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Original Research Article

Assessment of hydroxymethylfurfural and furfural in commercial bakery products

C. Petisca [a], A.R. Henriques [a], T. Pérez-Palacios [a,b], O. Pinho [a,c], I.M.P.L.V.O. Ferreira [a,*]

[a] REQUIMTE, Laboratório de Bromatologia e Hidrologia, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 4050-313 Porto, Portugal
[b] Tecnología de Los Alimentos, Facultad de Veterinaria, Universidad de Extremadura, Avda. de la Universidad s/n, 10071 Cáceres, Spain
[c] Faculdade de Ciências da Nutrição e Alimentação, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

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ABSTRACT

A survey was conducted on the presence of hydroxymethylfurfural (HMF) and furfural in bread and bakery products; for this purpose a reliable extraction procedure followed by high performance liquid chromatography (HPLC) was applied. The performance of the method was evaluated in terms of linearity (r always > 0.99); detection limits (0.001 mg L^{-1} for furfural and 0.006 mg L^{-1} for HMF); recovery percentages (98.5–100.5% for HMF and 94.9–98.9% for furfural); intraday precision (<4.65%) and interday precision (<7.51%). Two batches of a wide variety of products commercially available were analysed (a total of 88 samples). HMF and furfural levels presented high variability between products and batches of the same product. Cake/pastry samples showed the lowest HMF content (3.0 mg kg^{-1} fw) while biscuits showed the highest content (7.8 mg kg^{-1} fw) ($p < 0.05$). Regarding furfural, bread samples presented the highest furfural content (5.3 mg kg^{-1} fw) ($p < 0.05$), cake/pastry and biscuits showed the lowest content (1.9 and 3.0 mg kg^{-1} fw, respectively). Chocolate containing samples presented higher amounts of furfural (>20 mg kg^{-1}). These results indicate that special attention should be given to furfural content of bread (due to its daily high consumption) and re-evaluation of dietary exposure.

1. Introduction

The largest bakery product marketed in Europe is bread. It plays a notable role in the diet, with per capita annual consumption of bread in the EU around 62 kg; in Portugal, annual bread consumption stands slightly higher, at 70 kg per capita (Quilez and Salas-Salvado, 2012). Biscuits are the second largest bakery product consumed, followed by industrial pastries/cakes.

The term "bread" refers to a very broad range of products, but in general the basic ingredients are cereal flour, water, yeast and sodium chloride. The dough can also be prepared with sugar, fat and eventually, chocolate and dried fruits among other ingredients. Biscuits and cakes/pastry contain eggs and/or milk, and chemical leavenings (such as sodium bicarbonate) instead of yeast. The bakery industry uses different kinds of organic acids, such as citric acid or ascorbic acid to stabilize and regulate dough properties and prevent microbiological contamination (Nanditha and Prabhasankar, 2009). The main difference between biscuits and cakes/pastry is the moisture content and texture. Biscuits present high surface exposure of dough to heat during cooking; consequently, the moisture content is low and the texture is hard.

Chemical reactions such as the Maillard reaction and caramelization during baking are essential for final sensorial attributes, like surface colour, texture and flavour, which are the main features influencing a consumer's preference for bakery products (Abdullah, 2008). However, these reactions also lead to the formation of harmful components such as furanic compounds (Ameur et al., 2006; Ciesarová et al., 2009; Gökmen et al., 2007). Hydroxymethylfurfural (HMF) and furfural are commonly studied as Maillard reaction and sugar pyrolysis intermediates formed during the thermal processing of foods (Capuano et al., 2009; Ferrer et al., 2002; Morales et al., 1992). HMF and furfural are formed by the decomposition of hexoses and pentoses, respectively, during heating.

The generation of HMF and furfural is influenced by the concentration and type of sugar; lower pH, low water activity and high baking temperatures contribute to the formation of furanic

* Corresponding author. Tel.: +351 220428642; fax: +351 226093390.
E-mail address: isabel.ferreira@ff.up.pt (I.M.P.L.V.O. Ferreira).

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compounds (Ameur et al., 2006; Gökmen et al., 2007; Purlis, 2010). These compounds have been evaluated as indicators of the severity of heat treatment or length of storage in several foods including fruit juices (Gökmen and Acar, 1999); ultrahigh-temperature-treated milks (Ferrer et al., 2000; Morales et al., 1992, 1997); breakfast cereals (Rufián-Henares et al., 2006); honey, wine and other alcoholic beverages, vinegars, coffee, breads and baby cereals (Teixidó et al., 2006, 2011). Although the toxicological relevance of HMF and furfural is not clear, several *in vitro* studies point out that these compounds are suspected of genotoxic and mutagenic effects and their presence is undesired in thermally processed foods (Abraham et al., 2011; Arts et al., 2004; Capuano and Fogliano, 2011; EFSA, 2005). This deserves further attention and maximum concentration limits for certain foods might be considered (Janzowski et al., 2000).

Regarding bakery products, in the scientific literature there are works focused on the amounts of HMF and furfural in model systems to study the influence of different ingredients on their formation (Ameur et al., 2007, 2008; Gökmen et al., 2007, 2008; Ramírez-Jiménez et al., 2000a; Zhang et al., 2012). However, assessment of these harmful compounds in commercial samples is of major relevance to understand their contribution to daily intake. HMF quantification in commercial breads and biscuits are scarce (Ameur et al., 2006; Delgado-Andrade et al., 2009; Ramírez-Jiménez et al., 2000b). No recent studies have been found for furfural content in commercial bakery products.

The main objectives of this work were: (i) to analyse the HMF and furfural content in bakery products commercially available on the Portuguese market, namely, bread, biscuits, and cakes/pastry samples using an effective extraction methodology, followed by HPLC analysis; (ii) to investigate the effect of type of bakery product on the amounts of HMF and furfural; (iii) to check the relationship between HMF and furfural content and formulation information provided by the manufacturer.

2. Materials and methods

2.1. Chemical and reagents

HMF (98% purity) was supplied by Sigma–Aldrich (Steinheim, Germany). Furfural was purchased by MERCK (99% purity) (Darmstadt, Germany). Methanol (LiChrosolv) and sodium acetate (analytical grade) were supplied by MERCK (Darmstadt, Germany) and ultrapure water (0.055 $\mu S\ cm^{-1}$) was obtained by using a Serial Milli-Q system for Millipore (Supor DCF, Gelman Sciences, Chentelham, Australia). For protein precipitation, Carrez I and II containing, respectively, 15% potassium hexacyanoferrate (w/v) and 30% zinc acetate (w/v) analytical grade were used.

2.2. Sampling

Forty-four different bakery products were selected for this study, and 2 different batches of each product were analysed, for a total of 88 samples randomly purchased on the Portuguese market. They were clustered in 3 major types and randomly codified: bread (BR1 to BR10, $n = 20$), biscuits (BI1 to BI12, $n = 24$), and cakes/pastry (CA1 to CA22, $n = 44$). Total content of each package was ground to powder in a commercial grinder (Flama, Model 1705 FL, Portugal), homogenized, stored in flasks at 4 °C and analysed within 2 working days.

2.3. Extraction method

The extraction procedure was based on the method proposed by Rufián-Henares et al. (2006) with some modifications. Ten grams of sample was suspended in 5 mL water:methanol (70:30). The mixture was thoroughly stirred during 1 min and then 2.0 mL Carrez I and Carrez II solutions were added and centrifuged at 5000 rpm (4 °C) during 15 min, recovering the supernatant to a 15 mL flask. Two more consecutive extractions were made with 2 mL of water:methanol (70:30) until collecting 10 mL of supernatant. Two millilitres of this solution were centrifuged at 8000 rpm for 15 min before being analysed.

2.4. HPLC-DAD methodology

A 20 μL portion of the final extract was injected into the HPLC system using an autosampler (Jasco AS-2057 Plus, Tokyo, Japan) onto an Ultracarb ODS column (5 μm, 250 mm length, 4.6 mm i.d.). An analytical HPLC unit (Jasco, Tokyo, Japan) equipped with Jasco PU-2080 HPLC pumps and MD-910 Plus multi-wavelength detector was used. A Borwin PDA Controller Software (JMBS Developments, Le Fontanil, France) was also used. Peak identification in the chromatograms was carried out by comparing retention times and spectra of unknown peaks with reference standards. The mobile phase was composed of sodium acetate (0.04 M) and methanol (70:30), adjusted to pH 4.0 with acetic acid (99.8%) (Fisher Scientific, Leicestershire, UK). The flow rate was 0.8 mL min^{-1}. All analyses were performed in triplicate. Results are expressed as mg kg^{-1} fresh weigh (fw).

2.5. Quality control and criteria

Calibration curves were constructed for HMF and furfural. Limit of detection (LOD) and quantification (LOQ) based on a signal-to-noise ratio of 3:1 and 10:1, respectively, were determined using standard solutions ($n = 5$).

To ensure the reliability of the analytical method, validation was performed according to EURACHEM Guide (1998). The recovery experiments were carried out using 3 different samples in order to assess the accuracy of the method. Each sample was analysed in triplicate. Intraday precision ($n = 3$) and interday precision ($n = 9$) were also assessed.

2.6. pH analyses

For pH measurement, 1.0 g of sample was mixed with 20 mL of water and vortexed for 2 min. The mixture was held at room temperature (25 °C) for 30 min to separate solid and liquid phases. pH was measured after appropriately removing the supernatant layer by using a potentiometer (Micro pH 2001, Crison, Barcelona, Spain).

2.7. Statistical analyses

To ensure that parametric methods could be reliably applied normal distribution, and homoscedasticity of data were checked. *t*-test was carried out to ascertain the variability of HMF and furfural content between the two batches of the same product, purchased and analyzed separately. One-way ANOVA was performed to establish the effect of the type of product on HMF and furfural content of bread, biscuits and cakes/pastry. Pearson's correlation coefficient was used to search for correlations between HMF, furfural, pH and nutritional composition in bakery products. Principal component analysis (PCA) was performed using HMF, furfural, protein, carbohydrate, fat, fibre and moisture as variables to reduce dimensionality of data with no loss of significant amounts of information. Results from samples that contained chocolate or dried fruits were not included in bivariate and multivariate statistical analyses, except *t*-test. Statistical analyses were carried out using SPSS (v.20.0, IBM, Armonk, NY, USA).