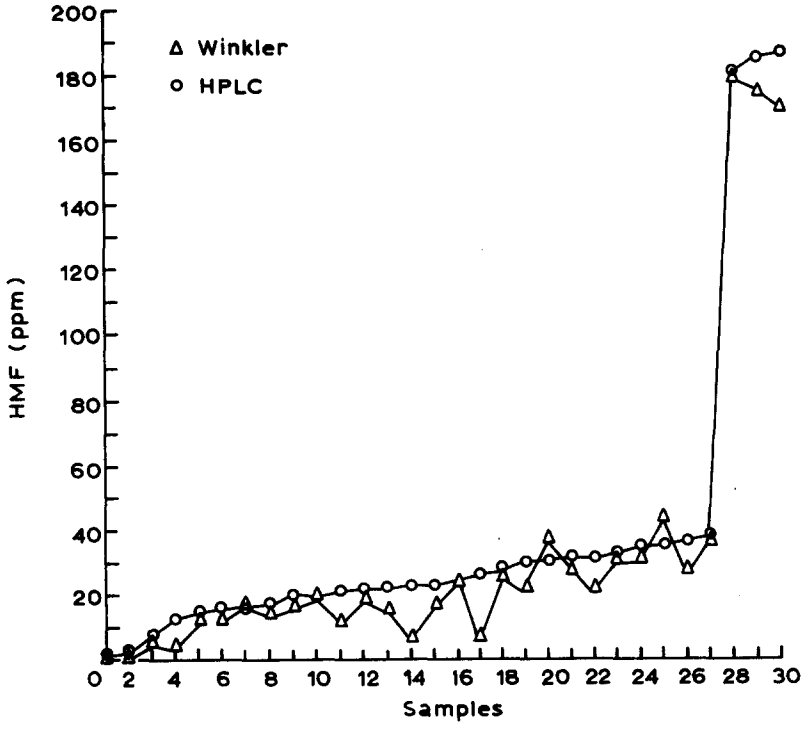

Fig. 1. HMF content values obtained by the two methods.

With the colorimetric method, recoveries proved highly variable and in any case did not exceed 83%.

Under the condition adopted, the characteristic chromatographic peak of HMF appeared after 5·1 min (Fig. 2). Using only water as the eluent, it appeared after 16·6 min, while with water and methanol in an 80:20 ratio it occurred after 3·2 min but was masked by other components.

The first important limitation to the Winkler method is the difficulty in following accurately the colorimetric reaction over time due to the fact that maximum colour intensity is obtained within an interval of 1–4 min, so its optimum recording is not always possible when large quantities of samples are to be analysed.

Furthermore, the reagents for this method must be prepared daily and are so toxic as to require efficient laboratory equipment (fume hoods, gloves, etc.).

The linear regression equation (Fig. 3), ppm HMF (Winkler) = $-3{\cdot}30 +$ 0·96 ppm HMF (HPLC), of the type $Y = A + Bx$, though having an R of 0·99, is evidence of the difference between the two methods, since the conditions of equality did not occur contemporaneously, i.e. $R = 1$, $A = 0$, $B = 1$.

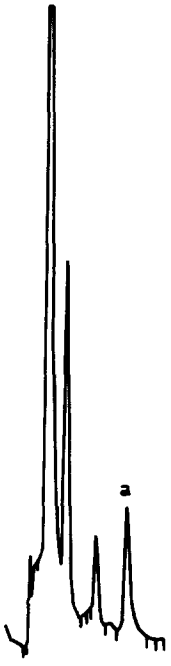

Fig. 2. HPLC of tomato paste. Time = 5·1 min, a = HMF.

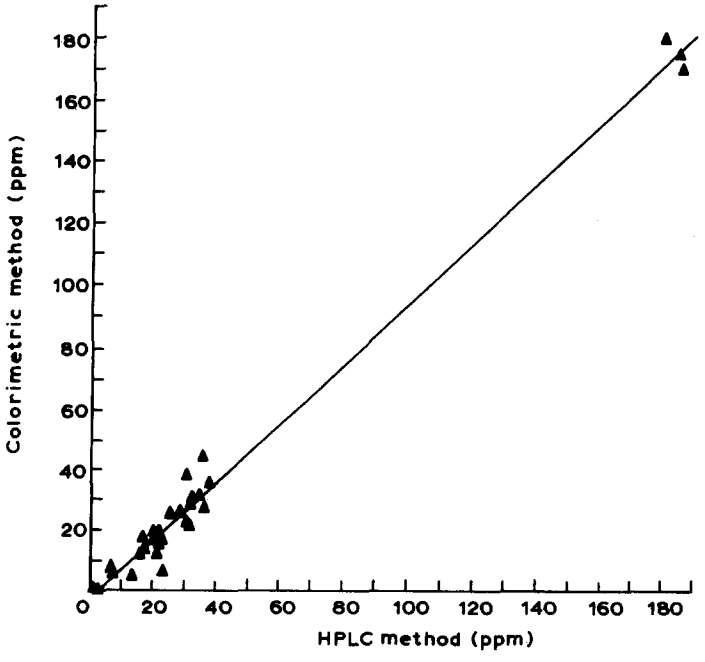

Fig. 3. Correlation between the colorimetric and the HPLC methods.

Indeed, the intervals of variation of A and B calculated by means of the t-test (the t value tabulated for 29 degrees of freedom is 2·04) are $-6{\cdot}13 < A < -0{\cdot}46$ and $0{\cdot}92 < B < 1{\cdot}00$ (the standard error for A is quite high, 1·39, and that for B is 0·022). This allows us to conclude that the colorimetric method is different from the proposed HPLC method.

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