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Study of hydroxymethylfurfural and furfural formation in cakes during baking in different ovens, using a validated multiple-stage extraction-based analytical method

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ABSTRACT

A procedure for extraction of hydroxymethylfurfural (HMF) and furfural from cakes was validated. Higher yield was achieved by multiple step extraction with water/methanol (70/30) and clarification with Carrez I and II reagents. Oven type and baking time strongly influenced HMF, moisture and volatile profile of model cakes, whereas furfural content was not significantly affected. No correlation was found between these parameters. Baking time influenced moisture and HMF formation in cakes from traditional and microwave ovens but not in steam oven cakes. Significant moisture decrease and HMF increase (3.63, 9.32, and 41.9 mg kg^{-1} dw at 20, 40 and 60 min, respectively) were observed during traditional baking. Cakes baked by microwave also presented a significant increase of HMF (up to 16.84 mg kg^{-1} dw at 2.5 min). Steam oven cakes possessed the highest moisture content and no significant differences in HMF and furfural. This oven is likely to form low HMF and furfural, maintaining cake moisture and aroma compounds.

1. Introduction

During baking complex reactions occur that involve physical, chemical, and biochemical changes promoted by dough heating (Sablani, Marcotte, Baik, & Castaigne, 1998), which are essential for the development of the aroma, taste and colour surface of the baked products. These reactions are mainly influenced by dough composition, baking conditions (temperature, time and heat transfer process) and evaporation of water (Gökmen, Açar, Köksel, & Açar, 2007; Purlis, 2010).

Baking can be performed using different types of oven, for example, static traditional oven, steam oven and microwave oven. In static traditional ovens the heat transfer to the baking food is through oven air (Walker, Seetharaman, & Goldstein, 2012). Steam oven uses steam as the source of heat transfer. Steam is discharged through channels that in turn heat the oven to the selected temperature to disperse steam throughout the entire oven (Isleroglu et al., 2012). Microwave oven uses high frequency microwaves. Microwaves cause food molecules to vibrate rapidly, creating friction that produces heat to cook the food (Malheiro, Casal, Ramalhos, & Pereira, 2011). The type of oven influences Maillard reactions and caramelisation; consequently, aroma generation of baked products, and nutritional aspects, such as the formation of undesirable compounds are affected (Isleroglu et al., 2012; Purlis, 2010).

Characterisation of physicochemical properties of food after the baking process (Chevallier, Della Valle, Colonna, Broyart, & Trystram, 2002; Walker et al., 2012) and the formation of undesirable heat generated compounds (Ait-Ameur, Trystram, & Birlouez-Aragon, 2006; Gökmen et al., 2007) are described by several authors. Among the many products formed, HMF and furfural are furanic compounds generated during the advanced stages of Maillard reaction and caramelisation that are commonly measured as quality parameters to evaluate the severity of the heat treatment, due to their accumulation during baking. There are several studies reporting analyses of HMF and furfural by HPLC with UV detection at 280 nm applied to food matrices that suffered heat treatment, namely, breakfast cereals (Rufián-Henares, Delgado-Andrade, & Morales, 2006a, 2006b, 2009), milk

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Table 1
Extraction methods for HMF and furfural analysis in model cakes baked in a convection oven at 160 °C for 50 min. Results are presented as mean (mg kg^{-1} dw) ± standard deviation.

	M1	M2	M3	M4	M5	M6	*p*
HMF	21.08 ± 0.06[a]	23.42 ± 0.07[b]	23.47 ± 0.10[b]	31.53 ± 0.05[c]	32.32 ± 0.03[d]	29.05 ± 0.05[e]	<0.001
Furfural	0.47 ± 0.022[a]	0.46 ± 0.012[a]	0.63 ± 0.021[b]	2.51 ± 0.020[c]	2.66 ± 0.010[d]	2.44 ± 0.004[e]	<0.001

M1 – extraction with oxalic acid and clarification with TCA; M2 – extraction with water and clarification with TCA; M3 – extraction with water and clarification with Carrez I and II reagents; M4 – extraction with water/methanol (60/40) and clarification with Carrez I and II; M5 – extraction with water/methanol (70/30) and clarification with Carrez I and II; M6 – extraction with water/methanol (80/20) and clarification with Carrez I and II.
[a–e]Means with different letters in the same line are significantly different ($p < 0.05$).

(Chávez-Servín, Castellote, & López-Sabater, 2006), coat deep-fried products (Pérez-Palacios, Petisca, Melo, & Ferreira, 2012, 2013), fortified wines (Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011), and different types of bakery products (Ait-Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008; Ait-Ameur et al., 2006). For simple matrices, such as alcoholic beverages and fruit juices, extraction with water and subsequent filtration are commonly applied before HPLC analyses. However, clean-up steps are needed for complex matrices. The use of solid-phase extraction (SPE) cartridges is appropriate to clarify some food extracts, such as honey or baby foods (Driffield et al., 2005; Gökmen & Şenyuva, 2006). In milk-based products, the extract can be heated for several minutes after the addition of oxalic acid (Chávez-Servín et al., 2006). In oils obtained from different roasted nuts and seeds, extraction of HMF and furfural is improved by the use of methanol or ethyl acetate, and subsequent evaporation and reconstitution of the extract with water (or mobile phase) prior to analysis (Durmaz & Gökmen, 2010). The use of Carrez I (15% $K_4Fe(CN)_6$) and Carrez II (30% $ZnSO_4$) solutions as clarifying agents instead of classical acids, such as trichloroacetic (TCA), metaphosphoric, or sulphosalicylic, is recommended for cereal and tomato products, due to the possible *in situ* production of HMF from glucose present in the food matrix at low pH (Mesías-García, Guerra-Hernández, & García-Villanova, 2010; Rufián-Henares et al., 2006a).

As mentioned by Durmaz and Gökmen (2010) the growing attention of the scientific community with regard to the potentially toxic effects of HMF and similar furan compounds requires new, rapid, and sensitive methods to determine these compounds in different food matrices. Each food matrix demands specific extraction conditions, in order to obtain reliable quantification of HMF and furfural. The use of inaccurate analytical methods or inadequate extraction procedures are a drawback to the establishment of a reliable database for HMF content in processed foodstuffs, pivotal for estimation of exposure and consequently a reliable risk assessment. Nowadays, data on dietary exposure are very limited; additional studies are therefore needed to obtain data on the concentrations of HMF and furfural in several foods when they are eaten (Capuano & Fogliano, 2011). Up to now there are no available mitigation strategies addressed to reduce HMF and furfural contents in baked foods. The aim is challenging since their formation follows the same pathways leading to brown and aroma compounds (Capuano & Fogliano, 2011).

As highlighted for other food toxicants, cooking conditions may strongly affect the actual exposure to HMF and furfural (Capuano & Fogliano, 2011). In general, lower baking temperatures and the use of sucrose over simple sugars minimises HMF formation (Zhang et al., 2012). No studies were found concerning the influence of oven type and respective baking time on the generation of HMF and furfural, and the effect on moisture and aroma compounds of cakes containing the usual ingredients, such as sucrose, flour, citrus juices, eggs, and oil. This evaluation is relevant to modulate HMF and furfural content of cakes, while keeping moisture and aroma responsible for consumer acceptability. Thus, the objectives of this work consisted in the (i) validation of an extraction methodology for reliable HPLC analyses of HMF and furfural in model cakes; (ii) evaluation of effects of baking process on formation of HMF and furfural in model cakes baked in static traditional oven, steam oven and, microwave oven, and search for baking conditions that produce low levels of HMF and furfural; (iii) evaluation of their impact on cake moisture and volatile profile, analysed by headspace solid-phase microextraction GC–MS.

2. Materials and methods

2.1. Chemical and reagents

HMF (98%) was supplied by Sigma–Aldrich (Steinheim, Germany). Furfural (99%) was purchased from Merck (Darmstadt, Germany). Methanol was supplied by Merck; ultrapure water (0.055 μS cm^{-1}) was obtained by using a Serial Milli-Q system for Millipore (Supor DCF, Gelman Sciences, Cheltenham, Australia). Reagents used for protein precipitation (15% potassium ferrocyanide (w/v) – Carrez I, 30% zinc acetate (w/v) – Carrez II), trichloroacetic acid solution (TCA) 40% (w/v), and oxalic acid solution (0.15 M) were from Merck.

Separate standard stock solutions of HMF and furfural containing *ca.* 1.0 mg mL^{-1} in methanol were prepared. A working solution (0.05 mg mL^{-1}) for each analyte was made by adding 0.25 mL of stock solution to 4.75 mL of HPLC-grade water. The working solution was stored at 0 °C and renewed daily.

2.2. Experimental design

The following recipe was chosen for model cakes: 80.0 g of sucrose, 90.0 g of wheat flour, 1.5 g of chemical leavening, 10.0 g of grated lemon peel, 1 medium egg (50.0 g), 30.0 g of orange juice and 40.0 g of sunflower oil. The ingredients were thoroughly mixed. Cake pH was 6.9 ± 0.2 evaluated using a pH meter (MicropH 2001; Crison, Barcelona, Spain). Dough was rolled out to two ceramic mugs (8 cm internal diameter and 10 cm height) to give *ca.* 150 g per mug. A total of 18 mugs was prepared for validation of the HPLC method used for quantification of HMF and furfural; nine mugs were cooked in the traditional oven (Model MF22VD, 22L; Electric Co., Portugal), 50 min at 160 °C, and nine mugs were cooked in the microwave (Haier, Model HR-6752T(E), Italy), 1.5 min at maximum power. Each mug was cooked individually.

The effect of baking process on HMF and furfural content, moisture and volatile profile was evaluated in model cakes prepared with the same recipe and cooked in traditional, steam and microwave ovens applying three different cooking times. Samples were (i) baked in a traditional and in a steam oven, at 160 °C for 20, 40 and 60 min, and (ii) microwaved at maximum power (1250 W), during 1.5, 2 and 2.5 min. Three mugs were analysed at each time point. Oven temperature was monitored using a cooking thermometer (Model 26003 Delta TRAK, USA). After baking, all samples were removed from the ceramic mug and crushed with a commercial mill (Flama, Model 1705 FL, Portugal). Dry matter was