FOOD COMPOSITION AND ADDITIVES

Application of a Fast High-Performance Liquid Chromatography Method for Simultaneous Determination of Furanic Compounds and Glucosylisomaltol in Breakfast Cereals

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The high-performance liquid chormatography method reported in the present paper provides a fast, low-cost, precise, and simple technique to analyze simultaneously 3 different indicators of nonenzymatic browning, i.e., hydroxymethylfurfural (HMF), furfural, and glucosylisomaltol (GIM), in breakfast cereals. These compounds were extracted in aqueous solution and separated on a reversed-phase C_{18} column with water–acetonitrile (95 + 5, v/v) mobile phase under isocratic conditions. Average recovery rates for HMF, furfural, and GIM were 99.1, 98.4, and 99.4%, respectively. The coefficients of variation were 3.67, 2.42, and 1.59% for HMF, furfural, and GIM, respectively. The detection limit was 0.01 mg/kg, and the quantitation limit was 0.05 mg/kg for the 3 studied compounds.

Heat treatment of foods rich in reducing sugars may result in nonenzymatic browning depending on the conditions applied. The chemical reactions involved in this process are essentially the Maillard reaction and caramelization, both dependent on the type of reagent, temperature, water activity, and pH. The Maillard reaction occurs between reducing sugars and amino acids, peptides, or proteins (1). This reaction is favored in systems with intermediate moisture content, temperatures over 50°C, and pH 5–7. Caramelization depends on direct degradation of sugars and requires more drastic conditions, such as temperatures higher than 120°C, 3 < pH > 9, and low water activity (A_w; 2).

Breakfast cereals, an important source of energy in human nutrition, are usually manufactured by extrusion, which is a well-established industrial process with beneficial effects on the nutritional properties and organoleptic characteristics (e.g., texture) of these products (3). During the drying and toasting steps, starch and nonreducing sugars, such as sucrose, may be hydrolyzed to form reducing sugars, which favor nonenzymatic browning linked to the pleasant flavor and color of these kinds of products (4). Recently Torbatinejad et al. (5) have shown that the Maillard reaction results in high lysine losses (20 to 54%) in breakfast cereals due to their manufacturing process.

Hydroxymethylfurfural (HMF) is formed from the degradation of hexoses heated in acid solution, even mild acid solution (6), and is also an intermediate product in the Maillard reaction (7). Furfural is another intermediate product of this reaction that is formed from the degradation of pentoses in acid solution (6). These indicators have been analyzed in different cereal products, such as baby and breakfast cereals (8, 9), pasta (10), and bakery products (11). Glucosylisomaltol (GIM) is a third intermediate product of the Maillard reaction that is generated principally from the reaction between maltose and glutamine (10). This indicator has been used as a control index of Maillard reaction development during pasta drying (10), storage of baby cereals, and bread baking (12).

Dehydrated fruits, caramel, and honey are commonly added to breakfast cereals. The dehydration process undergone by these ingredients during their processing results in nonenzymatic browning, mainly due to carbohydrate degradation, that produces HMF and furfural (13, 14). Thus, although HMF is the best-known indicator of the extent of the browning reaction, its application to monitor modifications during the manufacture and storage of ready-to-eat cereals is limited because HMF and furfural are already present in ingredients added to the cereals.

The present study aimed to optimize a previous methodology to analyze HMF and furfural in order to include the detection of GIM within the same analysis. In addition, based on the high proportion of maltose (15) and glutamine (16) in ready-to-eat cereals, the potential application of GIM as a new indicator of the Maillard reaction during breakfast cereals manufacture was also investigated.

Experimental

Reagents

(**a**) *HMF.*—Sigma (St. Louis, MO).

(**b**) *Furfural.*—Fluka Chemika (Buchs, Switzerland).

(**c**) *GIM.*—Kindly donated by Eduardo J. Guerra-Hernández (University of Granada, Spain).

(**d**) *Carrez I.*—Potassium ferrocyanide, 15%, w/v; Sigma.

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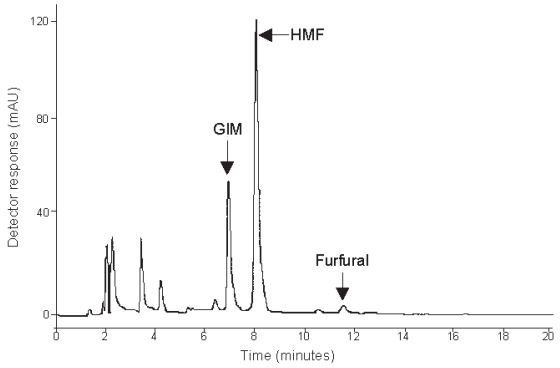

Figure 1. Typical chromatogram of furanic compounds and GIM in breakfast cereal.

(**e**) *Carrez II.*—Zinc acetate, 30%, w/v; Sigma.

(**f**) *Acetonitrile.*—Liquid chromatography (LC) grade; Scharlab (Madrid, Spain).

(**g**) *Water.*—Produced by a Millipore MilliQ Ultrapore Water System (Millipore Corp., Bedford, MA).

Apparatus

Analysis of furanic compounds and GIM was performed by using an LC Pump (MD-420) coupled to an ultraviolet-visible (UV-Vis) detector (MD-432) and a DT-450/MT-2 v.3.90 computing integrator connected to a personal computer (Kontron Instruments, Milan, Italy). The HPLC system for mass spectrometry (MS) detection consisted of an 1100 liquid chromatograph coupled to a quadrupole MS detector and a diode array detector (DAD; Agilent Technologies, Palo Alto, CA). For both systems, the column used was a Tracer Excel 120 ODSB 5 μm, 25 × 0.4 cm (Teknokroma, Barcelona, Spain).

Chromatographic Operating Conditions

(**a**) *LC/UV analysis.*—The LC mobile phase was water–acetonitrile (95 + 5, v/v) delivered at a flow rate of 1 mL/min under isocratic conditions through the analytical column thermostatted at 32°C. The UV detector was set at 280 nm, and 20 μL of sample was injected. HMF, furfural, and GIM were quantified by the external standardization method within the range 2–100, 1–20, and 1–50 μM, respectively.

(**b**) *LC/MS/DAD analysis.*—Data acquisition was performed in electrospray ionization (ESI) positive ion mode. Interface parameters were set as follows: needle and cone voltages 3.0 kV and 100 V, respectively. Nitrogen (99.999% high-purity) was used as the nebulizer gas at 12.0 L/h, and the source temperature was set at 300°C. Full-scan analyses were performed in the 50–350 *m/z* range. Single ion monitoring (SIM) analyses were performed at 97, 127, and 311 *m/z* for furfural, HMF, and GIM, respectively. The DAD spectra were collected in the range of 190–440 nm with a range step of 2 nm. The mobile phase was formic acid–acetonitrile–water (0.1 + 5 + 94.9, v/v) delivered at the flow rate of 1 mL/min under isocratic conditions.

Breakfast Cereal Samples

Eight different commercial breakfast cereals were purchased from supermarkets and representatives from Western retails. All brands selected are usually commercialized in many countries. They were named with initials BC (breakfast cereal), followed by a number. Sample composition covered the 4 different types of flour (wheat, rice, corn, and mixture) used during manufacture of breakfast cereals.

HMF, Furfural, and GIM Extraction

HMF, furfural, and GIM determination was based on the method of García-Villanova et al. (9) with some modifications. Ground sample (500 mg) was suspended in 5 mL deionized water in a 10 mL centrifuge tube. Subsequently, the tube was shaken vigorously for 1 min and clarified with 0.250 mL each of Carrez I and Carrez II solutions. The resulting mixture was centrifuged at 4.500 × *g* for 10 min at 4°C. The supernatant was collected in a 10 mL volumetric flask, and another 2 extractions were performed by

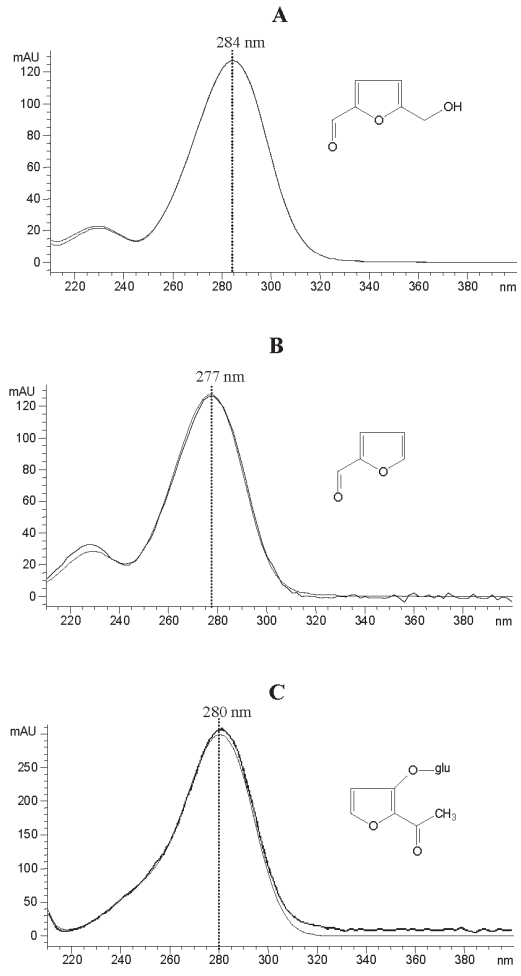

Figure 2. Overlapped spectra of HMF (A), furfural (B), and GIM (C) in Sample BC-7 and standards.

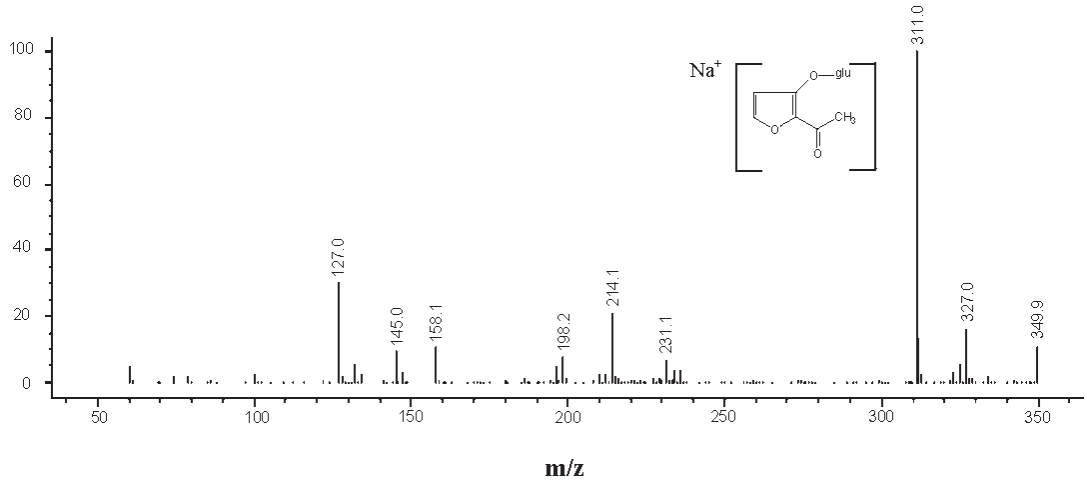

Figure 3. Mass spectrum of GIM in sample BC-7. Glucose (-glu). GIM sodium adduct ($C_{12}H_{16}O_8Na$) is at *m/z* 311.0.

adding 2 mL deionized water. The supernatants were mixed, and the volume was completed up to 10 mL with deionized water. Then 2 mL was filtered (0.45 μm) to analyze HMF, furfural, and GIM determination.

Results and Discussion

HMF and furfural are the furanic compounds most widely used for the assessment of nonenzymatic browning in foods (17). They have been successfully used in different cereal-based products (8–11), but their use as browning indicators is limited in products enriched in ingredients with a previous content of HMF or furfural, such is the case of breakfast cereals (12). In this case, GIM, previously applied as an indicator to control the manufacture of pasta (10) and storage of baby cereals or bread baking (12), could be useful in ready-to-eat cereals.

Chromatographic Conditions

As described by Guerra-Hernández et al. (12) for GIM determination in infant cereals and bakery products, a mobile phase composed of acetonitrile–water (5 + 95, v/v) was used. Because of the absence of peaks-interfering, it was not necessary to increase the acetonitrile proportion. As shown in Figure 1, chromatograms with good baseline resolution of the 3 analytes were obtained with this mobile phase. The retention times for GIM, HMF, and furfural were of 7.1, 8.4, and 11.6 min, respectively.

HMF, Furfural, and GIM Characterization

Peak purity was studied with a DAD, and spectra were collected between 190–440 nm. As shown in Figure 2, good peak purity was obtained for HMF (A), furfural (B), and GIM (C); these compounds showed absorption maxima at 284, 277, and 280 nm, respectively, which were used for sample analysis. In addition to UV spectral analysis, to achieve a more precise characterization of the analyzed compounds, HMF, furfural, and GIM were identified by means of MS. Full-scan analyses were performed in the 50–350 *m/z* range, and SIM analyses were performed at 97, 127, and 311 *m/z* for furfural, HMF, and GIM, respectively. As shown in Figure 3, a 100% relative abundance was

Table 1. HMF, furfural, and GIM recovery in the analysis of fiber-rich cereal

HMF				Furfural				GIM			
Added[a]	Total[a]	Detected[a]	Recovered, %	Added[a]	Total[a]	Detected[a]	Recovered, %	Added[a]	Total[a]	Detected[a]	Recovered, %
0.0	47.6	47.6	100.0	0.00	0.7673	0.7673	100.0	0.0	12.20	12.20	100.0
10.2	57.8	56.4	97.6	0.05	0.8173	0.8042	98.4	5.0	17.20	16.84	97.9
15.3	62.9	62.5	99.4	0.10	0.8673	0.8412	97.0	10.0	22.20	22.68	102.2
20.4	68.0	68.2	100.3	0.15	0.9173	0.9106	99.3	15.0	27.20	27.11	99.7
25.5	73.1	72.8	99.6	0.20	0.9673	0.9357	96.7	20.0	32.20	31.57	98.0
30.6	78.2	76.4	97.7	0.25	1.0173	1.0184	100.1	25.0	37.20	36.58	98.3
Mean			99.1				98.6				99.4

[a] mg/kg.

Table 2. Breakfast cereal composition

Sample	Flour type	Proteins, %	Carbohydrates, %	Fat, %	Fiber, %
BC-1	Rice	5.5	84.0	2.3	3.0
BC-2	Wheat and corn	10.8	49.2	2.1	31.3
BC-3	Wheat	12.5	47.0	4.5	28.0
BC-4	Wheat	7.0	84.0	0.4	3.0
BC-5	Wheat	7.8	81.0	3.5	4.3
BC-6	Corn	8.0	82.0	0.9	3.0
BC-7	Corn	7.4	83.5	1.5	1.7
BC-8	Corn	5.9	86.7	0.8	3.0

obtained for the molecular ion at *m/z* 311.0, corresponding to [GIM-Na^+], with the molecular formula $C_{12}H_{16}O_8Na$ as previously stated for GIM (12). Similar results were obtained for HMF and furfural (data not shown). In the method reported by Guerra-Hernández et al. (12), the inclusion of a solid-phase extraction (SPE) purification step was necessary to improve GIM resolution, which was low in infant cereals and bread because of the proximity of an unknown peak. However, in the method proposed here, no further purification steps were needed because HMF, furfural, and GIM are well resolved, with no interfering peaks and a high purity and certainty in the identification of these compounds.

Method Performance

In order to study the influence of the number of extraction steps and the matrix effect on the quantitation of HMF, furfural, and GIM, the extraction procedure was tested with 4 different types of ready-to-eat cereals based on rice, wheat, corn and corn-wheat flours mixture. When 1 extraction step was applied, recoveries of only 85% of GIM and 65% of HMF or furfural were obtained. This was probably due to the lower water solubility on these furanic compounds compared to GIM (17). By carrying out a second extraction with deionized water, higher recoveries (nearly 100%) were obtained for GIM, but they were still low for the furanic compounds (85–90%). Finally, a third extraction was applied, resulting in mean recoveries of 99.3, 99.0, and 99.7% for HMF, furfural and GIM, respectively. It was concluded that it is necessary to use 3 consecutive extractions to obtain the highest recoveries for GIM and furanic compounds.

Because of the absence of matrix effects during the extraction procedure, recovery assays were performed on a breakfast cereal enriched with bran fiber, which is probably the most complex cereal matrix. Recovery was determined by the standard addition procedure. HMF, furfural, and GIM standards were added to the BC-3 sample. As shown in Table 1, HMF recovery ranged from 97.6 to 100.3%, with an average value of 99.1%; furfural recovery varied between 96.7 and 100.1%, with an average value of 98.6%; and recovery of GIM ranged from 97.9 to 102.2%, with an average value of 99.4%. Three independent determinations were carried out for each addition level.

The intraday precision study for each compound was carried out on 8 sample preparations of the previously analyzed breakfast cereal. The relative standard deviation (RSD) values for HMF, furfural, and GIM were 3.67, 2.42, and 1.59%, respectively. The interday RSD was 4.01% for HMF, 2.87% for furfural, and 2.03% for GIM.

The detection limit was 0.01 mg/kg for the 3 compounds analyzed (calculated at a signal-to-noise ratio of 3). The quantitation was performed on concentrations above 0.05 mg/kg.

Sample Analysis

Eight different ready-to-eat cereals were analyzed in order to check the reported method. As stated in Table 2, where their composition is given, breakfast cereals were divided into 4 groups based on their flour composition (rice, wheat, corn, and corn-wheat mixture). As shown in Table 3, HMF, furfural, and GIM could be quantified in all samples. Levels of HMF ranged from 12.01 to 61.10 mg/kg (median 40.08 mg/kg) and are in accordance with those reported previously by García-Villanova et al. (9). Levels of furfural were lower than those of HMF, varying between 0.06 and 0.85 mg/kg (median 0.12 mg/kg). Values for furfural content in breakfast cereals have not been previously reported in the literature. Finally, GIM levels were usually lower than those of HMF, ranging from 6.87 to 18.67 mg/kg, except for sample BC-4 with a GIM content as high as 61.88 mg/kg (median 10.12 mg/kg). As stated in Table 3, the molar ratio between HMF and GIM ranged from 1.08 to 12.48. No correlation was observed between this ratio and the composition of the different ready-to-eat cereals analyzed. As expected, the higher the ratio value, the higher the HMF content.

The presence of HMF could be due not only to formation of this product during cereal processing (i.e., from caramelization as well as from the Maillard reaction), but also

Table 3. HMF, furfural, and GIM content (mg/kg) in commercial breakfast cereals

Sample	HMF	Furfural	GIM	Ratio HMF/GIM[a]
BC-1	49.19	0.06	9.1	12.34
BC-2	12.01	0.85	9.99	2.75
BC-3	47.6	0.77	12.2	8.91
BC-4	29.14	0.18	61.88	1.08
BC-5	37.54	0.1	6.87	12.48
BC-6	61.1	0.13	18.67	7.47
BC-7	28.44	0.09	10.24	6.34
BC-8	46.62	0.11	9.77	10.90

[a] moles HMF/moles GIM.

to the addition of thermally damaged ingredients, such as honey or sugar, that already contain HMF. However, the Maillard reaction is the sole pathway for GIM formation from 2,3-enolization (12), and it would be generated during thermal processing of breakfast cereals due to their high content of maltose and glutamine.

GIM has been evaluated in breakfast cereals for the first time in this study. Other authors (12) have found GIM values ranging from 0.48 to 4.54 mg/kg during accelerated baby cereal storage (4 weeks, at 55°C). In the case of bread baking (12, 18), GIM values were found to be between 0.82–20.90 mg/kg. Our results are in agreement with those obtained for bread baking, where the heat treatment applied is similar to that applied to cereal flours during ready-to-eat cereal manufacture.

Conclusions

The method reported in this paper provides a simple technique to analyze simultaneously 3 different indicators of nonenzymatic browning, i.e., HMF, furfural, and GIM, in ready-to-eat cereals. The method is fast, low-cost, and precise. Determination of HMF, furfural, and GIM as chemical heat-induced markers of the thermal processing applied is highly valuable for on-line control of product quality. However, because of the presence of HMF and furfural in some of the ingredients added for breakfast cereal enrichment, the determination of GIM could be useful to control their manufacture and/or storage. More detailed studies must be performed in order to assess the relationship between GIM and processing conditions.

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