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Ultrasound-assisted extraction of resveratrol from functional foods: Cookies and jams

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ABSTRACT

Rapid extraction techniques with ultrasonic systems have been established to extract resveratrol from two functional foods, namely cookies and jams. The ultrasound-assisted extraction (UAE) was optimized using a full factorial design with three variables: solvent composition (10–70% and 30–90% methanol in water for cookies and jams, respectively), solvent-to-solid ratio (10:1–40:1), and ultrasonic probe diameter (2 and 7 mm). The extraction kinetics (5–30 min) were studied to confirm the full recovery of resveratrol from the functional foods. The resveratrol was quantified by Ultrahigh Performance Liquid Chromatography with a Fluorescense Detector (UPLC-FD).

The solvent composition was found to have a significant effect on the recovery ($p < 0.005$) for both of the functional foods studied. The optimal UAE conditions were 90% methanol in water, an extraction time of 10 min, an ultrasonic probe with a diameter of 7 mm, and solvent-to-solid ratios of 40:1 and 10:1, respectively, for cookies and jams.

The methods developed in this study show a high precision in terms of Coefficient of Variance (CV), with values of less than 5% for both repeatability and intermediate precision. The applicability of the methods to real samples has been evaluated and it was confirmed that the methods are suitable for the extraction of resveratrol from cookies and jams.

1. Introduction

Trans-resveratrol is a phenolic phytoalexin (trans-3,5,4′-trihydroxystilbene) found in regular foods, including grapes, red wine, peanuts, and mulberry [1]. This compound is synthesized by plants as a means of protection against pathogenic attack, environmental stress, UV irradiation, fungal infections [2], the effects of ozone, heavy metal ions, and changes in climate (extreme cold) [3]. This active compound has important properties such as antioxidant, anti-inflammatory, analgesic, cardio- and neuro-protective, anti-aging, antitumor, and anticancer effects [4]. In research on neurodegeneration, resveratrol has proven to be beneficial against Alzheimer Disease through in vitro and in vivo models and, as a consequence, the regular intake of resveratrol is recommended [5]. The results of some studies suggest that resveratrol-rich supplements are effective in slowing progressive dementia [6]. In recent years, resveratrol has become a dietary supplement in several countries [3] with a dose between 1 and 8 mg by tablet. However, the resveratrol present in some functional foods would be a more convenient source for regular consumers and functional foods that contain resveratrol are of increasing interest in the market [7]. Resveratrol is highly sensitive to light and to high temperatures and its stability during functional food preparation is a prerequisite when developing new functional foods. In many cases baking steps and/or pasteurization are required during food processing and the availability of suitable analytical methods for these kinds of samples is necessary in functional food design.

Extraction is the first step in the isolation and determination of a compound in a solid or semisolid matrix. Several new and innovative techniques [8] have been used as extraction methods and these include ultrasound-assisted extraction, enzymatic extraction, supercritical fluid extraction, and microwave-assisted extraction. These methods are considered to be viable alternatives to traditional extractions [9] and they simplify the extraction process and reduce extraction times and solvent volumes [10].

Some phenolic compounds are photosensitive and thermo-labile. As a result, the extraction of such materials at high

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temperature for prolonged periods might lead to the degradation of the compounds [11]. Ultrasound-assisted extraction is an increasingly attractive approach due its inherent advantages; namely, high efficiency and reproducibility, ease of operation, and relatively short extraction time. The application of UAE therefore minimizes the degradation of bioactive compounds [3], reduces analysis costs, requires less laboratory work, and can produce high purity compounds in the extraction yield [8].

The objective of the study reported here was to develop a UAE method for the extraction of resveratrol from some recently developed functional foods, namely cookies and jams, produced from byproducts of the wine industry.

2. Materials and methods

2.1. Materials and chemicals

HPLC-grade methanol, acetic acid, and acetonitrile were purchased from Merck (Darmstadt, Germany). Trans-resveratrol standard was obtained from Sigma–Aldrich (St. Louis, MO, USA). Water was purified with a Milli-Q purification system (Millipore, Billerica, MA, USA). Commercial resveratrol Anti-Ox (nutritional supplement) (Acoforma, Terrassa, Spain) was purchased from a pharmacy in Spain.

2.2. Functional food preparation

The functional foods described in this work were previously designed by the Andalusian Center of Wine Research (CAIV). The foods were fortified with commercial resveratrol capsules, which were ground in a mortar and homogenized prior to use.

The following ingredients were used for the preparation of cookies: ground almonds, flour, oats, baking powder, salt, grape seed, oil, and honey, which were mixed for approximately 20 min in a mixer (KitchenAid® Artisan®, Canada). A circular mold was used to make regular cookies (approximately 7.9 g/cookie). The cookies were baked at 170 °C for 10 min in an electrical oven (Delonghi model EO 2131, Canada).

The jam was prepared using milled grapes, sugar, and pectin in a Thermomix (VORWEK) with the ingredients mixed for 6 min and then semi-pasteurized at 70 °C for 35 min in the same equipment.

In the two food matrices considered (cookies and jams), around 210 mg of commercial resveratrol was added as an ingredient per batch of food. As a consequence, resveratrol was exposed to semi-pasteurization during jam preparation and to a baking process during cookie preparation. Additionally, the same amount of resveratrol was added to the finished food samples prepared without resveratrol in order to generate reference samples containing resveratrol that had not been subjected to the processing steps. Each food sample (cookies and jams) was placed in a plastic cylinder and milled with an Ultraturrax homogenizer (IKA® T25 Digital, Germany) for 10 min (cookies) or 5 min (jams) prior to the addition of resveratrol. The fortified foods were then homogenized by stirring and were stored in a closed container.

2.3. Extraction of resveratrol

UAE was carried out using a UP200S ultrasonic system (Hielscher Ultrasonics GmbH, Teltow, Germany). This compact ultrasonic system is designed to be mounted on a stand and was equipped with a water bath coupled to a temperature controller (Frigiterm, J.P. Selecta, Barcelona, Spain) to maintain the desired extraction temperature in the range from −10 °C to 100 °C. Food samples (cookies and jams) (1 g) were accurately weighed and then placed in an extraction tube. Based on the experimental design, a set volume and type of solvent was added to the extraction vessel and the extraction was performed under controlled UAE conditions. The extraction was carried out for 10 min and the extract was then centrifuged for 10 min at 0 °C and 8000 rpm in a Microfriger-BL (10 × 10 mL, J.P. Selecta, SA mark) centrifuge. The supernatant was removed and adjusted to the appropriate volume based on the design of experiment. The extract was filtered through a cellulose filter (0.22 μm, Millipore) prior to injection into the UPLC-FD system.

2.4. Determination of resveratrol

Ultrahigh Performance Liquid Chromatography (UPLC) analyses were carried out on an ACQUITY UPLC® H-Class system coupled with a Fluorescence Detector (FD) and controlled by Empower™ 3 Chromatography Data Software (Waters Corporation, Milford, MA, USA). A reverse phase C18 column (ACQUITY UPLC®BEH C18 1.7 μm, 2.1 × 100 mm) from Waters (Ireland) was used and the temperature was set at 47 °C.

A gradient elution program was used with two phases: A (0.01% acetic acid in water) and B (2% acetic acid in acetonitrile). The applied gradient was as follows (time, B%): 0–1 min, 0%; 1–1.1 min, 0–10%; 1.1–2 min, 10%; 2–3 min, 10–20%; 3–3.5 min, 20–60%; 3.5–4 min, 60–100%. The column was subsequently washed with 100% B for 3 min and equilibrated with 0% B for 3 min. The flow rate was set at 0.6 mL/min. The excitation wavelength was set at 310 nm and the emission wavelength was set at 403 nm. The injection volume was set to 3 μL.

The chromatographic method used to determine resveratrol was performed according to the suggestions of ISO 17025 and the recommendations in the ICH Guideline Q2 (R1). The range, linearity, recovery, precision, detection, and quantification limits of the method were evaluated.

Linearity was estimated in order to express how the method provides linear responses from the analytical signal to the resveratrol concentration within the studied range. A series of dilutions from a stock solution of resveratrol were carried out to give concentrations ranging from 10 to 100 $\mu g\,L^{-1}$ and 100 to 700 $\mu g\,L^{-1}$. Gnumeric 1.12.17 was used to generate the regression analysis of the calibration curves. The standard deviation estimated for the response and the slope from the regression were then used to calculate the limit of detection (LOD) and limit of quantification (LOQ). The analytical properties for the chromatographic method for the determination of resveratrol are listed in Table 1.

The precision of the extraction method was evaluated by studying the repeatability (intra-day) and intermediate precision (extra-day). Repeatability was assessed by nine independent extractions of the same samples on the same day, while intermediate precision was determined by three independent extractions on three consecutive days. Precision was expressed as the coefficient of variance (CV) of the peak height. The acceptable CV limit is ±10% according to the AOAC manual for the Peer-Verified Methods program. The CV values with reference to the peak height for both repeatability and intermediate precision were less than 10%, thus showing that the method has a satisfactory precision.

Table 1
The analytical characteristic for determination of resveratrol.

Linear range ($\mu g\,L^{-1}$)	Linear equation	R^2	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
10–100	$y = 181.82x + 155$	0.998	12	37
100–700	$y = 288.67x - 13236$	0.997		