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Effect-directed analysis of ginger (*Zingiber officinale*) and its food products, and quantification of bioactive compounds via high-performance thin-layer chromatography and mass spectrometry

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ABSTRACT

Decision makers responsible for quality management along the food chain need to reflect on their analytical tools that should ensure quality of food and especially superfood. The "4ables" in target analysis (stable, extractable, separable, detectable) focusing on marker compounds do not cover all relevant information about the sample. On the example of ginger, a streamlined quantitative bioprofiling was developed for effect-directed analysis of 17 commercially available ginger and ginger-containing products via high-performance thin-layer chromatography (HPTLC-UV/Vis/FLD-bioassay). The samples were investigated concerning their active profile as radical scavengers, antimicrobials, estrogen-like activators and acetylcholinesterase/tyrosinase inhibitors. The [6]-gingerol and [6]-shogaol content of the different products ranged 0.2–7.4 mg/g and 0.2–3.0 mg/g, respectively. Further, multipotent compounds were discovered, characterized, and for example, assigned as [8]- and [10]-gingerol via HPTLC-ESI-HRMS. The developed bioprofiling is a step forward to new analytical methods needed to inform on the true product quality influenced by cultivation, processing, and storage.

1. Introduction

Ginger (*Zingiber officinale* Roscoe) is a tropical plant indigenous to South East Asia, though its commercial cultivation is not restricted to Asia alone. In other tropical and subtropical countries of Africa and South America, the reed-like plant does also grow. The aromatic ginger rhizomes are widely used as spice, dietary supplement and medicine. Fresh ginger owes its characteristic scent and flavor to volatile compounds of the essential oil as well as to the non-volatile oleoresin fraction, in which about 25% of the compounds are responsible for the pungent taste. These compounds are phenolics, mainly gingerols, but also shogaols in smaller quantities. Shogaols are the degradation products of corresponding gingerols formed during storage or thermal processing. (Bartley & Jacobs, 2000; Chrubasik, Pittler, & Roufogalis, 2005; Semwal, Semwal, Combrinck, & Viljoen, 2015; Ternes et al., 2007).

The most abundant and the most pungent gingerol in ginger is [6]-gingerol, whereas gingerols with other chain lengths like [8]- or [10]-gingerol are found at lower contents. Its even more pungent dehydrated form, [6]-shogaol, is mainly responsible for an increased pungency after storage and in dried products. (Jolad et al., 2004; Ternes et al., 2007) Many beneficial properties were attributed to ginger like *e.g.* antioxidative (Murthy, Gautam, & Pura Naik, 2015; Nile & Park, 2015), anti-inflammatory (Jolad, Lantz, Chen, Bates, & Timmermann, 2005; Jolad et al., 2004; Nile & Park, 2015), cancer preventive (Bode, Ma, Surh, & Dong, 2001), antimicrobial (Gull et al., 2012; Murthy et al., 2015), antifungal (Ficker et al., 2003; Murthy et al., 2015), and acetylcholinesterase inhibitory (Oboh, Ademiluyi, & Akinyemi, 2012) to name a few. Also, its pharmacological properties are diverse. For example, ginger has been described to have positive effects on blood pressure, blood clotting, inflammation, and the gastrointestinal tract as well as antioxidant effects in *vitro* and in *vivo* (Ali, Blunden, Tanira, & Nemmar, 2008).

Chromatographic methods like high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC) or gas chromatography were used for analyses of ginger (Bartley & Jacobs, 2000; Jolad et al., 2004; Nile & Park, 2015). Depending on the chromatographic method, detection was mostly performed using UV/Vis detectors, diode array detectors, mass spectrometers or UV/Vis/FLD densitometers. Not only chemical marker compounds were analyzed by such chromatographic systems, but also the activity of ginger extracts was studied. For example, aqueous and methanolic extracts of ginger, as well as [6]-gingerol, [6]-shogaol, and [6]-paradol were investigated concerning their radical scavenging

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potential via 2,2-diphenyl-1-picrylhydrazyl radical (DPPH˙) or OH-radical, their ferric reducing antioxidant power, their xanthine oxidase, ß-glucosidase, hyaluronidase and lipoxidase inhibition as well as their anti-inflammatory activity (Nile & Park, 2015). Further, the acetylcholinesterase (AChE) inhibition of aqueous red *versus* white ginger extracts was studied and showed that AChE inhibition of white ginger was higher (Oboh et al., 2012). *In vitro* [6]-gingerol has been described to show butyrylcholinesterase inhibition activity, rather than to inhibit acetylcholinesterase at concentrations of 1 mM (Ghayur et al., 2008). *In vivo* however, it was shown that [6]-gingerol was able to decrease the whole brain acetylcholinesterase activity in mice (Joshi & Parle, 2006). The strong antioxidant activity of methanolic high-pressure carbon dioxide extracts of ginger was demonstrated using DPPH˙ and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) assays (Mošovská, Nováková, & Kaliňák, 2015; Somman & Siwarungson, 2015; Stoilova, Krastanov, Stoyanova, Denev, & Gargova, 2007). The inhibition of tyrosinase was higher in fresh than in processed ginger (Somman & Siwarungson, 2015). The antimicrobial activity of ginger was determined against various pathogenic microorganisms, *e.g.*, *Escherichia choli*, *Staphylococcus aureus*, *Lysteria monocytogenes*, *Bacillus cereus* and *Bacillus subtilis* bacteria (Gull et al., 2012; Murthy et al., 2015). Huang, Chiu, and Chang (2014) showed that [6]-gingerol was capable of suppressing the murine tyrosinase activity and decreasing the melanin content in a dose-dependent manner.

Current target analyses or sum parameter assays do not sufficiently cover the product quality in a satisfying way along the whole food chain. In contrast to the given methods, the objective of this study was to develop a fast quantitative method for bioprofiling of ginger and ginger-containing food products focused on their single bioactive components. Bioactivity patterns inform more comprehensively on product quality, as all compounds contributing to the effect attract attention, and not only the targeted. Such an effect-directed analysis (EDA) was performed via HPTLC separation combined with bioassays, *e.g.*, *Aliivibrio fischeri*, *B. subtilis* and planar yeast estrogen screen (pYES) bioassays, or with enzymatic assays, *e.g.*, AChE and tyrosinase assays, or with chemical effect-directed assays, such as the DPPH˙ assay. The powerful side by side comparability of the resulting effect-directed chromatograms allowed the streamlined identification of multipotent compounds, which can be characterized further by electrospray ionization high-resolution mass spectrometry (HPTLC-ESI-HRMS) via direct elution of zones of interest into the HRMS. For various effect-directed assays, LOD and LOQ values for [6]-gingerol and [6]-shogaol were determined via HPTLC-EDA, which to the best of our knowledge has not been performed so far.

2. Material and methods

2.1. Chemicals

[6]-gingerol (≥95%) and [6]-shogaol (≥90%) were obtained from PhytoLab (Vestenbergsgreuth, Germany). Bi-distilled water was generated using a Heraeus Destamat Bi-18E (Thermo Fisher Scientific, Schwerte, Germany). Ethyl acetate (≥99.7%) and *t*-butyl methyl ether (≥99.8%) were obtained from Th. Geyer, Renningen, Germany, and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH˙, 97%) and primuline (50%) from Sigma-Aldrich, Steinheim, Germany. Formic acid (≥98%), *n*-hexane (≥98%), ethanol (≥99.9%), petroleum ether (40–60 °C, ROTISOLV), 4-methoxybenzaldehyde (anisaldehyde, ≥97.5%) and sulfuric acid (96%) were purchased from Carl Roth, Karlsruhe, Germany. Diethyl ether (> 99%, stabilized with BHT) was obtained from ACROS Organics, Geel, Belgium. Methanol, HPLC (> 99.8%) and MS grade (> 99.9%), were purchased from VWR (Darmstadt, Germany). HPTLC plates silica gel 60 F_{254} (for quantitation and most assays), silica gel 60 (for *B. subtilis* bioassay) and silica gel 60 RP-18 WF_{254}s (for pYES bioassay) were from Merck, Darmstadt, Germany (all 20 cm × 10 cm). Plates were cut using the smartCUT Plate Cutter (CAMAG, Muttenz, Switzerland). For bioassay materials see respective sections.

2.2. Stock solutions and standard mixture solution

The ethanolic stock solutions of [6]-gingerol (5.0 mg/mL) and [6]-shogaol (11.6 mg/mL) were diluted with methanol 1:10 for [6]-gingerol and 1:20 for [6]-shogaol, resulting in concentration of 0.50 µg/µL and 0.58 µg/µL, respectively. For the standard mixture, 100 µL [6]-gingerol and 50 µL [6]-shogaol standard solution were pipetted together into a vial and filled up to 1.0 mL with methanol, resulting in concentrations of 50 ng/µL and 29 ng/µL for [6]-gingerol and [6]-shogaol, respectively.

2.3. Origin of samples and sample preparation

2.3.1. Extracts of fresh ginger

Fresh ginger rhizomes were purchased in a local store in Giessen in February and November 2016 (Table 2). For extraction, rhizomes with and without peel were cut into small pieces (2 × 2 mm) and ground in a mortar for about 2 min until a paste was produced. For each sample (I) with and (II) without peel, three different extracts with (1) ethyl acetate, (2) water – methanol (1:1, *V/V*) and (3) petroleum ether – *t*-butyl methyl ether (1:1, *V/V*) were prepared. Briefly, 10 mL extraction solvent were added to 0.5 g sample in a 15 mL falcon tube and mixed by vortexing and vigorous shaking. After centrifugation for 5 min at 3000 × *g*, 1 mL of the supernatant was transferred into a 2-mL sampler vial.

2.3.2. Extracts of dried ginger powder and tea bags

The content of tea bags was ground in a mortar and an aliquot of 0.1 g was extracted as described (Section 2.3.1.). Ginger powder (0.1 g) was extracted as described, but additionally with ethanol, methanol and combinations of both solvents with water (1:1, *V/V*), resulting in six different extracts.

2.3.3. Extracts of ginger ale and herb tea beverage

Two ginger ales and the herb tea beverage type "*lemon grass ginger*" (10 mL each) were extracted with 2 mL *n*-hexane. After centrifugation for 5 min at 3000 × *g*, about 1.5 mL of the upper organic phase were transferred into a 2-mL sampler vial.

All extracts were stored at −18 °C in the dark.

2.4. Sample application and development

Solutions were sprayed as 6-mm bands on HPTLC plates with the Automatic TLC Sampler 4 (ATS 4, CAMAG). Up to 24 tracks were applied with a distance from the lower edge of 8 mm, distance from the left edge of 15 mm and automatic distance between bands of 7.3 or 10 or 12 mm. For a four-point calibration, standard mixture volumes ranged from 1.0 to 10.0 µL/band (50 to 500 ng/band) for [6]-gingerol and 2.5 to 10.0 µL/band (72 to 289 ng/band) for [6]-shogaol. Sample volumes ranged from 3.0 to 40.0 µL/band. For the DPPH˙ assay, bioassays, and mass spectrometric experiments the application volume for all samples was 15.0 µL/band. For limits of detection (LOD) and quantification (LOQ), the standard mix applied in eight different volumes ranged between 0.3 and 2.0 µL/band. LODs of EDA were determined visually. Application volumes ranged from 0.3 to 1.5 µL/band for DPPH˙, 2.0 to 5.5 µL/band for AChE, 8.0 to 26.0 µL/band for tyrosinase, 0.4 to 4.0 µL for *A. fischeri* and 12.0 to 33.0 µL/band for *B. subtilis*.

Development was performed in a twin-through chamber with a mixture containing *n*-hexane and ethyl acetate (Ficker et al., 2003; Goel, Ahmad, Singh, Goel, & Singh, 2008) that was adjusted in the solvent ratio to be 13:7 (*V/V*). The migration time was 20 min up to migration distance of 70 mm from the lower edge of the plate. The chromatogram was dried in a stream of warm air for 2 min (hair dryer).