Food Research International 99 (2017) 821–827

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Growth suppression of *Fusarium culmorum*, *Fusarium poae* and *Fusarium graminearum* by 5-*n*-alk(en)ylresorcinols from wheat and rye bran

Hannes Patzke, Sabrina Zimdars, Nadine Schulze-Kaysers*, Andreas Schieber

University of Bonn, Institute of Nutritional and Food Sciences, Molecular Food Technology, Römerstraße 164, D-53117 Bonn, Germany

ARTICLE INFO

Keywords:
Alk(en)ylresorcinols
Wheat
Rye
Bran
Fusarium spp.
Antifungal activity

ABSTRACT

Alk(en)ylresorcinols (AR), a class of phenolic lipids, are regarded as antifungal compounds showing high potential for the use in plant protection, especially against *Fusarium* head blight (FHB). In view of the very limited knowledge of the activity of single AR against *Fusarium* species, the antifungal effect of crude extracts, fractions and isolated homologues from wheat and rye bran was determined. It was shown that the saturated AR are the active compounds in the extracts, whereas the presence of unsaturated molecules leads to an antagonistic effect. The activity of single saturated AR is dependent on the chain length, but for highest antifungal efficiency a mixture of saturated homologues is required. Affecting the stage of germination, these molecules reduce, and may even completely prevent, the growth of the tested *Fusarium* species.

1. Introduction

5-*n*-Alk(en)ylresorcinols (1,3-dihydroxy-5-alk(en)ylbenzenes, AR) belong to a group of phenolic lipids which are widely distributed in nature. Besides algae, bacteria, and molds, these substances are mainly found in higher plants, especially in the families Anacardiaceae and Poaceae (Landberg, Marklund, Kamal-Eldin, & Åman, 2014). Several *in vitro* studies have demonstrated that AR are biologically active, for example, they show antioxidative activity, inhibit enzymes such as lipase and lipoxygenase, prevent LDL from copper-induced oxidation, and inhibit colon cancer cell growth and DNA-strand scission (Landberg et al., 2014). In addition, AR display antimicrobial activity (Carpinella et al., 2011; Himejima & Kubo, 1991; Jin & Zjawiony, 2006), which is particularly expressed in the suppression of the germination and the mycelial growth of fungi (Ciccoritti, Pasquini, Sgrulletta, & Nocente, 2015; Droby, Prusky, Jacoby, & Goldman, 1986, 1987; Garcia, Garcia, Heinzen, & Moyna, 1997; Hassan, Dann, Irving, & Coates, 2007; Kienzle, Carle, Sruamsiri, Tosta, & Neidhart, 2014; Reiss, 1989; Suzuki, Esumi, Hyakutake, Kono, & Sakurai, 1996; Zarnowski, Kozubek, & Pietr, 1999). Because AR are located in an intermediate layer of the caryopsis (Landberg, Kamal-Eldin, Salmenkallio-Marttila, Rouau, & Åman, 2008), it is assumed that they are produced by the epidermal cells to protect the interior tissue (Ji & Jetter, 2008). Therefore, AR are regarded as phytoanticipins and might be used in the plant protection of mango fruits against *Alternaria alternata* and of wheat against *Fusarium* head blight (FHB) (Ciccoritti et al., 2015).

Despite their amphiphilic character, AR from cereal grains with at least 13 C-atoms in the side chain are insoluble in water (Ross, Åman, Andersson, & Kamal-Eldin, 2004), which makes bioactivity tests of these molecules difficult. Usually, AR are dissolved in small volumes of organic solvents, or the solvent is evaporated prior to performing the bioactivity assays. For the determination of the antimicrobial activity of AR, several organic solvents were used and a wide range of different methodologies was applied, which makes a comparison of the results challenging. Furthermore, the application of crude extracts cannot exclude that other compounds might be responsible for, or at least contribute to, the activity (Ciccoritti et al., 2015). This is supported through the general composition of AR-rich extracts from grain. It was shown that up to 74% of total extracted weight are co-extracted substances, mainly composed of fatty acids and glycerols (Landberg, Dey, Francisco, Åman, & Kamal-Eldin, 2007). Only a few studies employed isolated or commercially available standard AR (Carpinella et al., 2011; Ciccoritti et al., 2015; Himejima & Kubo, 1991; Jin & Zjawiony, 2006; Suzuki et al., 1996). Even if unsaturated AR are supposed to show higher biological activities compared to their saturated homologues (Knödler, Kaiser, Carle, & Schieber, 2008), no evidence has so far been provided for a stronger antifungal activity. Compared to wheat, rye contains higher amounts and a more complex profile of AR, with about 20% of unsaturated homologues, which amount to only 6–7% in wheat (Ross et al., 2003). Rye is more tolerant against FHB than wheat, and bread molds seem to form smaller colonies on rye bread (Reiss, 1989), which might be an indication of the influence of the unsaturated AR against *Fusarium* species and other fungi (Bottalico, 1998). With no unsaturated AR being available as standard substances, it is necessary

* Corresponding author.
E-mail address: nschulze@uni-bonn.de (N. Schulze-Kaysers).

http://dx.doi.org/10.1016/j.foodres.2017.05.022
Received 16 March 2017; Received in revised form 19 May 2017; Accepted 24 May 2017
Available online 26 May 2017

to isolate these homologues from natural sources or to synthesize them in a complex and costly manner (Ross et al., 2004).

The objective of this work was to determine the effect of AR from wheat and rye bran on the growth of *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium poae*. In a first step, the growth inhibitory activity of the crude extracts, as well as of their fractions containing saturated and unsaturated AR, was tested. On the basis of these results, the AR composition of the most active fraction was reconstituted with highly purified individual AR homologues. In addition, single AR were included to obtain insight into a structure-depending inhibitory activity of AR against *Fusarium* species.

2. Material and methods

2.1. Sample material, chemicals, and reagents

Wheat and rye bran were provided by Kampffmeyer Mühlen GmbH (Ellmühle Cologne-Deutz, Germany). The organic solvents acetone, *n*-hexane (VWR, Darmstadt, Germany), methanol, 2-propanol (Th. Geyer, Renningen, Germany) and water were of HPLC grade. Denatured ethanol (Schmittmann GmbH, Düsseldorf, Germany) was applied in the microbial tests. The purity of the commercially available AR 1,3-dihydroxy-5-pentadecylbenzene (ReseaChem, Burgdorf, Switzerland) was > 98% (HPLC, 220 nm).

2.2. Fractionation and isolation of 5-n-alk(en)ylresorcinols

The following chapter describes briefly the separation process from the source material to the isolated AR. For detailed information, we refer the reader to a previously published work (Patzke, Schulze-Kaysers, & Schieber, 2016).

2.2.1. Extraction of alk(en)ylresorcinols from wheat and rye bran

Quantities of 100 g of wheat bran or rye bran were first defatted with 750 mL *n*-hexane for 3 h under continuous shaking. The defatted bran was separated by filtration through filter paper, dried, and extracted twice with 750 mL acetone for 2 h under sonification. *n*-Hexane and acetone extracts were evaporated to dryness at 40 °C using a Rotavapor R-210 (Büchi, Flawil, Switzerland).

2.2.2. Separation of saturated and unsaturated alk(en)ylresorcinols through crystallization

The dried acetone extracts were made up to 500 mL with methanol, chilled at − 80 °C and deep-bed filtered through diatomaceous earth. After collection of the permeate containing the unsaturated AR, the retentate containing the saturated AR was recovered by flushing the filtration apparatus with methanol at room temperature. The rye bran permeate was adjusted to a volume of 150 mL with methanol, and the filtration process was repeated. Permeate and retentate were evaporated to dryness at 40 °C in a rotary evaporator. The same filtration process was conducted at − 25 °C to separate AR fractions C23:0/C25:1 and C25:0/C27:1, which were subsequently isolated using semi-preparative HPLC (Section 2.2.3).

2.2.3. Isolation of 5-n-alk(en)ylresorcinols from permeate and retentate

AR from rye bran permeate and retentate were isolated by semi-preparative HPLC using a Smartline semi-preparative HPLC system (Knauer, Berlin, Germany) equipped with a model Manager 5050 degasser, a model 1050 pump, a model 3950 autosampler, a Knauer Eurospher II 100-5C18 (250 × 16 mm, particle size 5 µm) column with a Knauer Eurospher C18 (particle size 5 µm) guard column, a model 2550 UV detector, and a model Foxy R1 fraction collector (Teledyne, ISCO, Lincoln, NE, USA). Injection volumes varied between 500 and 1000 µL. AR were separated at a flow rate of 5 mL·min^{-1}. Chromatographic runs were monitored at a wavelength of 273 nm.

The rye bran retentate was dissolved in 6 mL 2-propanol/water (90:10, v/v), and saturated AR were separated isocratically using 2-propanol/water (90:10, v/v) with a total run time of 35 min. The rye bran permeate was dissolved in 5 mL of methanol and AR were eluted using the following gradient program: 0 min, 20% A (water), 0% B (2-propanol), 80% C (methanol); 5 min, 10% A, 0% B, 90% C; 7 min, 10% A, 0% B, 90% C; 12 min, 0% A, 0% B, 100% C; 17 min, 0% A, 0% B, 100% C; 30 min, 0% A, 100% B, 0% C, 40 min, 0% A, 100% B, 0% C. Fractions containing the isolated AR were evaporated to dryness under vacuum at 40 °C.

2.3. Ultra high-performance liquid chromatography

The AR content in the extracts, fractions and isolated AR was determined using a UHPLC method described by Ross (2012) that was slightly modified. For this purpose, a Prominence UHPLC system (Shimadzu, Kyoto, Japan) was used, equipped with a Prominence DGU-20A$_{5R}$ degasser, Nexera *X2* LC-30AD pumps, a Nexera SIL-30AC Prominence autosampler, a Nucleodur C18 Pyramid column (150 × 2.0 mm, particle size 1.8 µm) from Macherey-Nagel (Düren, Germany), a CTO-20 AC Prominence column oven, and a SPD-M20A Prominence DAD detector. The following gradient program was applied at a flow rate of 0.5 mL·min^{-1} using methanol/water (89:11, v/v) as solvent A and methanol/water (99:1, v/v) as solvent B: 0 min, 0% B; 1 min, 0% B; 2 min, 25% B; 3 min, 25% B; 8 min, 100% B; 11 min, 100% B; 13 min, 0% B; 16 min, 0% B. The column temperature was 50 °C and the injection volume was 2 µL. Quantification of the AR content in the extracts, fractions and isolated homologues was accomplished using an external standard AR C15:0 under consideration of the respective molecular weight.

2.4. Determination of the antifungal activity of AR

2.4.1. Spore suspension

Fusarium graminearum and *Fusarium poae* obtained from the collection of the Institute of Crop Science and Resource Conservation (INRES), University of Bonn, and *Fusarium culmorum* (DSM 1094) were cultivated separately in 200 mL potato dextrose broth with 20 mg of chloramphenicol in a 500 mL Erlenmeyer flask for 4 days at 25 °C under continuous shaking. Subsequently, 2 mL of the broth was distributed on half concentrated potato dextrose agar plates, dried for approximately 45 min under sterile laminar flow and further incubated for 2 days under ultraviolet light at 25 °C. Plates were covered with approximately 15 mL of sterile water and after 10 min, the spores were removed from the mycelium with a scoop. The suspension was filtered through double-layered cheese cloth. Spores were washed and separated twice by centrifugation (10 min; 150 s^{-1}), and adjusted to a final concentration of about 5·10^5 spores·mL^{-1}. Spore suspensions were stored at − 20 °C.

2.4.2. Adjustment of the concentration of AR containing solutions

n-Hexane and acetone extracts (Section 2.2.1) as well as permeate and retentate (Section 2.2.2) from wheat, as well as rye bran, were adjusted to a final AR concentration of 4 mg·mL^{-1} in ethanol. These solutions were diluted geometrically until a concentration of 0.25 mg AR·mL^{-1} was reached, resulting in 5 different dilutions each. Isolated AR from the retentate (AR C17:0, C19:0, C21:0, C23:0, C25:0) and permeate (AR C19:1, C21:1, C19:2 and C21:2) were adjusted to a concentration of 2.0 and 4.0 mg AR·mL^{-1} in ethanol. Furthermore, the 5 individual saturated AR were combined to represent the relative composition in rye bran retentate (Table 1) at a concentration of 4 mg AR·mL^{-1} in ethanol. The method applied for the determination of the antifungal activity includes the previous evaporation of the solvent (Section 2.4.3) and, therefore, the AR concentration is expressed per area of the culture medium. Thus, the highest extract concentration is 72.2 µg AR·cm^{-2}.