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Accumulation of 5-hydroxymethyl-2-furfural in cookies during the backing process: Validation of an extraction method

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Abstract

During baking, complex chemical reactions take place in the cookies, such as the Maillard reaction (MR) and caramelisation (CR). Both reactions involve glucose and fructose, generated from starch and sucrose hydrolysis during baking. Among the many products formed, HMF a possible mutagen, seems particularly interesting because of a rapid accumulation during the process.

No validation of HMF quantification method has been proposed for cereal products. Our objectives were to validate a simple HPLC method with UV detection and study the kinetic of HMF accumulation in a cookie model during baking at three temperatures. Solubilization in water followed by protein precipitation in trichloroacetic acid was selected as the best extraction procedure avoiding interferences with the matrix. During the baking process, HMF accumulated exponentially with an activation energy of 10.6 kJ mol^{-1}, once the water activity decreased from 0.40 downwards. HMF in commercial cookies ranged from 0.5 to 74.6 mg kg^{-1}.

Keywords: Hydroxymethylfurfural; HPLC-UV; Cookies; Baking

1. Introduction

The major components of cookies are cereal flour, sugars and fats. The dough is conventionally baked at high temperature (up to 200 °C) for few minutes (<5 min), in order to obtain a low final water content (<10%) and a brown surface. Many complex phenomena occur in the dough during preparation and baking, such as protein denaturation, loss of starch granular structure, fat melting and finally development of a brown surface resulting from both caramelisation (CR) and Maillard reaction (Chevallier, Colonna, Buléon, & Della Valle, 2000).

The loss of available lysine and accumulation of undesirable compounds generated during the advanced stages of MR, such as furfurals, are commonly measured to evaluate the severity of the heat treatment applied and the effect of storage (Rada-Mendoza, Luz Sanz, Olano, & Villamiel, 2004; Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2001). Upon heating at high temperature, sugars decompose into furfural compounds by two possible pathways, both involving a first step of sucrose hydrolysis: (i) the caramelization (CR), where the reducing carbohydrates, including maltose and maltotriose (Kroh, 1994) directly suffer 1–2 enolisation, dehydration and cyclization reactions; and (ii) the Maillard reaction (MR), where the Amadori product, formed by reaction with the amino group of free amino-acids or proteins – is submitted to enolisation and subsequent dehydration of the sugar moiety while releasing the amino-acid intact (Cardenas Ruiz,

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Guerra-Hernandez, & García-Villanova, 2004; Ferrer, Alegría, Farré, Abellán, & Romero, 2002; Kroh, 1994; Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2000b). But caramelisation requires higher temperatures than MR to develop (Kroh, 1994).

5-Hydroxymethyl-2-furfuraldehyde (HMF) is a common product of these two reactions. It is formed from 3-deoxyhexosulose, the dehydration product derived from 1,2 enolization of glucose and fructose (Ferrer et al., 2002; Kroh, 1994; Ramírez-Jiménez, García-Villanova, & Guerra-Hernández, 2000a). HMF is considered as the essential decomposition product of hexoses, especially when the pH is low (Akkan, Özdemir, & Ekiz, 2001; Espinosa Mansilla, Salinas, & Berzas Nevado, 1992; Ferrer et al., 2002; Xu, Templeton, & Reed, 2003), whereas F and MF are supposed to be mainly produced from pentoses (Ledl & Sevrin, 1978). Moreover Kroh (1994) demonstrates that at the pyrolysis temperature of glucose (300 °C), HMF is degraded into F, MF and furyldialdehyde (FDA) by decarboxylation, oxidation and reduction, respectively.

HMF is not present in fresh, untreated foods, but rapidly accumulates during the heat treatment and storage of carbohydrate-rich products, sometimes exceeding 1 g kg^{-1} in certain dried fruits and caramel products (Akkan et al., 2001; Ibarz, Pagán, & Garza, 2000; Rada-Mendoza et al., 2004). The toxicological relevance of HMF is not clear as in vitro studies on genotoxicity and mutagenicity have given controversial results (Cuzzoni, Stoppini, Gazzani, & Mazza, 1988; Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Lee, Shlyankevich, Jeong, Douglas, & Surh, 1995). However, HMF is considered as an undesirable compound.

That is why HMF is considered to be a good indicator of quality deterioration due to excessive heating or storage for a wide range of carbohydrate-containing foods such as processed fruits (Ibarz et al., 2000; Rada-Mendoza, Olano, & Villamiel, 2002; Rada-Mendoza et al., 2004), honey (Fallico, Zappalà, Arena, & Verzera, 2004; Tosi, Ciappini, Ré, & Lucero, 2002) and milk (Morales & Jiménez-Pérez, 2001; Van Boekel, 1998; Van Boekel & Rehman, 1987). HMF is also used for monitoring the heating process applied to cereal products such as pasta drying and cookies or bread baking (Ramírez-Jiménez et al., 2001; Ramírez-Jiménez et al., 2000a, 2000b; Sensidoni, Peressini, & Pollini, 1999), as well as extrudated baby cereals (Fernandez–Artigas, Guerra-Hernández, & García-Villanova, 1999; Ferrer et al., 2002; Ramírez-Jiménez, Guerra-Hernández, & García-Villanova, 2003) and breakfast cereals (García–Villanova, Guerra-Hernández, Martinez Gomez, & Montilla, 1993).

Various methods have been used to extract and quantify HMF in foods. In complex matrices like cereal, milk and fruit products, extraction in water followed by clarification by Carrez (I and II) or trichloroacetic acid is commonly used, before quantification by HPLC–UV (Ramírez-Jiménez et al., 2000a, 2000b; Van Boekel, 1998). In simple matrices, such as honey and fruit juices, a colorimetric method based on the reaction of HMF with thiobarbituric acid (TBA) has been proposed (Espinosa Mansilla et al., 1992; Fallico et al., 2004; Tosi et al., 2002). But this method lacks specificity as other carbonyl compounds present or formed in the food during the process may also react with TBA. This probably explains the overestimation of HMF reported in some papers by (Ferrer et al., 2002; Van Boekel & Rehman, 1987). HPLC techniques produce more accurate results and allow analysing various furfural compounds in a same run. However, no precise validation of the method has been proposed for cookies.

The aim of our study was to select and validate an extraction procedure and an HPLC–UV method for quantifying HMF and its degradation products in cookies. For that purpose, a cookie model was baked at various temperatures (200, 250 and 300 °C) for different times. Kinetic analysis and HMF levels in 17 commercial cookies are presented and discussed.

2. Materials and methods

2.1. Samples

Cookies (CK) were prepared from wheat flour (60%), sucrose syrup (30%) and palm fat (10%). After mixing the products for 4 min in a bawl (Hobart, USA), the dough was allowed to rest for 30 min in the oven at 25 °C, and was rolled mechanically to reduce the dough thickness to 3 mm.

Baking kinetics were realized by cooking the dough in an oven (SPAG – ENSIA, France) set at 200, 250 and 300 °C for maximum 15, 12 and 10 min, respectively, to obtain a comparable surface color. Various baking times, from 2 to 15 min, were applied to realize the kinetic model.

The temperature in the cookie was measured using sensors placed horizontally in the core of the cookie, and directly connected to a computer to allow online monitoring. Analyses were done exclusively on the two central cookies of a cooking grid (12 cookies), because these two samples were associated to the lowest error of reproducibility (lowest interaction with the oven heated walls).

HMF extraction and analysis was validated on two cereal products: a commercial toasted sliced bread (SB) and the cookie model (CK) baked for 10 min at 250 °C. The samples were crushed with a commercial crusher (Bioblock, France) and stored in glass bottles at −18 °C.

Seventeen commercial biscuits were purchased at local markets. The sugar content in the cookies varied be-

tween 5 and 77 g per 100 g, and the lipid content between 8.7 and 29.1 g per 100 g. However, these information were not available for almost half of the samples.

2.2. Analytical methods

Determination of furfurals: The chromatographic method was adapted from that proposed by (Van Boekel et al., 1987) for milk products.

Reagents: Only analytical-grade chemicals were used. For protein precipitation, Carrez I and II containing, respectively, 15% potassium ferrocyanide (w/v) (Prolabo, France) and 30% zinc acetate (w/v) (Prolabo, France) were compared to a trichloroacetic acid solution (TCA) 40% (w/v) (Prolabo, France). The three extractants used were sodium acetate 0.04 M, pH 4.6 (Prolabo, France) (SA), sodium borate pH 8.3 (Flucka, France) (BOR), and milli-Q water (W). The standards 5-hydroxymethyl-2-furfural, 2-furaldehyde and 5-methylfurfural were purchased from Fluka (Switzerland).

Apparatus: The HPLC system consisted in a Kontron instrument (France) with a 20 µl injection loop, a Kontron auto-sampler and a C_{18} reversed-phase (EQUISIL ODS 5 µm, 250 × 4.6 mm, CLUZEAU, France) column connected to a UV detector set at 284 nm (waters 486, France). The identity and purity of HMF, F and MF were confirmed by a photodiode array detector (Waters – 2996). The mobile phase was composed of sodium acetate (0.04 M) (Prolabo, France) and methanol (80:20) (Prolabo, France), adjusted to pH 3.6 with acetic acid (99.8%) (Sigma–Aldrich, Germany). The percentage of methanol in the eluant was optimised for a complete separation of furfural compounds in an acceptable time (12 min). The flow rate was 1 ml min^{-1}. All analyses were performed in triplicate, including the extraction procedure. The quantification used an external calibration. Results are expressed as mg kg^{-1} cookie.

Extraction procedure: Three extraction solutions proposed in many papers (Birlouez-Aragon & Leclerc, 2001; Cardenas Ruiz et al., 2004; Cuzzoni et al., 1988; Ramírez-Jiménez et al., 2000a; Van Boekel & Rehman, 1987) were compared according to the repeatability of analysis and HMF recovery rate. The final procedure selected was based on water and TCA and was applied as follow.

The ground sample (1 g) was weighed into a 25-ml cup and suspended in 10 ml of milli-Q water and 2.5 ml of a 40% (w/v) TCA solution. The mixture was thoroughly stirred for 5 min. The suspension was adjusted to 25 ml with milli-Q water, centrifuged for 5 min at 5000 rpm and 20 °C. Two millilitres aliquot of this solution was filtered through a 0.45 µm nylon filter (Waters) and injected on the HPLC system.

3. Results and discussion

3.1. Extraction of HMF from the cereal matrix

The use of Carrez I and Carrez II as a clarifying agent instead of classical acids (trichloroacetic, metaphosphoric, sulfosalycilic) is recommended in many papers for cereal products, because of the possible production of HMF from glucose present in the food matrices at low pH (Cardenas Ruiz et al., 2004; Ramírez-Jiménez et al., 2000b). However, TCA has been used in other foods such as milk (Morales & Jiménez-Pérez, 2001; Van Boekel & Rehman, 1987) and fruit preparations (Ibarz et al., 2000; Rada-Mendoza et al., 2002). But, no indication is given of the artefactual production of HMF during sample preparation in the presence of TCA. In order to precisely verify this aspect, pure flour plus glucose and fructose were submitted to the procedure using TCA as clarifying agent and HMF was measured. No trace of HMF was detected. On the other hand, the ability of the different buffers and clarifying solutions to totally extract standard HMF added to the cereal products were compared.

Tables 1a and 1b show the repeatability error of analysis determined from six independent extractions as well as the HMF recovery rate depending on the extraction

Table 1a
HMF recovery in the toasted bread (TB)

Extractant/clarifying agent	Sample plus standard[a]	Sample alone[a]	HMF recovery[b]
BOR/TCA	188.65	148.39	74.71 ± 0.27
BOR/CARR	175.60	137.90	76.05 ± 0.30
W/TCA	*230.81*	*181.42*	*95.59 ± 0.00*
W/CARR	194.93	140.00	94.92 ± 0.04
SA/TCA	213.70	171.27	92.44 ± 0.23
SA/CARR	194.11	143.45	73.44 ± 0.23

Note: BOR, sodium borate; TCA, trichloroacetic acid; W, water; CARR, Carrez I and Carrez II; SA, sodium acetate.
[a] mg kg^{-1} of biscuit powder.
[b] Mean of the percentage of recovery.

Table 1b
HMF recovery in the cookie (CK)

Extractant/clarifying agent	Sample plus standard[a]	Sample alone[a]	HMF recovery[b]
BOR/TCA	145.70	108.02	79.83 ± 0.25
BOR/CARR	140.66	102.30	74.73 ± 0.30
W/TCA	*174.01*	*126.11*	*97.91 ± 0.01*
W/CARR	149.83	100.46	107.64 ± 0.10
SA/TCA	157.98	113.01	77.72 ± 0.12
SA/CARR	143.03	108.76	93.47 ± 0.10

Note: BOR, sodium borate; TCA, trichloroacetic acid; W, water; CARR, Carrez I and Carrez II; SA, sodium acetate.
[a] mg kg^{-1} of biscuit powder.
[b] Mean of the percentage of recovery.

procedure in toasted bread (TB) and in cookies (CK). Recovery was determined by the ratio between the theoretical HMF quantity added to the products (50.44 mg kg^{-1}) and the difference in HMF concentration measured between the cereal products with and without added HMF.

The recovery rates ranged from 73.45% to 95.60% for the TB samples (Table 1a) and from 74.73% to 107.64% for CK samples (Table 1b). The best results (closer to 100%) were obtained when using the W/TCA solution mixture (95.60% for TB and 97.91% for CK). The lowest error of repeatability was also obtained with this reagent mixture (0.18% and 0.00% for TB and CK, respectively) (Tables 1a and 1b). This procedure was therefore used for further analysis.

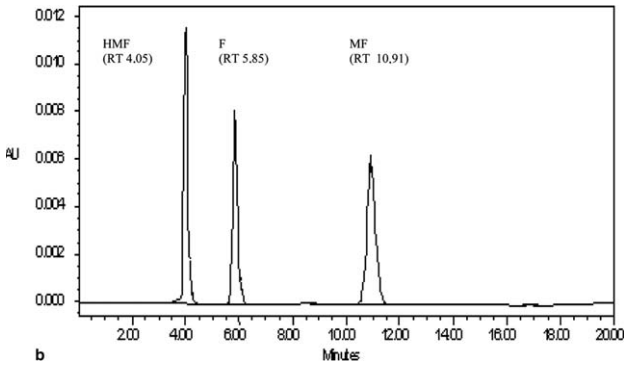

Fig. 1(b). Chromatogram of a mixture of furfural standards (HMF, F and MF).

3.2. Characterization of HMF peak purity

Many authors use 280 nm as an optimal wavelength to quantify HMF (Birlouez-Aragon & Leclerc, 2001; Morales & Jiménez-Pérez, 2001; Van Boekel & Rehman, 1987). However, on our samples, a maximal absorption of HMF was observed between 283 and 285 nm using the diode array detection as reported by many authors (Fallico et al., 2004; Fernandez–Artigas et al., 1999; Ferrer et al., 2002; Rada-Mandoza et al., 2002; Ramírez-Jiménez et al., 2000a, 2000b).

Fig. 1(a) shows the good superposition of the UV spectra of HMF and F peaks for the cookie and the pure standard. MF spectrum of the standard only is presented because only traces of this compound could be detected in any samples.

Ferrer et al. (2002) analysed these furfural compounds in infant formulas. They found that the maximal absorbance wavelength for HMF and F was 284 nm, and for MF 293 nm. On the other hand, Kermasha, Goetghebeur, Dumont, and Couture (1995) determined by diode array detection the optimal wavelengths for the detection of HMF, F and MF in apple juice. They found a unique absorbance maximal at 291 nm for MF, but two maximal absorbance wavelengths at 222 and 278 nm, and 220 and 285 nm for HMF and F, respectively. Our results are somewhat different: we found a maximal wavelength at 284, 276.9 and 292.3 nm for HMF, F and MF, respectively (Fig. 1(a)). The effect of the eluant (variable in the different papers) could explain some differences.

With the present method the three furfural compounds are separated in 12 min (Fig. 1(b)), with retention times of 4, 6 and 11 min approximately for HMF, F and MF, respectively.

3.3. Matrix effect and linearity

To evidence an eventual matrix effect, a concentration range between 0.5 and 504.4 mg kg^{-1} was added to a cookie sample for building a calibration curve and comparing it to that obtained with the pure standard. The response was linear in this range of concentration, which is compatible with the levels expected in cereal products. The slopes of the regression lines obtained for the pure standard ($a = 0.55$) and for CK ($a = 0.56$) or SB ($a = 0.56$) added with the standard were not significantly different ($p = 0.95$, $n = 6$, paired Student's t test). This allows concluding that there is

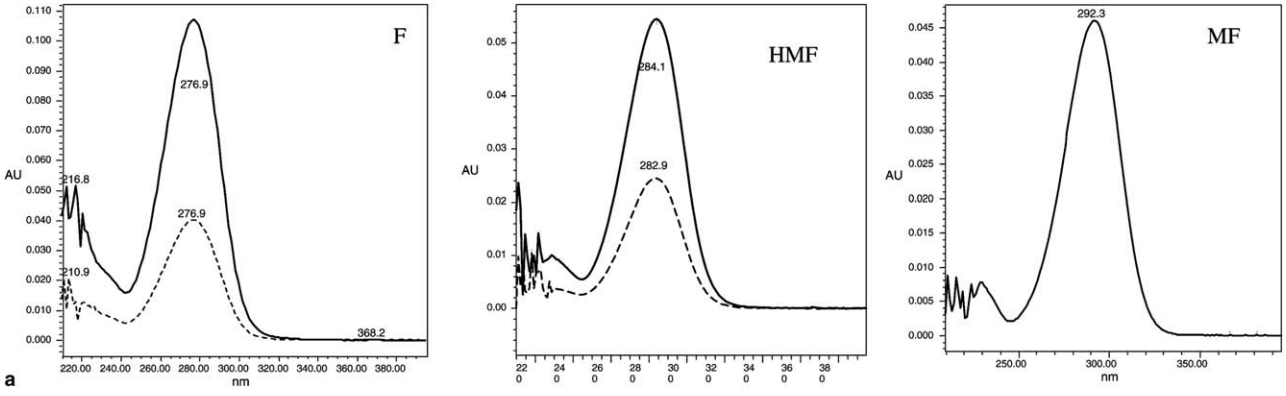

Fig. 1(a). Superposition of UV spectra of the chromatographic peaks for furfural standards and cookies. (—), Standards. (----), Cookie extract.

no interference between HMF and the food matrix using this analytical procedure. The detection limit was defined as three times the standard deviation of the noise integrated at the HMF retention time and quantified in the control sample without HMF (Feinberg, 1996). This limit was calculated to be 0.36 mg kg^{-1}.

3.4. HMF kinetic during baking at 200, 250 and 300 °C

HMF formation in cookies followed a first order kinetic. The rates of HMF formation during the baking process were 0.0028, 0.0067 and 0.0082 s^{-1} at 200, 250 and 300 °C, respectively (Fig. 2). The activation energy was then calculated using the Arrhenius equation as 10.63 kJ mol^{-1}. Gentry and Roberts (2004) reported that the activation energy of HMF formation during the pasteurization of apple cider was around of 27.3 kJ mol^{-1}, i.e., two times higher than the activation energy found in the cookies. This difference is probably due to the low water content in cookies (99% water in milk and; <10% in cookies), which strongly favors HMF formation explaining the lower activation energy in our case. The curve between HMF concentration in the cookie and the water activity (Fig. 3) indicates that HMF formation starts from an average water activity of 0.40 whatever the temperature in the cookie. Eichner and Karel (1972) reported the same limit value for the development of browning in water-soluble polymers model system. The water activity is a fundamental parameter in HMF production (Kroh, 1994), as on the one hand, the water content must be sufficient to allow a substrate solubilisation and sucrose hydrolysis, but on the other hand, the formation of one mole of HMF from one mole of fructose or glucose needs the release of three moles of water, explaining that too much water induces an inhibition of the reaction by the product. This is also valid for most Maillard products (which always needs at least one dehydration step during formation of the Amadori product), but to a lesser extent than for HMF.

After 10 and 12 min at 250 and 300 °C, respectively, a decrease in HMF was observed, indicating that HMF was degraded into secondary products. But we detected only traces of F and no MF after 10 min baking at 200 °C (Fig. 4) even despite overcooking conditions, suggesting that F and MF, reported by Kroh (1994) as a possible HMF degradation products are marginally formed, or are volatile.

3.5. HMF analysis in commercial cookies

As indicated in Table 2, we observe a strong variation in HMF concentration within the 17 commercial cookies, from 0.5 to 74.6 mg kg^{-1}. Similarly, the HMF concentrations found by others in cereal products are highly variable : between 0.4 and 65.5 mg kg^{-1} in infant cereals (Fernandez–Artigas et al., 1999; Ramírez-Jiménez et al., 2003), between 3.7 and 193.3 mg kg^{-1} in breakfast cereals (García–Villanova et al., 1993), between 2.20 and 87.70 mg kg^{-1} in bread (Cardenas Ruiz et al., 2004; Ramírez-Jiménez et al., 2001, 2000a, 2000b) and between 0.08 and 7 mg kg^{-1} in pasta (Sensidoni et al., 1999). No data are available on HMF in cookies.

In conclusion, our study demonstrates that the use of water as extracting medium, followed by TCA addition

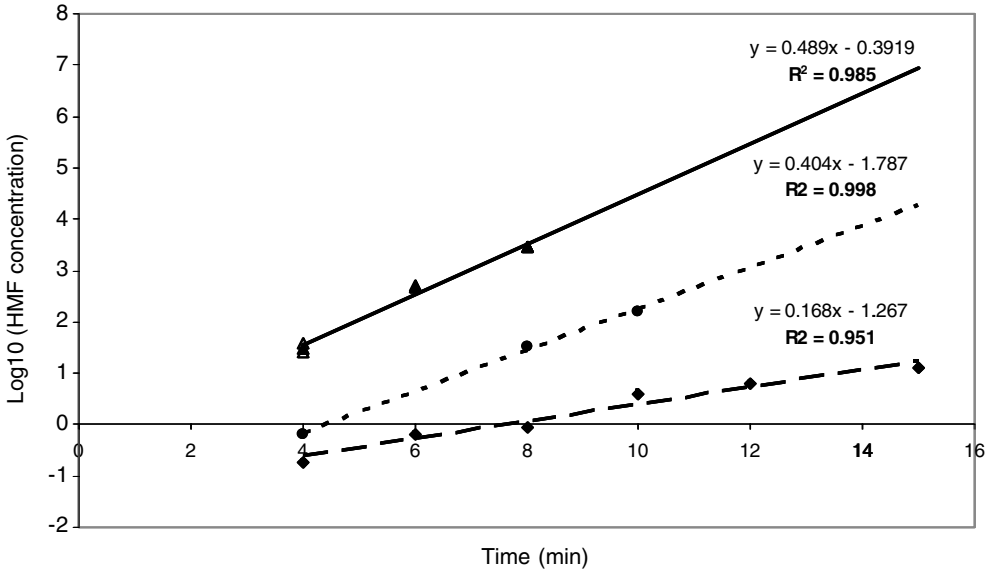

Fig. 2. Accumulation of HMF (mg kg^{-1}) (in its logarithm form) in cookies during baking at 200, 250 and 300 °C. (——), Kinetic at 300 °C. (-----), Kinetic at 250 °C. (– – – – –), Kinetic at 200 °C.

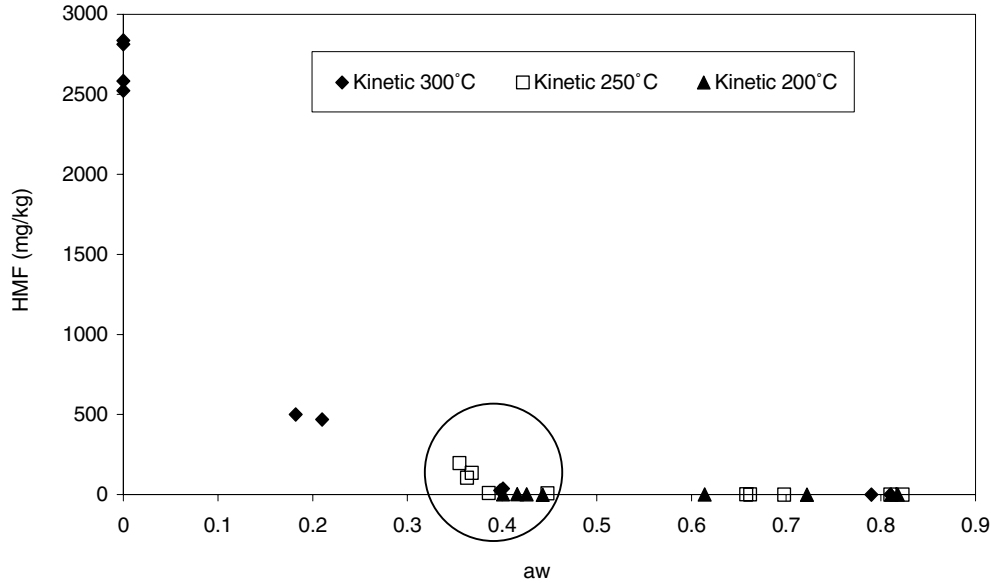

Fig. 3. Evolution of the water activity as a function of HMF concentration in cookies baked at 200, 250 and 300 °C.

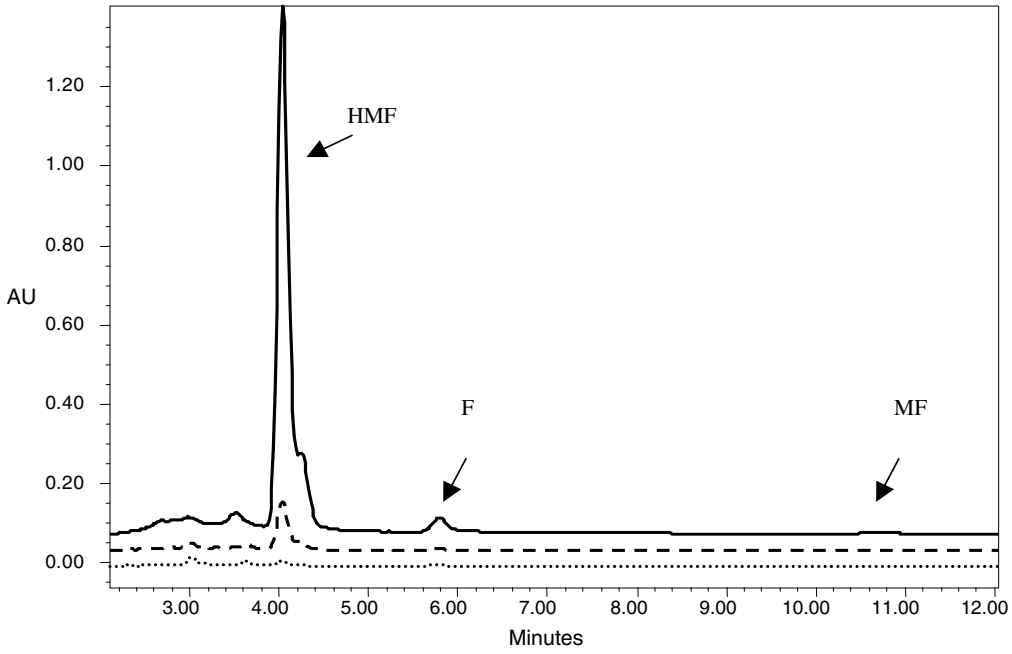

Fig. 4. Evolution of HMF, F and MF chromatogram during the baking process. (——), 200 °C at 15 min; (----), 200 °C at 10 min; (·····)200 °C at 4 min.

is a good way to extract HMF from cereal products, allowing a 97% recovery and a very low repeatability error (0.18%). HMF accumulation follows a first-order kinetic during baking, and is highly dependent on the water activity, which must reach levels lower than 0.4 for allowing a significant formation of HMF. F and MF, were present only at trace concentrations and not clearly related to the baking process.

During the last step of baking, when the temperature at the surface of the biscuit strongly increases, HMF rapidly accumulates. The high variability in HMF concentration in commercial cookies not only results from a variation in the baking process but also from differences in the dough formulation. The precise relationship between the sugar type and content in the formula and the HMF content of the cookie is under consideration.

Table 2
HMF content in commercial cookies

	HMF[a]
C1	74.60 ± 0.09
C2	26.38 ± 0.09
C3	19.84 ± 0.07
C4	7.03 ± 0.39
C5	0.49 ± 0.29
C6	3.31 ± 0.00
C7	17.29 ± 0.17
C8	57.45 ± 0.21
C9	13.02 ± 0.67
C10	32.65 ± 1.49
C11	34.03 ± 2.28
C12	19.15 ± 0.35
C13	11.78 ± 0.74
C14	19.33 ± 0.13
C15	10.34 ± 0.07
C16	16.33 ± 0.60
C17	46.63 ± 0.46

[a] mg kg^{-1}.

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