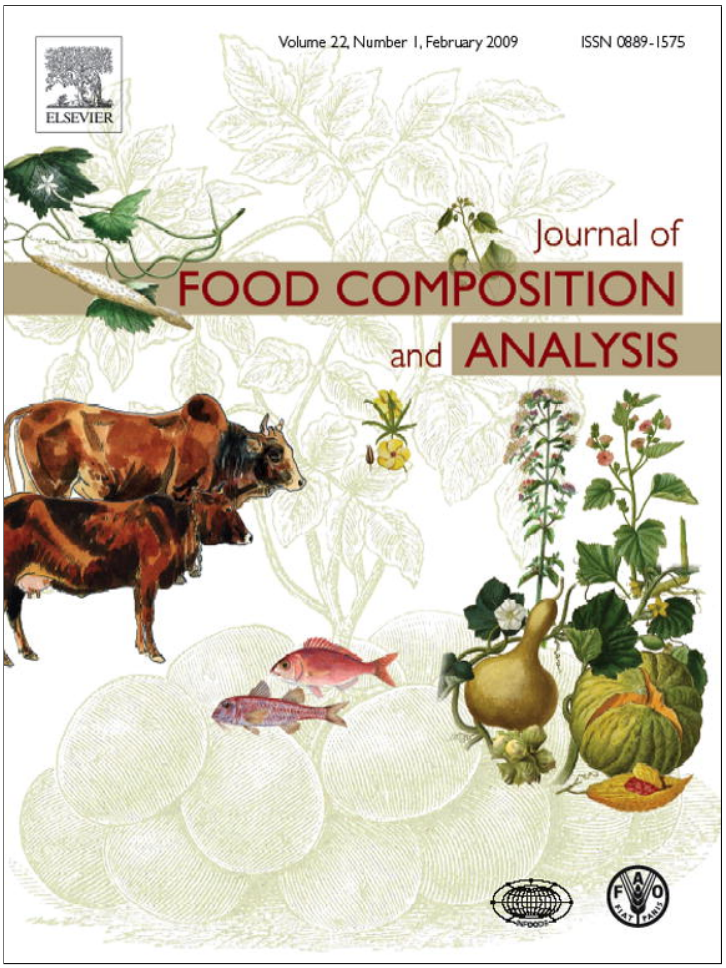

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Short Communication

Determination of furan by headspace solid-phase microextraction–gas chromatography–mass spectrometry in balsamic vinegars of Modena (Italy)

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ABSTRACT

An isotope dilution method for quantification of furan by internal standardization was adopted, using head space solid phase microextraction (HS-SPME) technique, to evaluate various samples of "aceto balsamico di Modena" (ABM), in order to verify if this seasoning may contribute to the intake of furans in the human diet. Matrix-matched calibration curves were adopted, and furan levels in the majority of the samples purchased on the Italian market ranged between 4 and 26 ng/g. Considering that furan levels in ABM derive from the heat-concentrated must of grapes and from the additive caramel E 150d often used in the production technology, the level of furan in the concentrated must appears, in some samples, not to be the only important factor affecting the risk assessment of furan linked to the use of ABM.

1. Introduction

Furanic compounds occur widely in foods and beverages (Maga, 1979) and the unsubstituted furan (C_4H_4O), considered as a processing contaminant, was previously detected in foods that had undergone thermal treatment (Becalski et al., 2005; Bianchi, Careri, Mangia, & Musci, 2006; Goldmann, Perisset, Scanlan, & Stadler, 2005; Ho, Yoo, & Tefera, 2005; Holscher & Steinhart, 1992; Kallio, Leino, & Salorinne, 1989; Persson & von Sydow, 1973; Qvist & von Sydow, 1974; Shimoda & Shibamoto, 1990). The literature indicates multiple sources of furan formation, and possible furan building blocks generated from different precursors have been identified (Becalski & Seaman, 2005; Perez & Yaylayan, 2004). The US Food and Drug Administration (FDA) found levels up to approximately 100 ng/g for approximately 300 food samples (US FDA, 2004a; US FDA, 2004b); additional data were presented from the Swiss Office of Public Health (Reinhard, Sagar, Zimmermann, & Zoller, 2004). A wide selection of food items was analysed to identify those products which contribute most to the human intake of furan (Zoller, Sager, & Reinhard, 2007).

Recently the European Food Safety Authority affirmed that the current limited availability of occurrence data in food does not allow a sound assessment of dietary exposure to furan, and a call for more information on the occurrence of furan in foods has thus been issued (EC-Commission Recommendation, 2007).

Among heat-processed foods, the Italian wine vinegars have not yet been analysed for furan content. The vinegar known as "aceto balsamico tradizionale di Modena" (ABTM) is produced by a long fermentation process from the concentrated must of white grapes, with several changes of the types of wood barrels in which it is stored. In the first step, the fresh grape must is concentrated by up to half of its original volume by a slow heating process. This sugar-rich solution serves as a substrate for bacteria, and acetic acid is the principal by-product of microbial fermentation. The total acidity of ABTM represents one of the most important chemical parameters of the product for both marketing and biological safety. Another balsamic vinegar is produced by blending the concentrated must with wine vinegar, and this mixture is then allowed to ripen for several months in wooden barrels to develop the typical sensorial characteristics of "aceto balsamico di Modena" (ABM).

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This work reports the results of the first analytical screening of 21 commercial samples of ABM purchased on the Italian market. The results make it possible to obtain a global risk assessment linked to home consumption of this product. The isotope dilution methods used in these evaluations are known for general robustness, and the quantification by internal standardization using isotopically labelled furan was adopted by the majority of authors, using simple headspace (HS) method or headspace solid phase microextraction (HS-SPME) analysis. This work was carried out using a HS-SPME-based analytical method, using matrix matched calibration curves.

2. Materials and methods

2.1. Reagents and standards

Furan and [$^2H_4$] furan (furan-d_4) were obtained from Sigma–Aldrich (Steinheim, Germany) at a purity higher than 99%. All stock solutions of furan and furan-d_4 used for spiking the samples were prepared in methanol and stored at −18 °C for no longer then 2 weeks. Intermediate standard solutions were prepared from the stock standard solutions by dilution with methanol. Aqueous working standard solutions were prepared daily by spiking water with appropriate volumes of the intermediate standard solutions, which were stored at 4 °C.

Furan ≥99% chemical purity (CAS 110-0-9) and furan-d_4, isotopic purity >99 atom% D, were from Sigma–Aldrich (Milan, Italy). Methanol and water were from Merck (Darmstadt, Germany). All solvents used were of analytical grade.

2.2. Sample and sample preparation

Twenty-one balsamic vinegars (ABM) produced from various manufacturers were purchased from different local supermarkets in Milan (Italy). The list of the producers, randomly placed, is reported in Table 1. The data reported in this paper cover only a limited number of brands, because the aim of this work is to obtain exploratory data and not to perform an absolute comparison or to obtain statistical data representative of the standard products.

Six aliquots of 1 g of each ABM sample, directly collected from original bottles at ab. 10 °C previously shaken, were mixed with 2 g of sodium chloride and 4 mL of water in 20 mL screw-cap glass vials fitted with silicone-PTFE septa, and were prepared for analysis by adding an appropriate amount of the aqueous furan working standard solution through the septum. Fortified samples were prepared in duplicate by adding the standard furan working solution (50, 100 and 150 μL) via a chilled gastight syringe through the septum to afford concentrations of added furan in the matrix equivalent to 5, 10, and 15 ng/g, respectively. The standard furan working solution in purified water (100 ng/mL) was prepared daily from an intermediate water standard solution (1 μg/mL) derived from a stock solution of furan in methanol (1 mg/mL).

Table 1
List of "aceto balsamico di Modena" samples from the Italian market analysed to detect furan levels.

Denomination	Trademark	Lot	Ingredients
Aceto balsamico di Modena	Ponti S.p.A.	L0955	Wine vinegar, grape must, artificial colouring agent E 150d, antioxidant E 220
Aceto balsamico di Modena invecchiato	Terre d'Italia	LB3M042	Wine vinegar, concentrated grape must, colouring agent E 150d, antioxidant: potassium metabisulphite
Aceto balsamico di Modena maturato in botti di rovere	Monari Federzoni S.P.A	LB4NO52	Wine vinegar, concentrated grape must, cooked grape must, artificial colouring agent E 150d, antioxidant E 224
Aceto balsamico di Modena del Duca	Aceto balsamico del Duca srl	L5081	Wine vinegar, grape must, artificial colouring agent E 150d
Aceto balsamico di Modena affinato	Fini	L19905	Wine vinegar, concentrated grape must, cooked grape must, artificial colouring agent E 150d
Aceto balsamico di Modena del Duca da uve di Agricoltura Biologica	Aceto balsamico del Duca srl	L5118	Wine vinegar, grape must
Aceto balsamico di Modena Casato Estense	C.D.E. S.r.l.	L08805	Wine vinegar, grape must, artificial colouring agent E 150d, antioxidant E 220
Aceto balsamico di Modena Fattorie Giacobazzi maturato	Fattorie Giacobazzi S.r.l.	L5/024	Wine vinegar, concentrated grape must, cooked grape must
Aceto balsamico di Modena	Maletti	L347	Wine vinegar, concentrated grape must. No caramel colouring added. No preservatives added
Aceto balsamico di Modena	Monari Federzoni S.P.A	L8344	Wine vinegar, concentrated grape must, artificial colouring agent E 150d, antioxidant E 224
Aceto balsamico di Modena Sigillo ligneo Sec.XVI della Comunità di Vignola	Ponti S.p.A.	–	Wine vinegar, grape must, artificial colouring agent E 150d, antioxidant E 220
Aceto Balsamico di Modena	Acetaia la Villa	L5010	Wine vinegar, concentrated grape must, artificial colouring agent E 150d
Aceto Balsamico di Modena	Acetaia Due Madonne	L5090	Wine vinegar, cooked grape must, artificial colouring agent E 150d, antioxidant E 220
Aceto Balsamico di Modena	Mazzetti l'originale	L2974	Wine vinegar, grape must, artificial colouring agent E 150d
Aceto Balsamico di Modena	Dìperdì	L-D069	Wine vinegar, grape must, artificial colouring agent E 150d, antioxidant: sulphur dioxide
Aceto balsamico di Modena maturato in botti di rovere	Acetaia Bellei	L4353	Wine vinegar, cooked grape must, artificial colouring agent E 150d
Aceto balsamico di Modena maturato in botti di rovere	Ortalli	L5040.2	Wine vinegar, cooked grape must, artificial colouring agent E 150d, antioxidant E 224
Aceto balsamico di Modena	Villa Ballentani	L5017	Wine vinegar, grape must, artificial colouring agent E 150d, antioxidant E 224
Aceto Balsamico di Modena invecchiato 3 anni	I Sapori delle Regioni	L4204	Wine vinegar, cooked grape must, artificial colouring agent E 150d, antioxidant E 224
Aceto Balsamico di Modena	Acetaia Bellei	L3037	Wine vinegar, cooked grape must
Aceto balsamico di Modena invecchiato in botti di rovere	Acetaia Bellei	L3247	Wine vinegar, cooked grape must

A similar procedure was used to prepare the working water standard furan-d_4 solution. A fixed volume (100 μL of a 100 ng/mL solution) was added to all vials to obtain a concentration corresponding to 10 ng/g in the matrix.

2.3. GC–MS conditions

The determination of furan was carried out using a Shimadzu 2010 gas chromatography coupled to a Shimadzu 2010 MSD quadrupole mass spectrometer (Shimadzu, Italy). Helium was used as the carrier gas at a flow rate of 1 mL/min. Suitable identification of the analyte was achieved using a Supelcowax™-10 silica capillary column, 30 m length, 0.25 mm i.d., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA, Milan, Italy). The oven temperature programme was 35 °C (held for 5 min) to 240 °C at a rate of 3 °C/min (held for 5 min). The injector temperature was 220 °C, and the splitless injection mode (1 min) was used. The ion source and transfer line temperatures were 200 and 280 °C, respectively. The MS operating conditions were the following: positive electron ionization mode (EI+) using automatic gain control with 70 eV of electron energy and 250 μA of emission current. The mass spectrometer was operated in selected-ion monitoring mode (SIM) by recording the current of the following ions: for furan and furan-d_4 determination, m/z 68 $[M]^+$ and m/z 72 $[M]^+$, respectively. For confirmation of furan and furan-d_4 the ions, m/z 39 $[M–CHO]^+$ and m/z 42 $[M–C_2HO]^+$ were monitored.

2.4. Headspace-SPME procedure

SPME experiments were performed using a manual fibre holder supplied from Supelco (Bellefonte, PA, USA).

Commercially available fibres DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane), 50/30 μm, were purchased from Supelco. Before use, the fibre was conditioned in the GC injection port under helium flow in accordance with the temperature and time recommended by the manufacturer. Fibre blanks were run between each sample to reduce memory effects.

Before HS-SPME analysis, each sample vial was vortex mixed for 3 min, and conditioned for 15 min in a thermostatic water bath at temperature of 25 °C. The fibre was exposed to the headspace of the 20 mL sample vial operating under standard conditions, i.e. extraction temperature 25 °C and extraction time 30 min, under constant slow agitation on magnetic stirrer. Thermal desorption of analytes was carried out by exposing the fibre in the GC injector port at 220 °C for 10 min.

2.5. Quality parameters

To evaluate the linearity, the correlation coefficients, R^2, of the matrix matched calibration curves were determined. The R^2 values ranged from 0.9972 to 0.9990.

Precision of our measurements was evaluated using three replicate solutions of a the same sample of ABM (code M8), which were prepared as previously described and spiked with two different levels of furan (low level, 5 ng/g and high level, 30 ng/g). Three replicate determinations for each spiked sample were consecutively carried out in 1 day and on three non-consecutive days for run-to-run and day-to-day precision, respectively. Good precision was achieved with a relative standard deviation (R.S.D.) of 4–6% for run-to-run and 5–7% for day-to-day determinations.

The limit of detection (LOD), with a signal/noise ratio of 3:1 and the limit of quantification (LOQ) with signal/noise of 10:1, were evaluated to be 0.4 and 1.5 ng/g, respectively.

Table 2
Furan concentration in "aceto balsamico di Modena" samples.

Sample code	Furan (ng/g)
C3	7 ± 0.7
F7	12 ± 1.4
H2	13 ± 1.6
L5	8 ± 0.8
B9	4 ± 0.4
K7	5 ± 0.5
D2	26 ± 3.6
M8	4 ± 0.4
T6	13 ± 1.6
A7	8 ± 0.8
N4	85 ± 11.9
P6	7 ± 0.7
Z9	10 ± 1.2
O4	20 ± 2.4
I5	4 ± 0.4
E7	8 ± 0.8
R1	8 ± 0.8
T1	5 ± 0.5
G6	10 ± 1.2
Q3	11 ± 1.3
S4	7 ± 0.7

LOD = 0.4 ng/g.

3. Results and discussion

The results obtained for the analysis of 21 samples of ABM from the Italian market, using the method of "calibration spiking", are reported in Table 2.

The data were calculated from the values resulting on negative X-axis (furan, ng/g), when the values of the ratio of [furan added]/[furan-d_4 I.S.] are reported on the Y-axis in correspondence to the values of furan spiking shown on the positive X-axis. Alternatively, the furan concentrations can be calculated in each sample from the corresponding equation of the curve $y = ax + b$, setting $y = 0$.

The furan content in the sample N4 was calculated, with the same method, after dilution 1:3 of the original sample with water, and addition of tartaric acid and inverted sucrose to reach acidity value of 6% at about 35° *Bx* value.

The furan concentrations of ABM were found to range between 4 and 26 ng/g, with a single sample reaching 85 ng/g (code N4). Our earlier research indicates that the levels of furan in some ABTM samples (Tateo & Bononi, 2005) exceed the levels of this potentially hazardous chemical detected in ABM in the present study.

In the labels of most of the samples considered in this work, the colouring agent caramel E 150d appeared as an additive provided for in the production specifications of D.M. 03.08.06 (MiPAAF, 2006) in concentrations not exceeding 2% (v/v) of the final product. From previous investigations (Tateo & Bononi, 2007), the furan level measured in various types of commercial caramel E 150d ranges between 58 and 165 ng/g and consequently the contribution of furan deriving from caramel varies from 0.58 to 1.65 ng/g in the final product, if 1% caramel is added. Thus, the furan derived from caramel in various ABM is high enough not to be considered negligible, and is all the more so if the added caramel reaches levels of 2%.

4. Conclusions

The furan and furan derivatives have long been known to occur in heat-treated foods and to contribute to the sensory properties. The furan levels in "aceto balsamico di Modena", arising from the furan levels in concentrated must of grapes and from the additive caramel E 150d, ranges between 4 and 26 ng/g.

The "aceto balsamico di Modena" is prepared by a long and slow heating process and should be considered as a product which

contributes to the human intake of furan. We report here the first results concerning the level of furan in several commercially available ABM products on the Italian market. Since the European Food Safety Authority has called for more information on the occurrence of furan in foods, this work contributes to the limited available data about the levels of furan in a popular seasoning in wide use today.

In order to give a contribute to the evaluation of the human intake for this seasoning, it may be useful to compare the data reported in this paper with the content of furan in various food recently evaluated, for example, from Heppner and Schlatter (2007) and Morehouse, Nyman, McNeal, Dinovi, and Perfetti (2008).

It is evident that, not in all cases is the furan content in ABM negligible, but for an evaluation of range of intake, it is necessary to consider data concerning average consumption. These data not known at this point by the authors of this short communication. In conclusion, it would be preferable to wait until EFSA has given its opinion on this study.

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