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Fast determination of intact glucosinolates in broccoli leaf by pressurized liquid extraction and ultra high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

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ABSTRACT

In this study, we investigate for the first time the efficiency of an environmentally sustainable extraction technique (pressurized liquid extraction, PLE) in conjunction with a fast separation technique (ultra-high performance liquid chromatography, UHPLC) coupled to a selective mass spectrometry (MS) detector (quadrupole time-of-flight, qTOF) to extract, separate and quantify fifteen intact-glucosinolates (GLSs) in broccoli leaves. Firstly, we have developed and optimized by means of an experimental design an efficient extraction procedure based on PLE (using ethanol/water as a solvent), giving complete extraction within 15 min; meanwhile, the average analyte recoveries were between 85% and 96% in all cases. Chromatography was performed on a UHPLC BEH Shield RP18 1.7 μm 110 Å (2.1 × 100 mm) analytical column with a mobile phase composed by formic acid in water (0.5%, v/v) and formic acid in acetonitrile (0.5%, v/v) in gradient elution mode at 0.3 mL/min, resulted in baseline-separated peaks and a run time of 13 min. The method was fully validated in terms of selectivity, limits of detection (LOD) and quantification (LOQ), linearity, precision, and trueness; meanwhile a study of the matrix effect was also performed. A good selectivity, low LODs and LOQs, ranging from 2 to 26 μg/g, wide linear ranges from LOQ to 2500 μg/g, and satisfactory precision and trueness with relative standard deviation and relative error values lower than or equal to 9%, were obtained for the studied GLSs. Finally, the proposed method was successfully applied to the analysis of intact-GLSs in fifteen broccoli leaf samples from three different cultivars (*Parthenon*, *Nubia*, and *Naxos*). Nine intact-GLSs were detected in all the varieties, although in different concentrations, which ranged between 14 and 1136 μg/g, depending on the broccoli cultivar. In addition, the highest total content of GLSs was found in broccoli leaf samples from *Parthenon* cultivar, being the *Naxos* cultivar the poorest in GLS content. This study demonstrates the efficiency of PLE as an environmentally sustainable alternative to extract intact-GLS from broccoli leaves, and that UHPLC–qTOF-MS allowed a rapid, selective and sensitive determination of intact-GLSs in this matrix.

1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is highly valued by large groups of the population not only due to its flavor, but also due to some health promoting effects, such as anticancer, antibacterial, antimicrobial, or antioxidant properties (Ares, Nozal, & Bernal, 2013). In addition, the chemopreventive effects associated to broccoli consumption have been attributed to glucosinolates (GLSs) and their degradation products (Latté, Appel, & Lampen, 2011). GLSs are β-thioglucoside N-hydroxysulfates, with a side chain (R) derived from amino acids and a sulfur-linked β-D-glucopyranose moiety (see Table 1), which could be found in all broccoli parts. Following tissue damage, these compounds undergo enzymatic hydrolysis by myrosinase to glucose and a variety of degradation products (isothiocyanates, nitriles, thiocyanates, epithiocyanates, epithionitriles and oxazolidines) (Latté et al., 2011), which are responsible for flavor and most biological activities related to GLSs (Bones & Rossiter, 2006). Intensive broccoli cultivars generate a high amount of waste products, mainly leaves, which are usually discarded despite their similar composition to the edible parts of the plant. Although most of the investigation has been focused on broccoli edible parts (sprouts, florets, heads, stems, roots), some research has been

Abbreviations: ALY, glucoalyssin; CCO, Central Composite Orthogonal; GBC, glucobrassicin; GBN, glucobrassicanapin; GER, glucoerucin; GIB, glucoiberin; GLS, glucosinolate; GNA, gluconapin; GNL, napoleiferin; GRA, glucoraphanin; NAS, gluconasturtiin; GTL, glucotropaeolin; 4-OH, 4-hydroxyglucobrassicin; LOD, limit of detection; LOQ, limit of quantification; 4-ME, 4-methoxyglucobrassicin; MS, mass spectrometry; MS/MS, tandem mass spectrometry; NEO, neoglucobrassicin; PLE, pressurized liquid extraction; PRO, progoitrin; QC, quality control; RE, relative error; RSD, relative standard deviation; SIN, sinigrin; qTOF, quadrupole time-of-flight; UHPLC, ultra-high performance liquid chromatography.

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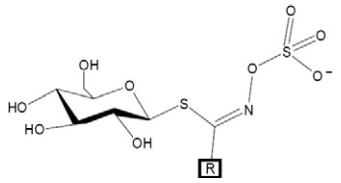
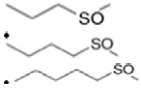
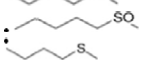
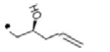
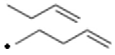
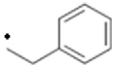
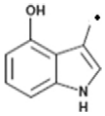
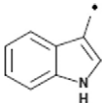
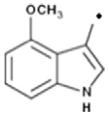
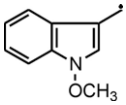
Table 1
List of intact-GLS investigated in the present study.

General chemical structure of GLSs



Compound name (abbreviation)	Molecular weight, g/mol	Side chain (R)	Precursor ions, m/z (Quantification, negative mode)	Fragments, m/z (Confirmation, negative mode)
Aliphatic GLS				
Alkyl GLS				
Glucoiberin (GIB)	423		422.0216	259.97
Glucoraphanin (GRA)	437		436.0412	372.97
Glucoalyssin (ALY)	451		450.0551	386.97
Glucoerucin (GER)	421		420.0411	259.97
Alkenyl GLS				
Progoitrin (PRO)	389		388.0375	259.97
Sinigrin (SIN)	359		358.0227	259.97
Napoleiferin (GNL)	403		402.0523	259.97
Gluconapin (GNA)	373		372.0387	259.97
Glucobrassicanapin (GBN)	387		386.0580	285.97
Aromatic GLS				
Glucotropaeolin (GTL)	409		408.0448	259.97
Gluconasturtiin (NAS)	423		422.0553	259.97
Indole GLS				
4-Hydroxyglucobrassicin (4-OH)	464		463.0479	259.97
Glucobrassicin (GBC)	448		447.0560	259.97
4-Methoxyglucobrassicin (4-ME)	478		477.0633	259.97
Neoglucobrassicin (NEO)	478		477.0677	446.97

conducted with the aim of determining nutritional ingredients from broccoli leaves in order to give them certain economic value, as nutraceutical reservoirs, and at the same time to reduce the environmental impact. In the scarce published scientific literature related to this matrix, they could be found some works where GLSs were studied (Ares, Nozal, Bernal, & Bernal, 2014a,b; Branca, Li, Goyal, & Quiros, 2002; Domínguez-Perles, Martínez-Ballesta, Carvajal, García-Viguera, & Moreno, 2010; Hennig, Verkerk, Bonnema, & Dekker, 2012; López-Berenguer, Martínez-Ballesta, García-Viguera, & Carvajal, 2008; Sasaki, Neyazaki, Shindo, Ogawa, & Momose, 2012). Different methods for the analysis of GLSs in broccoli leaves according to the presence (intact or non-intact desulfo-derivatives) of a sulfate group have been reported in literature. Although the desulfation step decreases the polarity of GLSs and improves their chromatographic resolution in reversed-phase liquid chromatography, it is a time consuming procedure, and faster methods have been proposed for the direct analysis of intact-GLSs (Glauser, Schweizer, Turlings, & Reymond, 2012; Mohn, Cutting, Ernst, & Hamburger, 2007). Typically, extraction of intact-GLSs is carried out in heated water (Ares et al., 2014a,b) or heated methanol and water mixtures (Domínguez-Perles et al., 2010; López-Berenguer et al., 2008; Sasaki et al., 2012). As can be seen all these treatments include heating, which is necessary to inactivate myrosinase and avoid the degradation of the intact-GLSs. It must be commented that similar sample treatments (solid–liquid extraction protocols) were used in most cases to extract those compounds from other broccoli parts. Nowadays, traditional extraction techniques, which usually require long extraction times, large amounts of samples, sorbents