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Analytical Methods

Determination of 5-hydroxymethyl-2-furfural and 2-furfural in oils as indicators of heat pre-treatment

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ABSTRACT

This study describes a method for the determination of 5-hydroxymethyl-2-furfural (HMF) and 2-furfural (F) in oils. The method entails liquid–liquid extraction and reversed-phase liquid chromatography. Spiked hazelnut oil was used to test the accuracy and reliability of the method. Furan compounds were extracted from oil using 70% methanol. Mean recoveries were 97 ± 2% and 99 ± 1% for HMF and F, respectively. To investigate the presence of HMF and F in oils, seven oily nuts and seeds were roasted at 180 °C for 30 min. Different oils were found to contain HMF and F ranging from 0.8 to 13.8 and 1.4 to 8.7 mg/kg, respectively. Increasing solvent polarity also increased the rate of HMF transferred to the oil. Spectral analyses of the 70% methanol extracts indicated that absorbance at 285 nm may be used to monitor the accumulation of furan compounds in oil phase during the roasting process of nuts.

1. Introduction

Chemical indicators for assessing the quality of over processed foods have proved to be useful. 5-Hydroxymethyl-2-furfural (HMF) and 2-furfural (F) are commonly studied as the Maillard reaction and sugar pyrolysis intermediates formed during thermal processing of foods (Capuano et al., 2009; Ferrer, Alegría, Farré, Abellán, & Romero, 2002; Kroh, 1994; Morales, Romero, & Jiménez-Pérez, 1992). These furan compounds have been evaluated as indicators of the severity of heat treatment or length of storage in several foods including spirits and honey, wine and other alcoholic beverages, fruit juices, vinegars, ultrahigh-temperature-treated milks, coffee, breakfast cereals, breads and baby cereals (Gökmen & Acar, 1999; Morales, Romero, & Jiménez-Pérez, 1997; Teixidó, Santos, Puignou, & Galceran, 2006). Even though they widely occur in aqueous foods, HMF and F are relatively less polar compared to water. It was previously shown that HMF strongly adsorbed to hydrophobic functional surfaces where they could only be desorbed by using non-polar solvents like diethyl ether (Gökmen & Şenyuva, 2006). Due to the absence of hydroxyl group attached to the furan ring, F is even less polar compound compared to HMF (Ferrer et al., 2002). Thus it is conceivably normal to expect HMF and F to be dissolved in oil phase if the major part is lipid in a food matrix.

Nuts and seeds are generally rich sources for protein and carbohydrates as well as edible oil. Roasting is applied to nuts before oil extraction to improve oil yield or sensorial and textural properties before consumed as snack foods (Durmaz & Alpaslan, 2007; Şenyuva & Gökmen, 2007). During roasting, Maillard reaction products are formed by the interaction of amino acids with reducing sugars or lipid oxidation products. Presence of HMF has been previously reported in roasted hazelnut (Fallico, Arena, & Zappalà, 2003), cocoa (Oliviero, Capuano, Cämmerer, & Fogliano, 2009) and coffee (Murkovic & Bornik, 2007), which contain significant amount of oil. Palm oil obtained from sterilized fruits has been analysed for F content (Arifin et al., 2009). However, to authors' knowledge there is no report showing the presence of furan compounds in the oils obtained from pre-roasted seeds and nuts.

In this study, we aimed to develop an analytical method for the determination of HMF and F in oils. Extraction recoveries of HMF and F were determined using stripped oils spiked with different levels of these compounds. Oils obtained from different roasted nuts and seeds were analysed to confirm the presence of HMF and F. Change of HMF and F concentrations of hazelnut oil as a function of roasting time was also determined.

2. Materials and methods

2.1. Chemicals

All solvents used were HPLC grade and purchased from Merck (Darmstadt, Germany). The HMF and F standards were purchased

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from Sigma (St. Louis, MO, USA). Ultrapure water was used throughout the experiments (Milli-Q system, Millipore, Bedford, MA, USA). Activated carbon and alumina were purchased from Merck (Darmstadt, Germany).

2.2. Analytical column

An Atlantis dC18 column (4.6 × 250 mm, 5 μm) that supplied by Waters Corporation (Milford, MA, USA) was used for chromatographic separations.

2.3. Materials

Seven nuts and seeds (hazelnut, pistachio nut, walnut, peanut, sunflower seed, pumpkin seed, almond) were obtained from local markets in Ankara. After roasting, oil was extracted from the samples. Extracted oils were analysed for their HMF and F contents.

2.4. Roasting of nuts and seeds

The seed and nut samples were roasted in a temperature controlled oven (Memmert, Germany) at 180 °C for 30 min prior to oil extraction. In another set of experiment, hazelnut samples were roasted at 180 °C for 5, 10, 15, 20 and 30 min to determine the effect of roasting time on the amounts of furan compounds present in resulting oils.

2.5. Extraction of oil from roasted samples

Roasted samples were crushed by using a domestic coffee grinder prior to oil extraction. A 10 g of ground sample was mixed with 100 ml of *n*-hexane and further crushed by using an ultra-turrax homogenizer for 2 min. The suspension was filtered through double layer filter paper (Whatman No. 1) and the extraction procedure was repeated thrice. Filtrates were combined and evaporated *in vacuum* at 40 °C under nitrogen atmosphere. Resulting oils were used to analyse furan compounds by HPLC and UV–Vis spectrometry. Different solvents such as *n*-hexane, diethyl ether and ethyl acetate were used to determine the effect of solvent polarity on the extraction of HMF and F to resulting oils from roasted samples.

2.6. Analysis of HMF and F in oil

Liquid–liquid extraction procedure was applied to determine HMF and F concentrations of oil samples. A mixture of methanol and water was used to extract furan compounds from oil samples. Different solvent compositions (50% and 70% methanol in water, or 100% methanol) were tested as the extraction solvent in terms of extraction yield and presence of interferences. The use of 70% methanol in water was found successful for the extraction of HMF and F prior to HPLC analysis. A 0.5 g portion of oil sample was transferred to a test tube for analysis. It was extracted with 1 ml of 70% methanol in a vortex mixer for 1 min. The mixture was centrifuged at 10,000*g* for 5 min. After centrifugation, upper phase was separated from the oil. The extraction was repeated three times under the same conditions. The combined upper phases were diluted to 5 ml by 70% methanol and filtered through 0.45 μm nylon Acrodisc syringe filters (Waters Corporation, Milford, MA, USA).

A 10 μl portion of the final extract was injected onto an Atlantis dC18 column for HPLC analysis. An Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a quaternary pump, an autosampler, a diode array detector and a temperature-controlled column oven was used. An isocratic mixture of 10 mM aqueous formic acid solution and acetonitrile (90:10, v/v) was used as the mobile phase at a flow rate of 1 ml/min at 25 °C. Data acquisition was performed acquiring chromatograms at a detection wavelength of 285 nm.

The quantitations of HMF and F were performed using calibration curves of each compound. Stock solutions of HMF and F were prepared at a concentration of 1 mg/ml in methanol. Working standards were prepared by diluting the stock solutions to concentrations of 0.10, 0.25, 0.50, 1.0 and 5.0 μg/ml with mobile phase. Each sample was analysed in triplicate and the mean values were reported.

2.7. Recovery of HMF and F

Stripped hazelnut oil (SHO) was used to determine the recovery of HMF and F from oil samples. Oil samples were spiked with HMF and F at final concentrations of 1, 2, 5, 10 and 20 mg/kg. A stock solution of HMF was prepared in ethyl acetate to a concentration of 0.1 mg/ml. Different volumes of this stock solution were added to oil to obtain certain concentrations of HMF. Ethyl acetate was removed under a gentle stream of nitrogen at room temperature. Due to its volatility, appropriate amounts of F were directly added to the oil in order to prevent any evaporative lose during nitrogen flushing treatment. The SHO samples spiked with different concentrations of HMF and F were extracted with 70% methanol in water as described above.

2.8. Oil stripping

The hazelnut oil was stripped using a procedure described elsewhere (Karabulut, Topcu, Basar, Onal, & Lampi, 2008). A 100 g of crude hazelnut oil was mixed with 10 g of activated carbon under nitrogen overnight with stirring. Excess of activated carbon was separated by centrifugation at 8000*g* for 10 min. The upper oil phase was taken, diluted twofold with *n*-hexane and subjected to alumina column chromatography. Alumina was activated by heating subsequently at 100 °C for 16 h and at 220 °C for 6 h. A 150 g of cooled activated alumina was slurried with 250 ml *n*-hexane and a glass column (300 × 28 mm i.d.) was filled with this slurry. Approximately 150 ml of pure *n*-hexane passed from the column to ensure proper packaging. Diluted oil was loaded to the column followed by washing with 150 ml of *n*-hexane. Solvent in collected micelle was removed *in vacuum* at 40 °C (Bibby RE 100, UK) under a stream of nitrogen to obtain SHO.

2.9. UV–Vis spectra of oils

Spectral characteristics of oils obtained from hazelnuts roasted under different conditions were determined. UV–Vis spectra of 70% methanol extracts of oils were recorded in a wavelength range of 250–400 nm using a double-beam spectrophotometer (Shimadzu 2010, Japan).

2.10. Statistical analysis

All data were subjected to analysis of variance (ANOVA). The SPSS statistical package was used for the evaluation of statistical significance of the differences between mean values three replicates by Duncan test.

3. Results and discussion

3.1. Analytical method's performance

Mixture of methanol and water was considered to be suitable solvent for the extraction of polar compounds from oils. Fig. 1 de-