Food Chemistry 233 (2017) 60–68

Contents lists available at ScienceDirect

Food Chemistry

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

The mechanism of deterioration of the glucosinolate-myrosynase system in radish roots during cold storage after harvest

Jeong Gu Lee [a], Sooyeon Lim [a], Jongkee Kim [b], Eun Jin Lee [a,*]

[a] Department of Plant Science, Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea
[b] Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Republic of Korea

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 13 April 2017
Accepted 17 April 2017
Available online 19 April 2017

Keywords:
Cold storage
Glucosinolate
Isothiocyanate
Myrosinase
Raphanus sativus

ABSTRACT

The hydrolysis of glucosinolates (GSLs) by myrosinase yields varieties of degradation products including isothiocyanates (ITCs). This process is controlled by the glucosinolatemyrosinase (G-M) system. The major ITCs in radish roots are raphasatin and sulforaphene (SFE), and the levels of these compounds decrease during storage after harvest. We investigated the GM system to understand the mechanism behind the decrease in the ITCs in radish roots. Six varieties of radish roots were stored for 8 weeks at 0–1.5 °C. The concentrations of GSLs (glucoraphasatin and glucoraphenin) were maintained at harvest levels without significant changes during the storage period. However, SFE concentration and myrosinase activity remarkably decreased for 8 weeks. Pearson correlation analysis between ITCs, GSLs, and myrosinase activity showed that a decrease of SFE during storage had a positive correlation with a decrease in myrosinase activity, which resulted from a decrease of ascorbic acid but also a decrease of myrosinase activity-related gene expressions.

© 2017 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Radish (*Raphanus sativus* L.), a popular vegetable cultivated around the world, is a root vegetable of the *Brassicaceae* family that has numerous varieties. Radish roots are good sources of vitamin C, folic acid, minerals, polyphenols, and glucosinolates (GSLs) (Hanlon & Barnes, 2011). Radish roots can be consumed in different forms, both processed and fresh. Before radish roots are consumed, sometimes they are stored for 1–4 months. For long-term storage, cold storage (Thompson, 2003) or modified atmosphere packaging (MAP) (Schreiner, Huyskens-Keil, Krumbein, Prono-Widayat, & Lüdders, 2003) is recommended. The MAP material can help maintain appropriate oxygen and carbon dioxide levels (Sandhya, 2010).

GSLs are nitrogen and sulfurcontaining secondary metabolites found in the *Brassicaceae* family (Rosa, Heaney, Fenwick, & Portas, 1997). Although the isothiocyanates (ITCs) have been found to be more than one thousand times more cytotoxic than the GSLs (Musk, Smith, & Johnson, 1995), intact GSLs also have direct biological activity to impact on the chemopreventive activity linked to cruciferous vegetable consumption (Abdull Razis, Bagatta, De Nicola, Iori, & Ioannides, 2010). All GSLs have a core structure like a β-thioglucoside moiety, a sulfonated oxime aglycone, and a variable side chain derived from amino acids. To date, approximately 130 different side chains of GSLs have been reported. GSLs are classified by precursor amino acids and types of side chains. GSLs derived from methionine, leucine, or isoleucine are classified as aliphatic GSLs, those derived from phenylalanine or tyrosine are classified as aromatic GSLs, and those derived from tryptophan are classified as indolyl GSLs (Fahey, Zalcmann, & Talalay, 2001).

In radish roots, the major GSLs are glucoraphasatin (GRH) and glucoraphenin (GRE), which are derived from methionine. GRH is the predominant GSL that accounts for approximately 74% of the total GSLs while GRE is the second most common GSL and accounts for less than 10% of the total GSLs in radish roots (Hanlon & Barnes, 2011; Yi et al., 2015). When plant tissue is damaged by mechanical treatments (chewing, chopping, or cutting) by insects or humans, myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1), separated from GSLs into vacuoles of myrosin cells (Bones & Rossiter, 2006), hydrolyzes GSLs to form ITCs, which have various biological functions in humans and plants. This overall process to form ITCs is called the glucosinolatemyrosinase (GM) system and described in Supplemental Fig. S1.

Myrosinase is the only known enzyme that cleaves a thio-linked glucose found in nature. In the presence of water, myrosinase cleaves off the glucose from GSLs (Holley & Jones, 1985). However, myrosinase and its substrate GSLs are stored in separate and different cell types (Winde & Wittstock, 2011). While GSLs are stored in

* Corresponding author.
E-mail address: ejinlee3@snu.ac.kr (E.J. Lee).

http://dx.doi.org/10.1016/j.foodchem.2017.04.104
0308-8146/© 2017 The Author(s). Published by Elsevier Ltd.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

"S-cells" (Koroleva et al., 2000), myrosinase is stored as the tonoplast-like membrane surrounding myrosin grains within myrosin cells (Höglund, Lenman, & Rask, 1992). The myrosinase enzyme is encoded by the thioglucoside glucohydrolase (TGG) gene family. TGG4 (Atlg47600) has been shown to encode functional myrosinase and seems to have root-specific expression patterns (Andersson et al., 2009). In addition, myrosinase-binding protein (MBP) can interact with myrosinase and form a myrosinase complex. MBP has been known to contribute to myrosinase activity in different *Brassica* species (Capella, Menossi, Arruda, & Benedetti, 2001; Rask et al., 2000). Ascorbic acid is a known cofactor of myrosinase, serving as a base catalyst in GSL hydrolysis. For example, myrosinase isolated from radish roots showed an increase in activity from 2.06 μmol/min per mg of protein in the absence of ascorbic acid to 280 μmol/min per mg of protein in the presence of 500 μM ascorbic acid on the substrate allyl glucosinolate (Shikita, Fahey, Golden, Holtzclaw, & Talalay, 1999).

The hydrolysis of GSLs by myrosinase can yield a variety of degradation products including ITCs, nitriles, thiocyanates, and epithionitriles, depending on pH and the presence of certain cofactors (Kissen, Rossiter, & Bones, 2009; Winde & Wittstock, 2011). ITCs are the most functional phytochemicals among GSL degradation products. In plants, ITCs are considered to play a role in the defense against herbivores (Beekwilder et al., 2008). ITCs have biological activity, such as antimicrobial (Esaki & Onozaki, 1982; Fahey et al., 2002; Lim, Han, & Kim, 2016), antioxidant (Barillari et al., 2006; Katsuzaki, Miyahara, Ota, Imai, & Komiya, 2004; Takaya, Kondo, Furukawa, & Niwa, 2003), and anticarcinogenic properties (Barillari et al., 2007; Hanlon, Webber, & Barnes, 2007; Yang, Teng, Qu, Wang, & Yuan, 2016) in human cell line and in mice. The major ITCs of radish roots, raphasatin (RH) and sulforaphene (SFE) (Scholl, Eshelman, Barnes, & Hanlon, 2011), are converted from GRH and GRE, respectively, by the activation of the G-M system (Hanlon & Barnes, 2011).

In our previous study (Lim, Lee, & Kim, 2015), an over 35% reduction in SFE in radish roots was observed during cold storage for 8 weeks. In this study, we first tried to clear up the cause of ITC reduction of harvested radish roots based on the G-M system. The objective of this study was to understand the changes of the G-M system in harvested radish roots. We analyzed the ITC concentrations of RH and SFE in six varieties of radish roots. Additionally, GRH and GRE concentrations, myrosinase activity, and myrosinase-related genes (TGG4 and MBP2) expression, and ascorbic acid were analyzed.

2. Materials and methods

2.1. Plant materials

Six varieties of radish roots (*Raphanus sativus* L.), 4 cultivars (Seoho, Cheonghwang, Junmuhumu, and Alpine), and 2 breeding lines (15FH352-1 and 15RA11-1), were harvested from an experimental field in Jeonju, Korea (Supplemental Fig. S2). After removing the leaves, radish roots were immediately transported to the laboratory.

2.2. Storage of radish roots

Harvested radish roots were washed with 0.01% sodium hypochlorite for 3 min, washed twice with tap water, wiped with a paper towel to dry up, and pre-cooled at 2 °C for 6 h. Radish roots were covered with polyethylene film during storage. The storage temperature was 0–1.5 °C, and the relative humidity was maintained at 85–90%. Three radish roots were sampled at 0 and 8 weeks, respectively, and analyzed for ITCs concentration, GSL concentration, myrosinase activity, ascorbic acid concentration, and expressions of myrosinase-related genes of TGG4 and MBP2. The middle area of the roots was sliced to a 1cmthick disk and sliced into quarters. Sliced radish disks were stored in plastic bags at −95 °C or freeze dried.

2.3. Extraction of ITCs

The ITCs in the radish roots were extracted using liquid-liquid extraction methods (Kim, Kim, & Lim, 2015) with slight modifications. Eight milliliters of methylene chloride (MC) and distilled water and 1 mL of 100 ppm benzyl ITC in MC as an internal standard were added to 500 mg of lyophilized radish root powder. To hydrolyze endogenous GSLs to ITCs by myrosinase, a mixture was placed in a water bath at 37 °C for 30 min. The hydrolyzed sample was added to 10 mL of MC and centrifuged at 2000×*g* for 10 min. The MC layer was collected and filtered with filter paper containing anhydrous sodium sulfate to remove any water. The MC extract was evaporated to remove the MC under a nitrogen evaporator at 30 °C, re-dissolved in 1 mL of MC, and finally filtered with a 0.45-μm syringe filter.

2.4. Analysis of ITCs

The ITC contents were analyzed by GC-MS using the method described by Kim, Lee, and Kim (1997). The analysis was performed on a TRACE1310 GC system (Thermo, USA) with ISQ LT mass spectrophotometer (Thermo, USA) and a DB-5 fused silica capillary column (0.25 × 30 mm, Agilent Technologies, USA). The oven temperature was set to increase from 50 °C to 310 °C at a rate of 5 °C/min. The injector was used in a split-less mode at 250 °C. The flow rate of the helium was 1 mL/min. The range of mass scan was from 35 *m*/*z* to 550 *m*/*z*.

2.5. Extraction of crude myrosinase

Crude myrosinase was extracted using a previously described method (Lim et al., 2015) with slight modifications. Fifty grams of radish root were homogenized with 80 mL of extraction buffer (10 mM potassium phosphate containing 3 mM DTT, 1 mM EDTA, and 5% glycerol, pH 7.2) for 1 min. The mixture was immediately filtered with six layers of gauze and centrifuged at 6700 × *g* for 30 min at 4 °C. The supernatant was saturated with ammonium sulfate to 55% and centrifuged at 6700 × *g* for 20 min at 4 °C. The supernatant was saturated with ammonium sulfate to 80% and centrifuged at 6700 × *g* for 50 min at 4 °C. The pellet was dissolved in 2 mL of desalting buffer (50 mM Tris-HCl, pH 8.6) and loaded onto a PD-10 column (GE healthcare, USA) equilibrated by 25 mL of desalting buffer. The desalted myrosinase was eluted with 3.5 mL of desalting buffer.

2.6. Assay of myrosinase activity

The myrosinase activity was investigated using a GO assay kit (Lim et al., 2015). One hundred microliters of crude myrosinase solution was added to 800 μL of 33 mM potassium phosphate buffer and 100 μL of 2 mM sinigrin. The mixture was incubated at 37 °C for 30 min, placed in a heat block at 95 °C for 10 min to stop enzyme activity, and centrifuged at 19,700 × *g* for 20 min. Fifty microliters of the supernatant was mixed with 100 μL of mixture regent in a 96-well plate, incubated at 37 °C for 30 min, and added to 100 μL of 12 N sulfuric acid to stop the enzyme activity. The absorbance of the mixture was measured by a Multiple Plate Reader Victor 3 (Perkin Elmer, USA) at 540 nm.

Download English Version:

https://daneshyari.com/en/article/5132802

Download Persian Version:

https://daneshyari.com/article/5132802

Daneshyari.com