View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Food Chemistry 133 (2012) 1640–1645

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Development of a fast MECK method for determination of 5-HMF in honey samples

Viviane Maria Rizelio [a], Luciano Valdemiro Gonzaga [a], Graciele da Silva Campelo Borges [a], Gustavo Amadeu Micke [b], Roseane Fett [a], Ana Carolina Oliveira Costa [a,*]

[a] Department of Science and Food Technology, Federal University of Santa Catarina – UFSC, Florianópolis, SC, Brazil
[b] Department of Chemistry, Federal University of Santa Catarina, Florianópolis, SC, Brazil

A R T I C L E   I N F O

Article history:
Received 10 May 2011
Received in revised form 30 August 2011
Accepted 6 November 2011
Available online 1 December 2011

Keywords:
Capillary electrophoresis
Factorial design
Honey
5-Hydroxymethylfurfural

A B S T R A C T

In this study, 5-hydroxymethylfurfural (5-HMF) determination was carried out by a micellar electrokinetic capillary chromatography (MEKC) methodology, using caffeine as the internal standard (IS). The optimisation of the electrolyte composition was approached using a 3^2 full factorial design with a central point to study the MEKC electrolyte components. Inspection of the response surface indicated that the optimal electrolyte composition was 5 mmol L^{-1} sodium tetraborate (STB, pH 9.3) containing 120 mmol L^{-1} sodium dodecyl sulphate (SDS). Under optimal CE conditions, separation of the investigated substance was achieved in less than 0.7 min. Quality parameters, such as linearity ($R^2 > 0.99$), precision (RSD < 5.41%), detection and quantification limits (3.37 and 11.24 mg kg^{-1} for honey samples) and recovery (96.37–99.56%). The proposed methodology was successfully applied to the analysis of 5-HMF in honey samples. The analytical performance of this method makes it suitable for implementation in food laboratories for the routine determination of 5-HMF in honey samples.
© 2011 Elsevier Ltd. Open access under the Elsevier OA license.

1. Introduction

Honey, produced by honeybees from nectar in flowers and plants, is an aqueous supersaturated sugar solution, mainly composed of fructose and glucose. Moreover, honey contains certain minor constituents like minerals, and other saccharides, proteins, enzymes, amino acids, vitamins, organic and phenolic acids, flavonoids, carotenoids, volatile substances and products of the Maillard reaction. During processing, honey is usually warmed in order to lower its viscosity, and to prevent crystallisation or fermentation. Temperatures of 32–40 °C do not affect honey quality; however, the use of higher temperatures leads to the formation of an important degradation product, 5-hydroxymethylfurfural (or 5-(hydroxymethyl)furan-2-carbaldehyde, 5-HMF) (Anklam, 1998; Turhan, Tetik, Karhan, Gurel, & Tavukcuoglu, 2008).

5-HMF is a furanic compound which is formed as an intermediate in the Maillard reaction (Ames, 1992) from the direct dehydration of sugars under acidic conditions (caramelisation) during thermal treatments applied to foods (Kroh, 1994). Under acidic conditions, 5-HMF can be formed even at low temperatures (Lee & Nagy, 1990), although its concentration significantly increases with an increase in the temperature of thermal treatments, or during long periods of storage. In addition to temperature, the amount of 5-HMF formation in foods is dependent on the type of sugar (Lee & Nagy, 1990), pH (Gökmen, Açar, Köksel, & Açar, 2007), water activity (Gökmen, Açar, Serpen, & Morales, 2008; Kroh, 1994) and the concentration of divalent cations of the media (Gökmen & Senyuva, 2006). The Codex Alimentarius of the World Health Organisation and the European Union have established a maximum quality level for the 5-HMF content in honey (40 mg kg^{-1}) (Alinorm 01/25, 2001; Directive 2001/110/EC, 2001). The Brazilian regulations set a maximum 5-HMF content of 60 mg/kg (Brasil, 2000). However, the toxicological relevance of 5-HMF has not been clearly demonstrated. Cytotoxic, mutagenic, carcinogenic and genotoxic effects are among the *in vitro* activities attributed to HMF (Murkovic & Pichler, 2006; Teixidó, Santos, Puignou, & Galceran, 2006).

The determination of 5-HMF in foods has been traditionally performed by the spectrophotometric method described by White (1979). Several other methods have been developed, employing high performance liquid chromatography (HPLC) with UV detection (Aquino, Rodrigues, Nascimento, & Casimiro, 2006; Gaspar & Lucena, 2009; Michail et al., 2007; Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011; Spano, Ciulu, Floris, Panzanelli, & Piloa, 2009; Xu, Templeton, & Reed, 2003; Zappaláet al., 2005). In addition, liquid chromatography with pulsed amperometric detection (Xu et al., 2003), refractive index detection (Xu et al., 2003) or coupled to mass spectrometry (LC–MS) (Gökmen & Senyuva, 2006; Teixidó, Moyano, Santos, & Galceran, 2008) has been used to analyse 5-HMF in several foodstuffs. Recently, techniques of gas chromatography coupled to mass spectrometry (CG–MS) (Teixidó et al.,

* Corresponding author. Address: Department of Science and Food Technology, Agricultural Science Center, Federal University of Santa Catarina – UFSC, Ademar Gonzaga 1346, 88034-000 Itacorubi, Florianópolis, SC, Brazil. Tel.: +55 48 3721 5374; fax: +55 48 3721 9943.
E-mail address: anacosta@cca.ufsc.br (A.C.O. Costa).

0308-8146 © 2011 Elsevier Ltd. Open access under the Elsevier OA license.
doi:10.1016/j.foodchem.2011.11.058

2006), and electrochemical biosensors (Lomillo, Campo, & Pascual, 2006) has been proposed for the analysis of 5-HMF in honey, baby foods, jam, orange juice and bakery products, among substances.

As an alternative technique, capillary electrophoresis has been occasionally selected for the analysis of 5-HMF in different foodstuffs such as breakfast cereals, toast, honey, orange juice, apple juice, jam, coffee, chocolate and biscuits (Silva, Schuch, Vainstein, & Jablonski, 2008; Teixidó, Núñez, Santos, & Galceran, 2011). Capillary electrophoresis has been applied to 5-HMF determination employing the micellar electrokinetic capillary chromatography (MEKC) mode. Morales and Jiménez-Pérez (2001) developed a procedure for determining HMF in milk-based products using MEKC and compared with the classical reversed-phase HPLC method it gives similar values of repeatability and recovery. The 5-HMF peak was resolved using an uncoated fused-silica capillary with phosphate buffer containing sodium dodecyl sulphate (SDS) (pH 7.5), and separation was completely achieved in 5 min. Silva et al. (2008) applied MEKC for the determination of 5-HMF in honey samples within 5 min. The recovery was 98% and the limit of detection was 0.025 mg kg^{-1}. More recently, Teixidó et al. (2011) found 5-HMF in several foodstuffs, and the MEKC method (analysis time of 6 min) was compared with the results obtained by liquid chromatography, coupled to tandem mass spectrometry. The sample limit of detection (LOD, 0.7 mg kg^{-1}) and limit of quantification (LOQ, 2.5 mg kg^{-1}) were established by preparing the standards in a blank matrix. This study attempted to design a rapid method for the determination of 5-HMF in honey samples, using a MEKC methodology with a 3^2 factorial design and electrolytes composed of tetraborate/SDS and modified by methanol. The method performed under the optimised conditions was validated and further applied in the determination of 5-HMF in honey samples of different geographical and botanical origins. No reports of a method faster than that presented in this paper using capillary electrophoresis can be found in the literature.

2. Experimental

2.1. Instrumentation and analytical procedures

The CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector set at 284 nm, a temperature control device maintained at 25 °C and acquisition and data treatment software supplied by the manufacturer (HP ChemStation, rev. A.06.01). An uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used, with dimensions of 32.0 cm total length, 8.5 cm effective length, an inner diameter of 50 μm and an outer diameter of 375 μm. The background electrolyte (BGE) was composed of 5 mmol L^{-1} STB at pH 9.3 containing 120 mmol L^{-1} SDS. At the beginning of each day, the capillary was conditioned by flushing with 1 mol L^{-1} NaOH (10 min) followed by a 10 min flush with deionised water and BGE solution (15 min). In between runs, the capillary was reconditioned with the background solution (1 min flush). At the end of each working day, the capillary was rinsed with 1 mol L^{-1} NaOH (5 min) and water (10 min) and then dried with air (2 min). Standard solutions and samples were introduced at the extremity of the capillary nearest to the detector and injected hydrodynamically (at 50 mbar for 3 s; 1 mbar = 100 Pa) with negative pressure. The applied separation voltage was 30 kV with positive polarity on the injection end.

The comparative method, using the LC/MS/MS analysis, was performed on chromatographic equipment consisting of a high-performance liquid chromatography (HPLC) system (Agilent Technologies – Germany). Separation was performed on an Atlantis HILIC Silica Column (30 mm, 2.1 mm ID, 2.0 μm particle size) Waters. A multi-step isocratic and linear gradient of solvent A (H_2O + 0.1% formic acid) and B (95:5 acetonitrile/H_2O + 0.1% formic acid) was applied. The runs were performed using a mobile phase as follows: 0–2.5 min, 90% solvent B (isocratic mode); The flow rate was set at 0.15 mL/min. In all instances, the injection volume was 0.5 μL. The column temperature was set to 30 °C. The LC system was coupled to a mass spectrometer system consisting of a hybrid triplequadrupole/linear ion trap mass spectrometer Q Trap 3200 (Applied Biosystems/MDS Sciex, Concord, Canada). Analyst version 1.5.1 was used for the LC/MS/MS system control and data analysis. The mass spectrometry was tuned in the negative and positive modes by infusion of polypropylene glycol solution. The experiments were performed using the TurboIonSprayTM source (electrospray-ESI) in positive ion mode. The capillary needle was maintained at 5500 V. MS/MS parameters: curtain gas, 10 psi; temperature, 400 °C; gas 1, 45 psi; gas 2, 45 psi; CAD gas, medium. Others parameters for the cone and collision energy are listed in Table 1. HMF was monitored and quantified using multiple reaction monitoring (MRM). Optimisation of the mass spectrometer was performed by the direct infusion of an aqueous solution containing HMF investigated here.

2.2. Reagents and solutions

All reagents were of analytical grade, solvents were of chromatographic purity and the water was purified by deionisation (Milli-Q system, Millipore, Bedford, MA, USA). 5-HMF, caffeine, sodium tetraborate (STB), methanol (MeOH) and sodium dodecylsulfate (SDS) were obtained from Sigma–Aldrich (Santa Ana, CA, USA). Sodium hydroxide was purchased from Merck (Rio de Janeiro, RJ, Brasil).

Stock solutions of 5-HMF (1000 mg L^{-1}) were prepared in MeOH:water (50:50, v/v) at a 1000 mg L^{-1} concentration and stored at 4 °C until analysis. Separate aliquots (0.1, 0.2, 0.4, 0.6 and 0.8 mL) of 5-HMF stock solution were transferred to a 10 mL volumetric flask and diluted with distilled water to make the concentrations: 10, 20, 40, 60 and 80 mg L^{-1}, respectively. Caffeine was used as internal standard (IS), and stock solutions (1000 mg L^{-1}) were prepared by dissolving 100 mg of caffeine in 100.0 mL of deionised water and stored it at 4 °C until analysis. The standard working solutions were prepared every day. In the direct analysis of 5-HMF the optimal electrolyte was composed of 5 mmol L^{-1} STB and 120 mmol L^{-1} SDS at pH 9.3 and stored at ambient temperature until analysis.

2.3. Honey samples

Seven multifloral honey samples were obtained directly from producers registered in local associations across the state of Santa

Table 1
Parameters of mass spectrometer.

Anaytes	Parent ion (*mz*)	Quantitative ion	DP*	EP*	CEP*	CE*	CXP*
HMF ($M+H^+$)	127.07	109.1	26	8	10	11	4
HMF ($M+H^+$)	127.07	81.1	26	8	10	21	4

* DP – Declustering potential; EP – entrance potential; CEP – collision cell entrance potential; CE – collision energy; CXP – collision cell exit potential.

Catarina (southern Brazil). The honey samples were harvested between 2009 and 2010, and were stored at ambient temperature, in the dark, until the experiment. Honey samples were accurately weighed (5 g), dissolved with deionised water in a 10 mL volumetric flask. Two millilitres of caffeine (1000 mg L^{-1}) were added, and the volume was properly figured out, to obtain a final concentration of 200 mg L^{-1} of IS. The honey sample solution was filtered through 0.45 μm membrane filters (Millipore, Bedford, MA, USA). An appropriate amount of the honey sample solution (0.5 mL) was placed in a CE vial, and this solution was injected into the CE equipment.

2.4. Linear range, accuracy, precision and sensitivity

The peak area for 5-HMF with and without IS was plotted against concentration to construct the calibration curves (six levels). The least squares method was employed to examine the linearity of the curve coefficient of determination. Both intra-day and inter-day precisions were determined, employing solutions prepared from a standard solution, as described in Section 2.2. The final filtrate was appropriately diluted to give two concentrations (20.0 and 40.0 mg mL^{-1}) on the calibration curve. Six separate solutions were prepared at each concentration; electropherograms were obtained within the same day to assess the intra-day precision, and over a period of 3 days (1–2 injections/day) to assess the inter-day precision. The limits of detection and quantification were taken as the concentrations at which the peak responses were 3 and 10 times the average noise level, respectively.

3. Results and discussion

3.1. Initial experiments

The pH value of the BGE is an important variable since it affects the charge of the compounds under investigation. In our study, pH has little effect on the mobility of the 5-HMF (pKa 12.82). The aim of the initial experiments was to establish the basic analytical requirements (the type of buffer, pH range of the BGE, SDS concentration range, temperature, voltage and injection time) of the method. The experiment was performed at a pH of 9.3 in the counter-electroosmotic mode with a borate buffer, because this achieved the lowest current and the most stable baseline. The separation was attempted in a micellar medium because at this pH, the 5-HMF is in the neutral form. The temperature was established at 25 °C; the maximum applied voltage was 15 kV without compromising the separation and without excessive current, due to the Joule effect. The effects of the injection time (1–9 s) on the peak characteristics were studied. An increase in the time over which the injection was made resulted in increasing peak heights up to a time of 3 s. The peak height remained stable when the injection time was longer than 3 s, but the shape of the 5-HMF peak broadened. Therefore, 3 s was chosen as the optimum injection time in all further experiments.

3.2. Experimental design

After the initial experiments, three relevant electrophoretic factors were investigated: the borate buffer, SDS concentration and MeOH percentage. The goal was to locate the optimum electrophoretic conditions that allow the minimal analysis time for the 5-HMF determination. A full factorial design (11 experiments) containing three selected factors, was chosen as a 3^2 full factorial design with three trials at the central point. The factors and their "low" (−) and "high" (+) levels are summarised in Table 2. The individual runs of the design were carried out in a randomised sequence. Randomisation offers some assurance that the uncontrolled variation of factors, other than those being studied, will not influence the estimation (Micke, Fujiya, Tonin, Costa, & Tavares, 2006). The replicate measurements were stable and the capillary was well-equilibrated after changing to new electrophoretic conditions. Multiple regression enabled the mathematical relationship between the responses and the independent variables to be determined.

Table 2
Levels of the design variables in the two-level factorial design with three centre points.

Variable	High level	Low level	Centre point
STB (mmol L^{-1})	15	5	10
SDS (mmol L^{-1})	120	100	110
MeOH (%)	10	0	5

The width and the migration time of 5-HMF and caffeine were computed as a function of the electrolyte composition according to the following empirical equation:

$$t_i \text{ or } R_{w1/2} = \text{constant} + a[\text{STB}] + b[\text{SDS}] + c[\text{MeOH}] \quad (1)$$

where, t is the migration time of the analyte i and w is the width of the analyte peak. The equations were solved numerically by means of the Solver algorithm (Microsoft® Excel 2007) and the coefficients are organised in Table 3.

The experimental results obtained from the factorial design were used for modelling the width and migration time of the peaks. With these data, it was possible to estimate the response provided by Eq. (2):

$$Resp. = \frac{R}{t_{caf}} \quad (2)$$

where R is the resolution between 5-HMF and caffeine, and t_{caf} is the migration time of caffeine (IS), the last peak on the electropherogram.

The resolution (R) was calculated using Eq. (3), where t_1 and t_2 are the migration times, and w_1 and w_2 the baseline widths of the HMF and caffeine peaks, respectively.

$$R = \frac{t_2 - t_1}{0.5(w_1 + w_2)} \quad (3)$$

The response function (Eq. (2) was calculated for the entire dataset, and a response surface was generated (data not shown) indicating the optimum conditions for separation with the electrolyte composed of 5 mmol L^{-1} STB and 120 mmol L^{-1} SDS, at pH 9.3. The corresponding electropherogram of a solution of 5-HMF and the caffeine standards under optimised conditions is shown in Fig. 1. The analysis time was successfully reduced using the short-end-injection mode (L_{det} 8.5 cm) and a high electrical field (468.8 V/cm). A baseline separation of 5-HMF and caffeine (IS) was achieved, with high resolution, within 42 s. This separation time is considerably shorter than that of other CE methods reported in the literature. The online acquired UV spectra are depicted in the insert of Fig. 1.

Table 3
Coefficients of the function, Eq. (1).

Compound		Constant	*a*	*b*	*c*
5-HMF	t_i	0.436	0.013	0.004	0.031
Caffeine	t_i	0.369	0.018	0.007	0.027

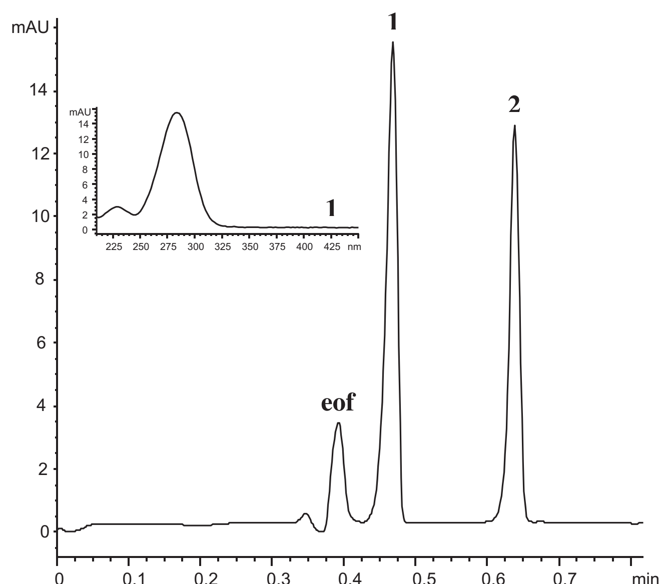

Fig. 1. Electropherogram of 5-HMF (1) and caffeine – IS (2) under optimal conditions. Separation conditions: 5 mmol L^{-1} STB (pH 9.3), 120 mmol L^{-1} SDS; injection, 50 mbar, 3 s; applied voltage, +15 kV; 32 cm (L_{tot}) × 8.5 cm (L_{det}) × 50 μm i.d. capillary; 25 °C; direct detection. Insert figure: on-line acquired UV spectra of the corresponding numbered peak. eof, electroosmotic flow.

3.3. Method validation

In order to validate the proposed analytical method for the determination of the 5-HMF content of Brazilian honey samples, the following validation parameters were evaluated: linearity, selectivity, precision, accuracy, limit of detection and limit of quantification.

3.3.1. Linearity

After the optimisation of the analytical conditions, the linearity of the analytical curves was studied. Five standard solutions in the concentration range of 10–80 mg L^{-1} for 5-HMF using IS (caffeine) were analysed, with triplicate injections at each concentration level. A linear relationship between the ratio of the peak area values (5-HMF/caffeine) and ratio of concentration (HMF/caffeine) was obtained with a satisfactory coefficient of determination (>0.99) and intercepts close to the origin.

3.3.2. Selectivity

The method indicates a significant degree of selectivity, since the main peak is separated from caffeine (IS). The purity of 5-HMF was assessed with the aid of the PDA detector. The peak slicing technique was employed with the aid of the PDA detector to check for peak purity. Detection was carried out at 284 nm, and the overlaid UV spectra obtained for the 5-HMF peak in the honey samples analysed were identical, indicating the purity of the peak and lack of interference from potentially interfering substances. Moreover, samples without 5-HMF (below LOD) were analysed and did not show any peak that might interfere in the analyses, verifying the selectivity of the method.

3.3.3. Precision

3.3.3.1. Repeatability of injection system. The repeatability of the injection system was examined by injecting 20 mg L^{-1} of 5-HMF and IS with 20 injections of the same solution. All determinations were carried out on the same day and under the same experimental conditions. The electropherograms were evaluated considering the migration time and the ratio of the peak area values (5-HMF/caffeine) and the calculated concentration. The RSD values were 2.40%, 4.91% and 4.55% for migration time, peak area ratio and calculated concentration, respectively, which verifies the acceptable repeatability of the method.

3.3.3.2. Intermediate precision. Repeatability (intra-day precision) was established by six consecutive injections of 5-HMF at 20 mg $L^{-1}$and the caffeine (IS) standard solution. The repeatability of the migration time, the peak area ratio and the calculated concentration were better than 0.60%, 1.07% and 0.91% RSD, respectively. Intermediate precision (inter-day precision) was established for the analysis of three preparations of standard solutions, over 3 days with six consecutive injections. The results ranged from 1.61% to 5.41% RSD. The data evaluated are summarised in Table 3. The obtained RSD values obtained indicate an acceptable level of inter-day and intra-day precision.

3.3.4. Accuracy

The method accuracy was investigated by analysing two final concentrations of 5-HMF (20 and 40 mg L^{-1}) added to honey samples not containing previously detectable concentrations of this substance (within the calibration range) which was been prepared as previously described (Table 4). Table 4 shows the results for the recovery tests. The recovery ranged from 96.37–99.56% for the analyte, demonstrating the good reliability of the method for the analysis of 5-HMF in honey samples.

3.3.5. Limit of detection (LOD) and limit of quantification (LOQ)

The signal to noise ratios (S/N) of 3 and 10 were considered to estimate the LOD and LOQ of 5-HMF, respectively. The LOD and LOQ values for the standard solution were respectively 0.09 and 0.31 mg L^{-1}. For the honey samples, the LOD and LOQ values were 3.37 and 11.24 mg kg^{-1}, respectively.

3.4. LC/MS/MS confirmation analysis

In order to show the CE–UV reliability of the HMF analysis in a real sample, a comparison was performed using the LC/MS/MS methodology analysis. Thus, a paired-samples t test was carried out, taking into account the HMF present in the honey sample. The statistical results (for $n = 7$) were p-value equal to 0.12 for the paired-samples t test. The Pearson correlation was 0.98, and this data, from the pairing (or matching), appears to be effective

Table 4
Analytical performance and figures of merit of the proposed electrophoretic method for determining levels of 5-HMF.

Parameter		Value
Instrumental precision (RSD%, $n = 20$)	Peak area ratio	4.91
	Migration time ratio	2.40
	Concentration ratio	4.55
Intra-day precision (RSD%, $n = 6$)	Peak area ratio	0.60
	Migration time ratio	1.07
	Concentration ratio	0.91
Inter-day precision (RSD%, $n = 18$)	Peak area ratio	1.61
	Migration time ratio	5.41
	Concentration ratio	3.79
Linearity	Slope	2.8190
	Slope – SD	0.0347
	Intercept	0.0204
	Intercept – SD	0.0047
	R^2	0.9940
Recovery (%)	At 20 mg L^{-1} level	99.56
	At 40 mg L^{-1} level	96.37
LOD (mg L^{-1})	In standard solution	0.09
LOQ (mg L^{-1})	In standard solution	0.31
LOD (mg kg^{-1})	In sample	3.37
LOQ (mg kg^{-1})	In sample	11.24

Table 5
Amounts of 5-HMF in the analysed honey samples.

Sample	mg kg^{-1}	RSD (mg kg^{-1})
A	17.91	1.56
B	11.97	0.86
C	16.32	1.46
D	127.25	8.97
E	12.87	1.18
F	82.80	5.63
G	12.96	2.02

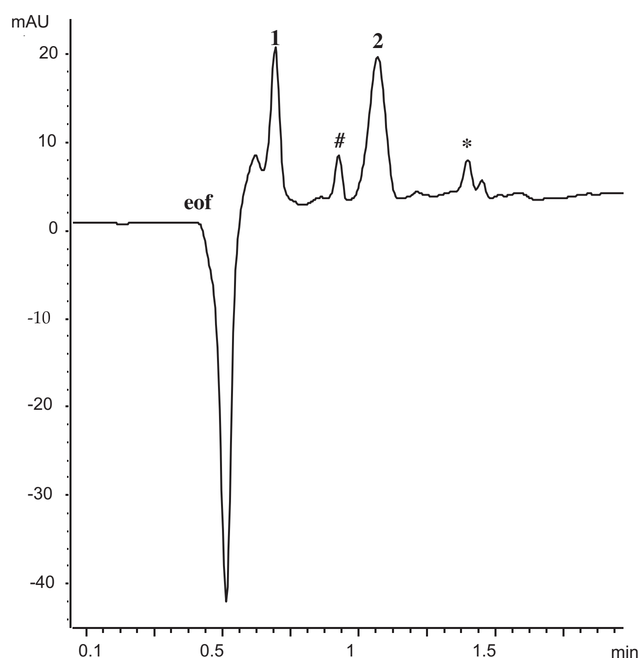

Fig. 2. Electropherogram of honey sample, 5-HMF (1) and caffeine – IS (2) under optimal conditions. Separation conditions: see eof, electroosmotic flow; #,* unknown peak.

with a *p*-value equalling 0.21 for Kolmogorov–Smirnov distance (normality test). As the *p*-value was higher than 0.05, no significant difference within the 95% confidence interval between CE–UV and LC/MS/MS methodologies was observed.

3.5. Sample analysis

The proposed method, after being optimised and evaluated in terms of the parameters described above, was successfully applied to determine 5-HMF in several commercially available honey samples ($n = 7$) which were prepared as indicated above. The honey samples were prepared in duplicate and injected in triplicate. The concentrations of 5-HMF determined for the samples are shown in Table 5.

All samples, with the exception of D and F, were below the concentration limit specified for this compound by Brazilian regulations (Brasil, 2000).The electropherogram of sample F is shown in Fig. 2.

4. Conclusions

A MECK–UV method was developed with the aid of an experimental design to rapidly optimise the analysis time and resolution for 5-HMF separation and determination of this compound in honey samples. Satisfactory results in relation to linearity, selectivity, precision and accuracy were obtained, which confirmed that the proposed method was suitable for this purpose. The analytical performance of the method, particularly the very short analysis time, low cost and simple sample pretreatment, verifies its potential applicability for routine and automated analysis of 5-HMF in the quality control of honeys. Overall, the results demonstrated that CE can be applied as an alternative (or complementary) technique to the recommended spectrophotometric method for application in food analysis.

Acknowledgements

The authors wish to acknowledge the government agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI), Instituto Nacional de Ciência e Tecnologia de *Catálise* em Sistemas Moleculares e Nanoestruturados (INCT Catálise) and Fundação de Apoio a Pesquisa Científica do Estado de Santa Catarina (FAPESC) for financial support and fellowships.

References

Alinorm 01/25 (2001). *CodexAlimentarius.*
Ames, J. M. (1992). The Maillard reaction. In B. J. F. Hudson (Ed.), *Biochemistry of food proteins* (pp. 99–153). London: Elsevier.
Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *FoodChemistry, 63*, 549–562.
Aquino, F. W. B., Rodrigues, S., Nascimento, R. F., & Casimiro, A. R. S. (2006). Simultaneous determination of aging markers in sugar cane spirits. *Food Chemistry, 98*, 569–574.
Brasil (2000). Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n. 11, de 2 de outubro de 2000. *Regulamento técnico de identidade e qualidade do mel.*
Directive 2001/110/EC of 20 December 2001 (2001).*Official Journal of the European Communities.*
Gaspar, E. M. S. M., & Lucena, A. F. F. (2009). Improved HPLC methodology for food control – furfurals and patulin as markers of quality. *Food Chemistry, 114*, 1576–1582.
Gökmen, V., Açar, Ö. C., Köksel, H., & Açar, J. (2007). Effects of dough formula and baking conditions on acrylamide and hydroxymehtylfurfural formation in cookies. *Food Chemistry, 104*, 1136–1142.
Gökmen, V., Açar, Ö. C., Serpen, A., & Morales, F. J. (2008). Effect of leavening agents and sugars on the formation of hydroxymethylfurfural in cookies during baking. *European Food Research and Technology, 226*, 1031–1037.
Gökmen, V., &Senyuva, H. Z. (2006), Improved method for the determination of hydroxymethylfurfural in baby foods using liquid chromatography-mass spectrometry. Journal of Agricultural and Food Chemistry, 54, 2845-2849.
Kroh, L. W. (1994). Caramelisation in food and beverages. *Food Chemistry, 51*, 373–379.
Lee, H. S., & Nagy, S. (1990). Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. *Journal of Food Processing and Preservation, 14*, 171–178.
Lomillo, M. A. A., Campo, F. J., & Pascual, F. J. M. (2006). Preliminary contribution to the quantification of HMF in honey by electrochemical biosensor chips. *Electroanalysis, 18*, 2435–2440.
Michail, K., Juan, H., Maier, A., Matzi, V., Greilberger, J., & Wintersteiger, R. (2007). Development and validation of a liquid chromatographic method for the determination of hydroxymethylfurfural and alpha-ketoglutaric acid in human plasma. *AnalyticaChimica Acta, 581*, 287–297.
Micke, G. A., Fujiya, N. M., Tonin, F. G., Costa, A. C. O., & Tavares, M. F. M. (2006). Method development and validation for isoflavones in soy germ pharmaceutical capsules using micellar electrokinetic chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1625–1632.
Morales, F. J., & Jiménez-Pérez, S. (2001). Hydroxymethylfurfural determination in infant Milk-based formulas by micellar electrokinetic capillary chromatography. *Food Chemistry, 72*, 525–531.
Murkovic, M., & Pichler, N. (2006). Analysis of 5-hydroxymethylfurfural in coffee, dried fruits and urine. *Molecular Nutrition and Food Research, 50*, 842–846.
Pereira, A., Albuquerque, F. M., Ferreira, A. C., Cacho, J., & Marques, J. C. (2011). Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions. *Food Research International, 44*, 71–76.
Silva, S. J. N., Schuch, P. Z., Vainstein, M. H., & Jablonski, A. (2008). Determinação do 5-hidroximetilfurfural em méis utilizando cromatografia eletrocinética capilar micelar. *Ciência e Tecnologia de Alimentos, 28*, 46–50.
Spano, N., Ciulu, M., Floris, I., Panzanelli, A., & Piloa, M. I. (2009). A direct RP-HPLC method for the determination of furanic aldehydes and acids in honey. *Talanta, 78*, 310–314.
Teixidó, E., Moyano, E., Santos, F. J., & Galceran, M. T. (2008). Liquid chromatography multi-stage mass spectrometry for the analysis of 5-hydroxymethylfurfural in foods. *Journal of Chromatography A, 1185*, 102–108.

Teixidó, E., Núñez, O., Santos, F. J., & Galceran, M. T. (2011). 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry, 126*, 1902–1908.

Teixidó, E., Santos, F. J., Puignou, L., & Galceran, M. T. (2006). Analysis of 5-hydroxymethylfurfural in foods by gas chromatography-mass spectrometry. *Journal of Chromatography A, 1135*, 85–90.

Turhan, I., Tetik, N., Karhan, M., Gurel, F., & Tavukcuoglu, H. R. (2008). Quality of honeys influenced by thermal treatment. LWT – Food Science and Technology, 41, 1396-1399.

White, J. (1979). Spectrophotometric method for hydroxymethylfurfural in honey. *Journal of the Association of Official Analytical Chemists, 62*, 509–514.

Xu, H., Templeton, A. C., & Reed, R. A. (2003). Quantification of 5-HMF and dextrose in commercial aqueous dextrose solutions. *Journal of Pharmaceutical and Biomedical Analysis, 32*, 451–459.

Zappalá, M., Fallico, B., Arena, E., & Verzera, A. (2005). Methods for the determination of HMF in honey: a comparison. *Food Control, 16*, 273–277.