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Analytical Methods

Development and validation of an HPLC method for the determination of alk(en)ylresorcinols using rapid ultrasound-assisted extraction of mango peels and rye grains

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ABSTRACT

Exhaustive extraction of alk(en)ylresorcinols (ARs) from biological matrices is a prerequisite for economic screening of extensive plant collections including their rapid quantitation. For this purpose, an ultrasound-assisted extraction protocol was developed to facilitate the liberation of ARs from mango peels (*Mangifera indica* L.) and rye grains (*Secale cereale* L.). While maintaining or even improving the extraction efficiency of the analytes, the duration of analytical extraction was shortened from more than 1 h to only 45 s as compared to previous methods. In addition, sample weight and solvent use were significantly reduced. Besides the validation of the extraction procedure, validation parameters for the HPLC–DAD–MS^n based characterisation and quantitation method are provided. In particular, fully satisfactory recovery rates and quantitation limits were achieved, and coefficients of variation (CV) for repeatability and reproducibility were $\leqslant$8 and <5%, respectively. Moreover, a high compound stability was shown for all ARs during storage in methanolic solution at room temperature for 48 h and at −80 °C for up to 13 months. The developed method was exemplified using two mango and three rye cultivars, and the results were compared to previously published data.

1. Introduction

Alk(en)ylresorcinols (ARs) represent a class of amphiphilic phenolic lipids (Fig. 1), which were found in cereals like rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), spelt (*Triticum spelta* L.), and barley (*Hordeum vulgare* L.) as well as in mango (*Mangifera indica* L.) peels and pulp, peas (*Pisum sativum* L.), and ginkgo (*Ginkgo biloba* L.) (Kienzle, Carle, Sruamsiri, Tosta, & Neidhardt, 2014; Knödler, Kaiser, Carle, & Schieber, 2008; Kozubek & Tyman, 1999; Ross, 2012a; Żarnowski & Kozubek, 1999). While several ARs were suspected to induce allergenic or sensitising reactions of mango peels upon human skin contact (Weinstein, Bassiri-Tehrani, & Cohen, 2004), health beneficial properties such as anti-microbial, anti-inflammatory, and anti-carcinogenic bioactivities were also described recently (Knödler, Conrad, et al., 2008; Kozubek & Tyman, 1999). For instance, ARs from wheat bran may contribute to colon cancer prevention, as indicated by *in vitro* and *in vivo* animal studies, respectively (Ross, 2012b). Besides their nutritional importance, AR analyses were used for the authenticity control of wheat products and as biomarkers of wheat and rye intake (Knödler, Most, Schieber, & Carle, 2010; Ross, 2012b). In contrast to cereals, by-products from industrial mango processing, i.e. peels and kernels, are a non-dietary source of ARs being so far under-utilised, although the extraction of these and other valuable phenolic compounds was proposed as plant-derived feed supplements and, moreover, for pharmacological applications (Geerkens et al., 2013). Reasonably, the AR content in mango peels is significantly higher than in the mesocarp, since their biological function in mango peels is believed to be associated with the protection against phytopathogens (Kienzle et al., 2014). High levels of the ARs 5-pentadecylresorcinol (C15:0) and 5-heptadecenylresorcinol (C17:1) were related with an increased resistance against black spot disease and anthracnose caused by *Alternaria alternata* and *Colletotrichum gloeosporioides*, respectively (Cojocaru et al., 1986; Hassan, Dann, Irving, & Coates, 2007).

Due to the above mentioned importance of ARs, both efficient extraction techniques and quantitative analytical methods are of

Abbreviations: ARs, alk(en)ylresorcinols; C15:0, 5-pentadecylresorcinol; C17:0, 5-heptadecylresorcinol; C17:1, 5-heptadecenylresorcinol; C17:2, 5-heptadecadienylresorcinol; C17:3, 5-heptadecatrienylresorcinol C19:0, 5-nonadecylresorcinol; C19:3, 5-nonadecatrienylresorcinol; C21:0, 5-heneicosylresorcinol; C23:0, 5-tricosylresorcinol; C25:0, 5-pentacosylresorcinol; CV, coefficient of variation; DL, detection limit; QL, quantitation limit; S/N, signal to noise; SPE, solid phase extraction.

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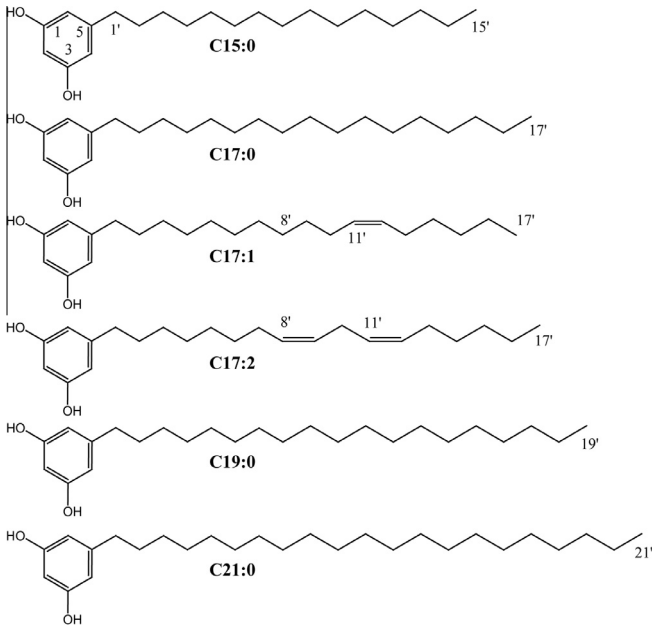

Fig. 1. Chemical structures of the major 5-n-alk(en)ylresorcinols present in *Mangifera indica* L. (C15:0, C17:1, C17:2) and *Secale cereale* L. (C17:0, C19:0, C21:0) as shown in our study and previous reports (Knödler, Conrad, et al., 2008; Knödler, Kaiser, et al., 2008). **C15:0** 5-pentadecylresorcinol, **C17:0** 5-heptadecylresorcinol, **C17:1** 5-(11′*Z*-heptadecenyl)resorcinol, **C17:2** 5-(8′*Z*, 11′*Z*-heptadecadienyl)resorcinol, **C19:0** 5-nonadecylresorcinol, **C21:0** 5-heneicosylresorcinol.

utmost interest. Therefore, our main objective was the development of a rapid, quantitative, and time- and solvent-saving extraction method. As previously described by Mason (1996) and Vinatoru (2001), ultrasound enhances the penetration depth of solvents into biological substrates and, thus, intensified mass transfer of soluble constituents into the solvents. Furthermore, cavitation phenomena lead to an extensive matrix disruption, thus facilitating the release of analytes such as proteins, lipids, and bioactive compounds like polyphenols and β-carotene into the extraction solvent (Mason & Lorimer, 2003; Toma, Vinatoru, Paniwnyk, & Mason, 2001; Vilkhu, Mawson, Simons, & Bates, 2008). Consequently, increased yields of several bioactives from vegetal matrices have been reported after ultrasonically assisted extraction as compared to conventional extraction procedures (Vinatoru, 2001). Besides validating our developed ultrasound-assisted extraction procedure, we sought to simultaneously verify the applicability and efficiency of our HPLC-DAD method for the post-extractive quantitation of individual ARs in the extracts of two different matrices, including the most common validation parameters as well as information about compound stability during short- and long-term storage at different temperatures.

2. Materials and methods

2.1. Raw material and chemicals

Mangoes (*M. indica* L.) of the cultivar Tommy Atkins originating from Brazil were purchased from a local market in Stuttgart, Germany. Fruits of cv. Kaew were obtained from a research orchard in Chiang Mai (Thailand). Rye grains (*S. cereale* L.) of the hybrid cultivars Bellami and SU Allawi, and the population cultivar Conduct were donated by the State Seed Breeding Institute at Hohenheim University (Stuttgart, Germany). The authentic standards 5-pentadecylresorcinol (C15:0), 5-heptadecylresorcinol (C17:0), and 5-nonadecylresorcinol (C19:0) were provided by Sigma–Aldrich (Steinheim, Germany), while 5-heneicosylresorcinol (C21:0), 5-tricosylresorcinol (C23:0), and 5-pentacosylresorcinol (C25:0) were from ReseaChem (Burgdorf, Switzerland). Dichloromethane was purchased from Merck (Darmstadt, Germany), while methanol and ethyl acetate were obtained from VWR (Leuven, Belgium). Deionized water was used throughout.

2.2. Preparation of dried mango peels and rye grain

Mangoes cv. Tommy Atkins were manually peeled. Subsequently, the peels were ground with liquid nitrogen and lyophilized. Peels of cv. Kaew were dried in a fluidized-bed dryer as described previously (Geerkens et al., 2013). After harvesting and threshing, rye grains were dried by circulating air at 40 °C to a final moisture content of 13–15%. Dried mango peels and integral rye grains were milled with a ZM 1 grinder (Retsch, Haan, Germany) obtaining a particle size of ⩽0.25 mm. The powdered peels were stored in a desiccator at room temperature, whereas milled rye grains were stored in a refrigerator at 7.5 °C prior to analyses.

2.3. Alk(en)ylresorcinol extraction

2.3.1. Solvent extraction under continuous stirring

Extraction of ARs under continuous stirring was conducted as described previously (Knödler, Berardini, Kammerer, Carle, & Schieber, 2007) with minor modifications. Briefly, 2.5 g of powdered mango peel were extracted with 50 mL dichloromethane for 1 h at room temperature under nitrogen atmosphere. After filtration, the solid residue was re-extracted with 50 mL dichloromethane for 10 min, and filtered again. The combined filtrates were evaporated *in vacuo* at 30 °C until dryness. Prior to HPLC analysis, the extract was dissolved in 1 mL methanol, filtered using a syringe filter (pore size 0.45 μm), and stored at -80 °C.

For evaluating the effect of purification by solid phase extraction (SPE), the above mentioned dried extract was dissolved in 10 mL dichloromethane and subjected to SPE using a polyamide sorbent as described by Knödler et al. (2007). Briefly, the re-dissolved extract was subjected to a column containing 2 g polyamide CC6 (Macherey–Nagel, Düren, Germany), which sequentially was conditioned with 20 mL methanol and 25 mL dichloromethane. After sample application, impurities were removed by washing the column with 25 mL dichloromethane, and elution of ARs was accomplished using 50 mL methanol. The methanolic eluate was evaporated and prepared for HPLC analysis as described above.

2.3.2. Ultrasound-assisted extraction

Ultrasound-assisted AR extraction was conducted using an ultrasonic homogenizer Sonopuls HD 3100 (Bandelin electronic, Berlin, Germany), consisting of a GM 3100 ultrasonic generator and UW 3100 transducer fitted with a VS 70 T 494 sonotrode (Bandelin electronic, Berlin, Germany). An aliquot of 1.0 g powdered mango peel and 20 mL dichloromethane were sonicated for 15 s at 50% amplitude. During ultrasonication, samples were cooled in an ice bath to avoid sample heating. The obtained suspension was filtered through a MN 615 filter (Macherey–Nagel, Düren, Germany). The separated residue was re-extracted two times, and the obtained filtrates were combined. After solvent evaporation *in vacuo* at 30 °C, the dry extract was dissolved in 1 mL methanol and filtered (0.45 μm) prior to HPLC analysis. The same extraction procedure was used for AR extraction from rye grain, except for the use of ethyl acetate instead of dichloromethane.

2.4. HPLC–DAD–MS^n analyses

The separation of ARs was performed using a series 1100 HPLC (Agilent, Waldbronn, Germany) equipped with a G1315B diode array detector. Operated at 25 °C, the column used was a Synergi