Hydroxymethylfurfural and Furosine Reaction Kinetics in Tomato Products

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The reaction kinetics of two heat damage indices, HMF and furosine, were examined in four tomato products with different dry matter contents (10.2, 25.5, 28.6, and 34.5%) over a temperature−time range of 80−120 °C and 0−255 min. The reactions followed pseudo-zero order kinetics. E_a and z-value were, respectively, 139.9 kJ/mol and 19.2 °C for HMF, and 93.9 kJ/mol and 28.4 °C for furosine. The analyses of both indices in several samples of commercial and industrial tomato products showed very low levels of HMF (from 1 to 42 ppm) and a lack of correlation between HMF and furosine mainly because of the different evolution of the two indices during storage. The HMF level of a tomato paste sample stored at 25 °C decreased from 609 to 17 ppm after 98 days, while furosine increased from 458 to 550 mg/100 g of protein.

Keywords: *Furosine; heat damage; HMF; reaction kinetics; tomato products*

INTRODUCTION

During processing and storage, tomato products undergo nutritional and sensorial quality changes. The parameter normally used by the industry to evaluate heat damage in these food products is 5-(hydroxymethyl)-2-furfural (HMF), an intermediate compound of the Maillard reaction. In a previous work (Hidalgo et al., 1998), the efficiency of this index was questioned because of its low heat damage evaluation ability. The authors studied a new descriptor, furosine [ϵ-*N*-(2-furoylmethyl-L-lysine)], produced by acid hydrolysis of the Amadori compounds and successfully used as a heat damage index in milk (Erbersdobler, 1987), pasta (Resmini et al., 1990a), and meat products (Pompei and Spagnolello, 1997). Hidalgo et al. (1998) concluded that furosine was a good heat damage index of tomato products, allowing the evaluation of both product quality and processing technology, but did not compare it experimentally with HMF.

The purpose of the present work was to study HMF and furosine content variation during heating of tomato products in order to compare the goodness of these two descriptors as heat damage indices as well as to describe the kinetic parameters of both indices.

MATERIALS AND METHODS

HMF and Furosine Reaction Kinetics. For this study, four tomato products with different dry matter contents (10.2, 25.5, 28.6, and 34.5%) were prepared in the laboratory and treated at 60 or 90, 80, 100, and 120 °C. HMF and furosine levels were determined on each sample at six different heat treatment times. The range of temperature and time studied covers the typical conditions used for the industrial processing of tomato products.

For tomato products preparation, tomatoes of the variety Roma, grown in Sicily, were manually washed, cool peeled with a knife, and cut. The recovered serum and the seeds were filtered through a 20-mesh sieve, the pulp was accurately ground and homogenized with the serum; this mix was finally sieved again. Tomato pulp was lyophilized using a freeze-dryer Edwards model Lyoflex 04 (West Sussex, England) at a temperature of 10 °C with a previous freezing phase at −45 °C. The freeze-dried product was stored at −20 °C under vacuum in plastic bags, until the experiments were performed. To obtain tomato products with different dry matter contents, the freeze-dried pulp was rehydrated in distilled water, accurately mixing with Ultra Turrax T25 (Janke & Kunkel, IKA Labortechnik, Postfach, Germany).

At each temperature, the heat treatment of every tomato product was conducted using six 30 mL Pyrex test tubes with screw caps (Schott GL 18) containing ca. 20 mL of tomato product and a thermostatic bath with agitation system (Haake L-Fisons, model D8, Germany) filled with diathermic oil, specific for medium and high temperatures. To fill the 24 test tubes required for the heat treatments of each product (six treatment times at each of the four temperatures), approximately 1 L of rehydrated tomato product was prepared and degassed immediately before the treatment. To prevent crack formation in tomato paste samples (dry matter content ≥ 24%) during heating, the material was pressed in the test tube with a silicone disk and a 15 cm stainless steel spring. Throughout the heat treatment, the temperature at the center of the product was registered at different times using a thermocouple (Ellab A-S, Copenhagen, Denmark) positioned inside one of the six test tubes. As an example, the profiles of the 10.2% dry matter content product are presented in Figure 1. The heating speed of the six test tubes containing water was controlled by computing the heating curve area subtended of each test tube. A homogeneous heating was assured by appropriately positioning the test tubes inside the thermostatic bath.

HMF and Furosine in Tomato Products of Market and Industry. To study the correlation between HMF and furosine, the following tomato products sampled in the Italian market and in industrial processing plants were analyzed: four samples of tomato pulp with a mean dry matter content of 9.0%; three, nine, and six samples of tomato paste with a mean dry matter content of 20.5, 28.8, and 35.2%, respectively.

From an industrial food plant processing tomato products, several 2 kg samples of the same tomato paste batch (dry

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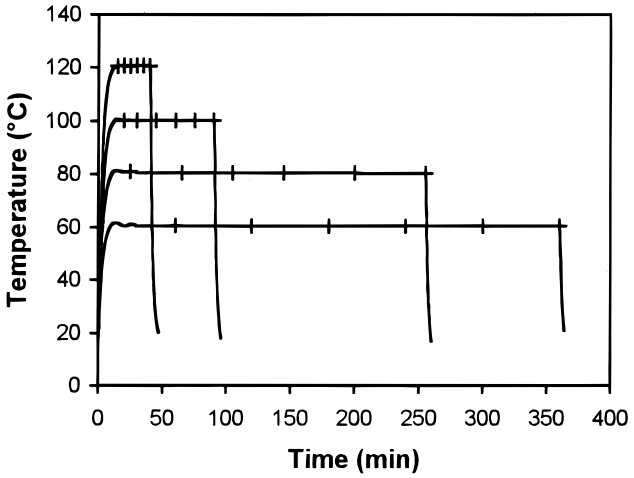

Figure 1. Time−temperature profiles of the heat treatments conducted on the tomato product with a dry matter content of 10.2%. Vertical lines indicate the sampling times.

matter content of 28.1%) were collected in aluminum−polyethylene aseptic bags. The samples were heat treated in autoclave at 103 °C for 120 min, cooled at room temperature, and stored at 25 °C. HMF and furosine were determined after different times.

Chemical Analyses. The following analyses were twice performed on the samples: dry matter content (g per 100 g of product), following the AOAC official gravimetric method n. 964.22 (AOAC International, 1995a); furosine content (mg per 100 g of proteins), following the HPLC method as proposed for milk by Resmini et al. (1990b), slightly modified for tomato products as described by Hidalgo et al. (1998); protein content (g per 100 g of product), using the Kjeldahl method (n. 920.152, AOAC International, 1995b); HMF content, determined by HPLC as follows: 2.5 g of tomato pulp (dry matter content ca. 10%) plus 2.5 g of redistilled water, or 0.5−1.0 g tomato paste (dry matter content ≥ 24%) plus 5 g of redistilled water, were accurately weighted in 10 mL plastic test tubes, vigorously shaken for ca. 1 min using a Vortex agitator and centrifuged at 12000 rpm for 11 min at 6 °C using a Centrikon T-42K centrifuge (Kontron Instruments). Afterward, the surnatant was filtered through a 0.45 μm Millipore GSWP membrane (Millipore, Bedford, MA) and immediately injected in a liquid chromatography apparatus consisting of a Millipore Waters Model 510 HPLC pump (Milford, MA), an injector with a 20 μL loop and a Millipore Waters 990 Series photodiode array detector (Milford, MA). Operative conditions were as follows: Waters Spherisorb S5 ODS2 (4.6 mm per 25.0 cm) column at room temperature; detection multiwavelength 282 nm; mobile phase 10% methanol in water; flow rate 1.2 mL/min; duration 16 min.

A calibration curve was built, using 13 different concentrations (between 0.10 and 20.28 mg/L) of the HMF standard (Merck, n. 820678) in water. On the basis of the calibration curve, the detection limit was calculated as the intercept value of the regression line plus 3 times the standard error of the estimate (Miller and Miller, 1988).

The repeatability of the HMF analytical method was assessed by performing, in each case, 10 replicate measurements on the same tomato pulp commercial sample and on the same rehydrated tomato paste sample (dry matter of 34.5%). The results were expressed in terms of standard deviation (SD) and of coefficient of variation (CV%).

RESULTS AND DISCUSSION

HMF Calibration Curve and Repeatability Test. The HMF calibration curve was linear in the range from 0.10 to 20.28 mg/L ($r^2 = 1.000$) showing a detection limit of 0.07 mg/L for the standard solution. The repeatability of the HMF analytical method, expressed in terms of mean ±SD and CV%, was 2.6 ± 0.4 (CV = 16.1%) in tomato pulp and 148.8 ± 2.2 (CV = 1.5%) in tomato paste. HMF method repeatability in tomato paste was better than in tomato pulp, maybe as a consequence of higher compound amounts and of better product homogeneity. The furosine method, instead, presented good repeatability in both tomato pulp (CV = 7.3%) and tomato paste (CV = 6.2%) products (Hidalgo et al., 1998).

HMF and Furosine Reaction Kinetics. Figures 2 and 3 show, respectively, HMF and furosine levels in four tomato products (dry matter contents of 10.2, 25.5, 28.6, and 34.5%) heat treated at 60, 80, 90, 100, and 120 °C for different times. The treatment at 60 °C was conducted only for the 10.2 and 34.5% dry matter content products. Because of the low levels found of HMF and furosine, this temperature was replaced by a heat treatment at 90 °C for the other two products. In fact, after 6 h of treatment at 60 °C, HMF and furosine levels were under the method detection limit (open symbols in Figures 2 and 3) with the exception of the 34.5% dry matter content tomato paste. For this product, furosine was detectable after 2 h of treatment, while HMF only after 5 h (solid symbols in Figures 2 and 3).

Under the conditions studied, both heat damage indices increased linearly ($p \leq 0.01$) as a function of the heat treatment duration and presented higher reaction rate at higher temperature and at higher product dry matter content. HMF and furosine reaction rate for each type of product and for each treatment temperature are: $v_{HMF} = k_1 DM$ and $v_{furosine} = k_2 DM$ where v = reaction rate, DM = dry matter and k = kinetic constant. Because the HMF and furosine formed are far less than the dry matter content, DM can be considered constant. In other words, under the time−temperature range studied, which is of practical interest in the tomato processing industry, the two reactions are in a very initial stage; hence, the reactions appear to follow pseudo-zero order kinetics; thus $v_{HMF} = k_{1app}$ and $v_{furosine} = k_{2app}$.

Because

$$\log k_{1app} = \log k_1 + \log DM = A - (E_{a_1}/RT) + \log DM$$

$$\log k_{2app} = \log k_2 + \log DM = A - (E_{a_2}/RT) + \log DM$$

where k_{app} = apparent kinetic constant, E_a = energy of activation, R = universal gas constant, T = absolute temperature, by applying Arrhenius equation the substantially parallel lines presented in Figure 4, whose distance is proportional to log DM, are obtained.

From the slopes of the Arrhenius lines, the energy of activation (E_a) and the z values [$z = 2.303 \times R \times \bar{T}^2/E_a$, where z represents the increase in temperature that causes a 10-fold increase in the reaction rate; R = universal gas constant (8.314 J/mol K); $\bar{T}^2$ = mean absolute temperature of the considered heat treatments temperature range (373 K)] were computed and are presented in Table 1. HMF and furosine E_a and z values for the four tomato products are similar on the basis of the coefficient of variation; it is thus possible to consider their mean values, and the differences may be attributed to the experimental error.

HMF mean activation energy ($E_{\bar{a}}$ = 139.9 kJ/mol) resulted very similar to the value of milk Ea (139.0 kJ/

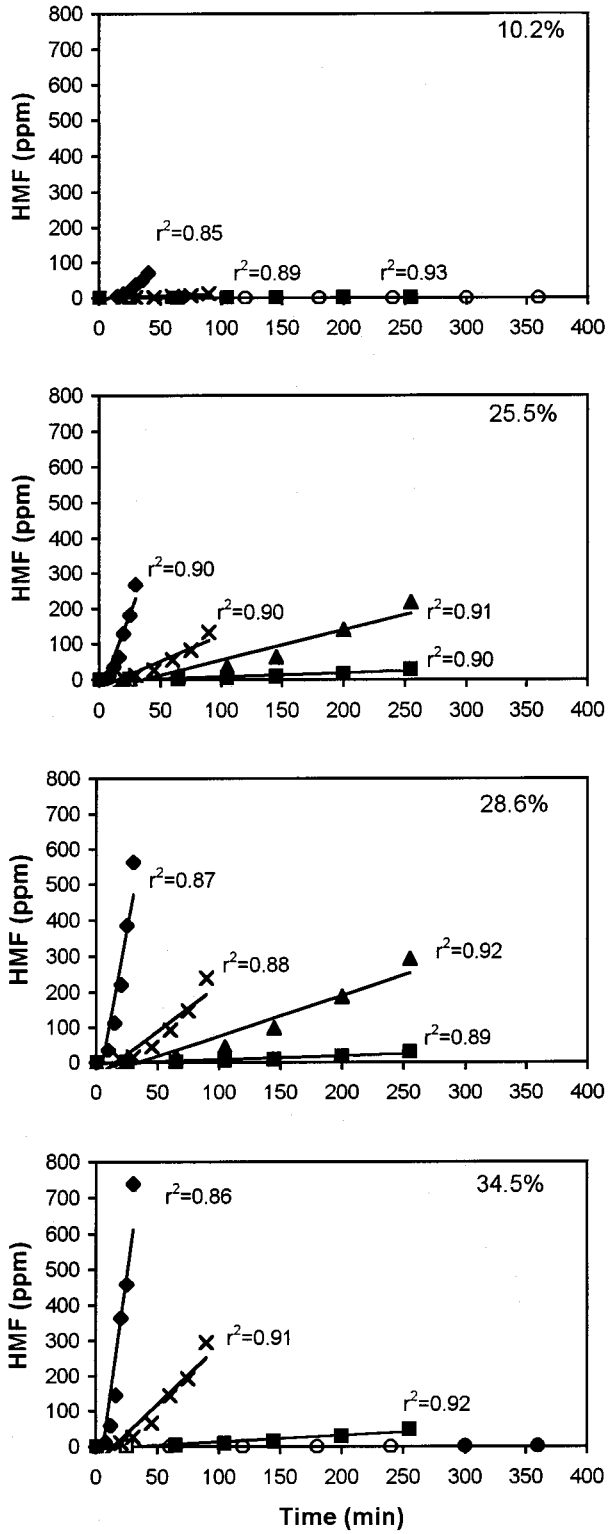

Figure 2. HMF content of four tomato products (dry matter contents of 10.2, 25.5, 28.6, and 34.5%) heat treated at 60 (●), 80 (■), 90 (▲), 100 (×), and 120 °C (◆) for different times. Open symbols represent the values beyond the method detection limit.

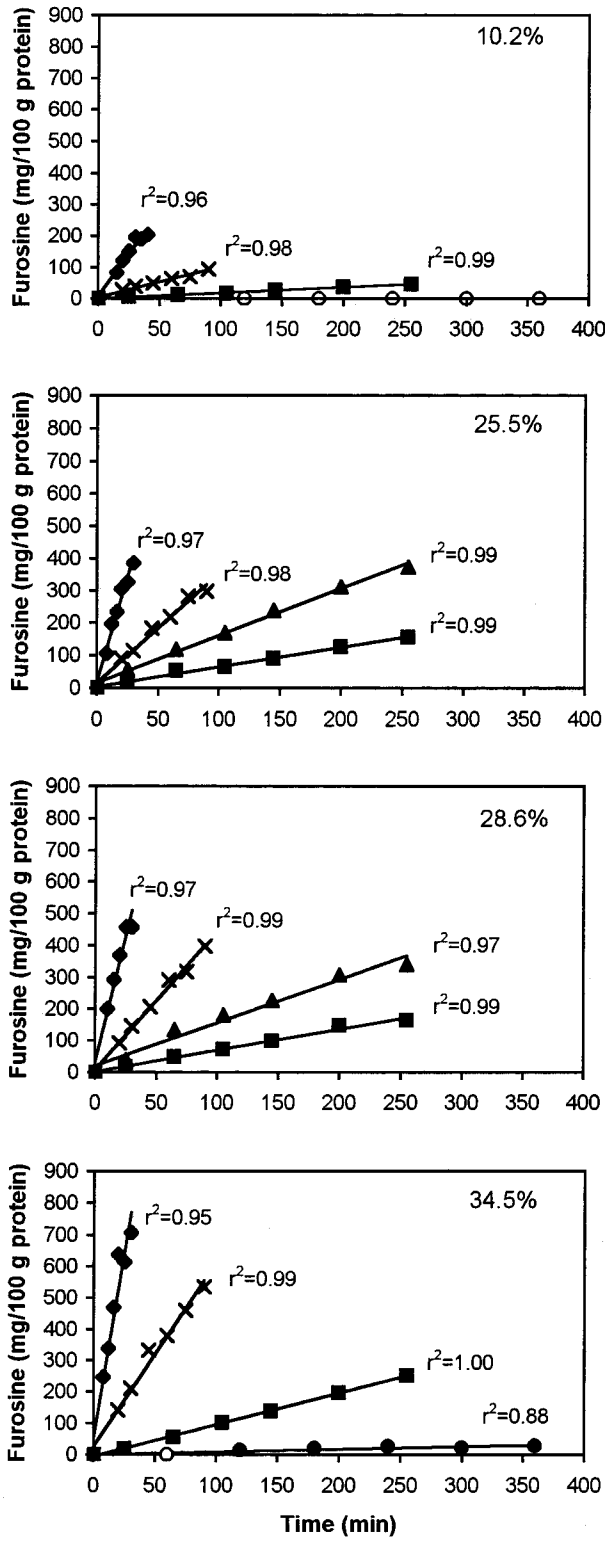

Figure 3. Furosine content of four tomato products (dry matter contents of 10.2, 25.5, 28.6, and 34.5%) heat treated at 60 (●), 80 (■), 90 (▲), 100 (×), and 120 °C (◆) for different times. Open symbols represent the values beyond the method detection limit.

mol) reported by Kessler and Fink (1986), while furosine mean E_a (93.9 kJ/mol) and mean z value (28.4 °C) were very similar to the values obtained by Pompei and Spagnolello (1997) for the raw (79.2 kJ/mol; 30.1 °C) and the tumbled pork muscle (81.7 kJ/mol; 29.2 °C). Finally, the HMF and furosine mean z values determined in this research are in the range of the values reported by Kessler (1981) for the formation of compounds related to the Maillard reaction.

To compare the heat damage of the four types of tomato products treated at different temperature−time conditions, HMF and furosine were standardized, as suggested by Hidalgo et al. (1998), by dividing HMF and furosine levels by the dry matter percentage of the

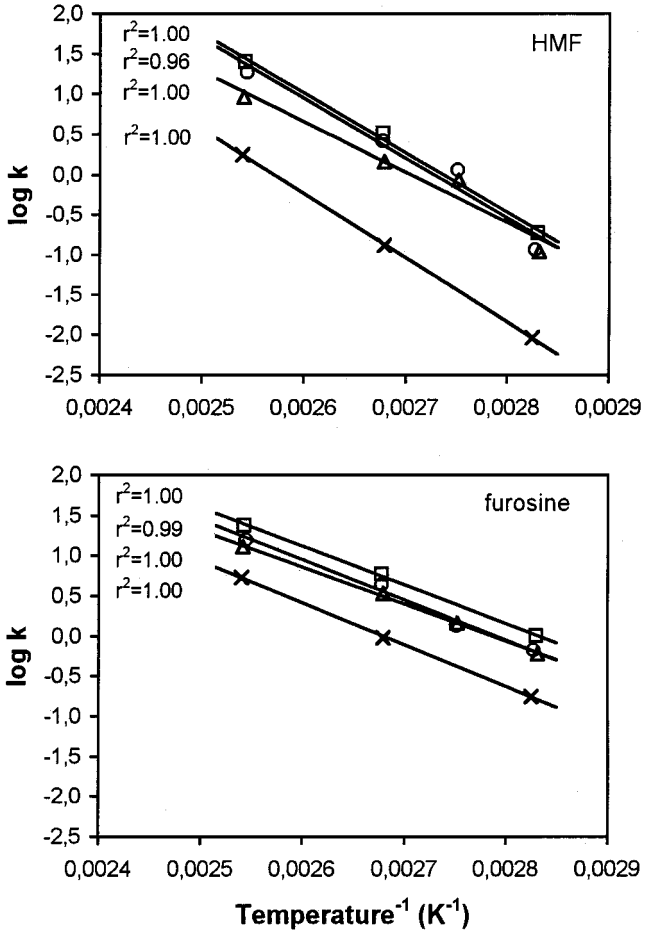

Figure 4. Arrhenius plots for the HMF and furosine formation in tomato products with 10.2 (×), 25.5 (△), 28.6 (○), and 34.5% (□) dry matter content.

Table 1. E_a and z Values for HMF and Furosine in Four Tomato Products with Different Dry Matter Contents

tomato product (% dry matter)	HMF		furosine	
	E_a (kJ/mol)	z (°C)	E_a (kJ/mol)	z (°C)
10.2%	153.9	17.3	99.9	26.7
25.5%	120.2	22.2	88.1	30.2
28.6%	142.8	18.7	95.5	27.9
34.5%	142.4	18.7	92.1	28.9
mean	139.9	19.2	93.9	28.4
SD[a]	14.2	2.1	5.0	1.5
CV[b]	10.1	10.8	5.3	5.3

[a] Standard deviation. [b] Coefficient of variation.

product. Simultaneously, the different heat treatment conditions were standardized by computing the *Co* value with the following equation: $Co = \int_0^t dt/10^{(T^*-T)/z}$, where *Co* is expressed as time at the reference temperature ($T^* = 80$ °C), t is the time of the treatment, T is the actual temperature of the treatment (°C) and z is the z value defined above ($z = 19.2$ °C for HMF and $z = 28.4$ °C for furosine). HMF and furosine levels increased linearly as a function of the *Co* values ($p \leq 0.01$) (Figure 5); however, the HMF determination coefficient ($r^2 = 0.63$) was much less than that of furosine ($r^2 = 0.91$).

HMF and Furosine in Tomato Products of Market and Industry. HMF and furosine levels of several commercial samples of four tomato products are shown in Figure 6. There is no clear correlation between the heat damage indices, and the HMF levels are too low (from 1 to 42 ppm), especially when considering the

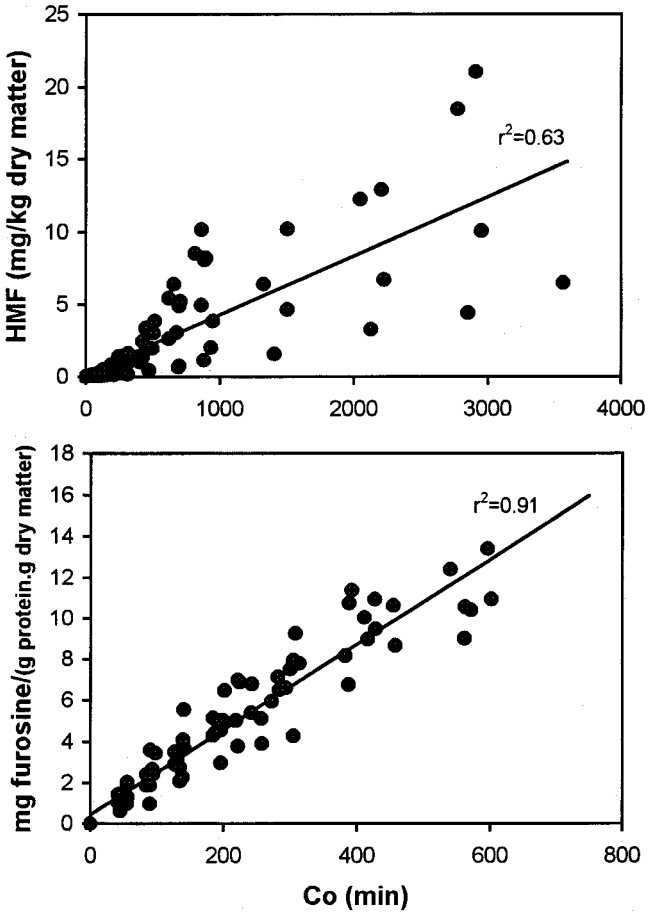

Figure 5. Furosine and HMF content as a function of *Co* value (equivalent time at a reference temperature of 80 °C).

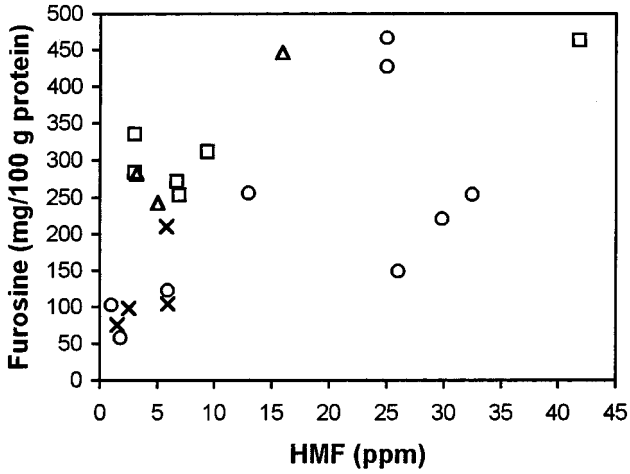

Figure 6. HMF and furosine level of different tomato products sampled in the Italian market and industrial processing plants: samples of tomato pulp with a mean dry matter content of 9.0% (×), samples of tomato paste with a mean dry matter content of 20.5 (Δ), 28.8 (○), and 35.2% (□), respectively.

values observed in the kinetics study (Figures 2 and 3). On the other hand, the furosine contents are in the range observed during the kinetics survey. The incongruence of these results suggests the possibility of a HMF decrease during storage.

The HMF level of a tomato paste sample (dry matter of 28.1%), treated, and stored at 25 °C as indicated in the Materials and Methods section, decreased from 609

to 339 ppm after 29 days and to 17 ppm after 98 days, while the furosine showed a slight increase from 458 to 504 and to 550 mg/100 g of protein after the same storage times.

The HMF decrease during room temperature storage is a limit for its use as an index of heat damage, because the true product damage caused by heat treatment is masked. The low correlation found between HMF and furosine in the commercial and industrial tomato products is thus mostly due to the different evolution of the two indices during storage.

Hence, on the basis of our results, HMF does not represent a valid heat damage or thermal history descriptor of tomato products, while furosine seems to be a more reliable index.

ABBREVIATIONS USED

Co, defined for a given heat treatment as the time required to get, at the reference temperature, the same chemical effect of the heat treatment, with reference to a well-defined reaction; CV, coefficient of variation; *DM*, dry matter; E_a, energy of activation; HMF, 5-(hydroxymethyl)-2-furfural; HPLC, high-performance liquid chromatography; *k*, kinetic constant; k_{app}, apparent kinetic constant; *p*, probability; *R*, universal gas constant; r^2, determination coefficient; SD, standard deviation; *T*, temperature (°C or K); *T**, reference temperature (°C); $\bar{T}^2$, mean absolute temperature of the considered heat treatments temperature range; *t*, time (s); *v*, reaction rate; *z* value, increase in temperature that causes a 10-fold increase in the reaction rate (°C).

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LITERATURE CITED

AOAC International. Vegetable Products, Processed. In *Official Methods of Analysis of AOAC International*; Cunniff, P., Ed.; AOAC International: Gaithersburg, MD, 1995a; Vol. II, Chapter 42, p 4.

AOAC International. Fruits and Fruit Products. In *Official Methods of Analysis of AOAC International*; Cunniff, P., Ed.; AOAC International: Gaithersburg, MD, 1995b; Vol. II, Suppl. March 1996, Chapter 37, p 10.

Erbersdobler, H. F.; Dehn, B.; Nangpal, A.; Reuter, H. Determination of furosine in heated milk as a measure of heat intensity during processing. *J. Dairy Res*. **1987**, *54*, 147−151.

Hidalgo, A.; Pompei, C.; Zambuto, R. Heat damage evaluation during tomato products processing. *J. Agric. Food Chem*. **1998**, *46*, 4387−4390.

Kessler, H. G. Pasteurization-Sterilization-Heating Methods. In *Food Engineering and Dairy Technology*; Verlag A. Kessler: Freising, Germany, 1981; Chapter 6, pp 139−207.

Kessler, H. G.; Fink, R. Change in heated and stored milk with an interpretation by reaction kinetics. *J. Food Sci*. **1986**, *51* (5), 1105−1111, 1155.

Miller, J. C.; Miller, J. N. Errors in Instrumental Analysis; Regression and Correlation. In *Statistics for Analytical Chemistry*; Ellis Horwood Limited: Chichester, West Sussex, England, 1988; Chapter 5, pp 101−136.

Pompei, C.; Spagnolello, A. Furosine as an index of heat treatment intensity in meat products: its application to cooked ham. *Meat Sci*. **1997**, *46*, 139−146.

Resmini, P.; Pagani, M. A.; Pellegrino, L. Evaluation of the thermal damage to pasta by determination of ϵ-furoylmethyl-lysine (furosine) by HPLC. *Tec. Molitoria* **1990a**, *October*, 821−826.

Resmini, P.; Pellegrino, L.; Battelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci*. **1990b**, *3*, 173−183.

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