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REVIEW PAPER

Horseradish (*Armoracia rusticana*), a neglected medical and condiment species with a relevant glucosinolate profile: a review

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Abstract *Armoracia rusticana* (horseradish), a member of the Brassicaceae family, has been known since ancient times as a folk medicinal herb and as a plant of nutritional value and culinary interest. Currently horseradish is cultivated for its thick, fleshy and white roots which have a delicious intense pungency and for its tender leaves which are frequently used for salad mixed to other vegetables. The traditions to use horseradish plant for medicinal purpose are still applied in many countries. Horseradish is a rich source of a number of bioactive compounds such as glucosinolates (GLSs) and their breakdown products. Sinigrin is the dominant glucosinolate in both leaves and roots. Recent studies have shown that crude plant extracts have a complex profile of naturally occurring GLSs, with particular regard to sprouts. The increasing interest in these secondary metabolites, associated to the long and diffuse tradition of using horseradish in food preservation and as condiment in many parts of the world, is generating new applications of this plant in several agro-industrial and pharmaceutical sectors and is encouraging the use of its roots and leaves in functional food and medicine for human health. A bibliography review is discussed on ethnobotanical aspects and uses of this plant, as well as knowledge about its flavour compounds and GLS content and composition. This study summarizes also the updated information concerning the influence of the genotype and environment on GLS profile in horseradish.

Keywords Brassicaceae · Ethnobotany · Ethnopharmacognosy · Glucosinolates · Horseradish · Isothiocyanates

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Introduction

Horseradish, *Armoracia rusticana* G. Gaertn., B. Mey. et Scherb., is an extremely hardy perennial plant, member of the Brassicaeae family (Weber 1949; Shehata et al. 2009). Its root and leaves have been used in antiquity as both a medicinal herb and a condiment (Rosengarten 1969), and the latter use is the principal nowadays. It is currently cultivated for its thick, fleshy and white roots that have a mix of delicious intense pungency and cooling taste which is caused by sulfur compounds, namely glucosinolates (GLSs) (Weber 1949; Balasinska et al. 2005; Walters and Wahle 2010).

The species has become naturalized in many parts of the world. Horseradish can be found in various environments including fields, home gardens, weedy areas, farmland, roadsides, ditches and disturbed areas throughout Europe (Al-Shehbaz 1988; Hammer et al. 1992; Luczaj and Szymanski 2007; Sampliner and Miller 2009). In open fields, horseradish is cultivated as an annual or perennial crop. As annual crop it requires a long growing season with high temperatures during the summer and fall to enhance root development; as a perennial it may stay productive for 10–20 years or more, thus requiring a careful field management (Walters and Wahle 2010).

The main areas of commercial cultivation are found in Europe and in North America (California, Illinois, and Wisconsin) (Sampliner and Miller 2009). In European countries, horseradish is especially produced in Germany, Hungary, and Poland (Shehata et al. 2009). Particularly, large scale productions can be found in certain regions of Germany, like Upper Franconia, Thuringia and the Spreewald, firstly documented in 1569 (Hanelt 2001). In the Serbian province of Vojvodina, the collection of wild horseradish began a long time ago, and the particular ecotype collected, called "Novosadski", was highly sought after by the Austro-Hungarian Empire court thanks to its exceptional quality (Perlaki and Djurovka 2009). In Italy this plant grows in the Northern part and in the Lazio and Basilicata regions (Hammer et al. 1990, 1992; La Rocca and Chisci 2005; Guarrera 2006; Sarli et al. 2012). Although the hypothesis needs to be confirmed by more detailed historic-botanical studies (Pieroni et al. 2005), it is probable that horseradish arrived in villages of inland Lucania (viz. Basilicata) via migrants from Swabia during the thirteenth century. Hammer et al. (2011) reported that it was possibly introduced as a traditional spice plant from Albania with Albanian immigrants to South Italy, where today is particularly diffused in the Arbëreshë areas of Basilicata, far away from North Italy. *A. rusticana* is cultivated also in Russia, the Caucasus, Asia and in some regions 1,000 m above sea level in tropical countries (Sampliner and Miller 2009). In some regions of Russia, it may still be possible to find horseradish landraces (Veteläinen et al. 2009). Recently, China has also begun commercial production of horseradish, although much of the crop is produced by small farmers (Shehata et al. 2009).

In this review we first present a botanical description of *A. rusticana* and its traditional uses in food and medical fields due to its peculiar chemical composition. Afterwards, we review knowledge on flavour constituents and profile, concentration and chemical form of GLSs and their breakdown products in roots and leaves. This review also stresses the influence of the genetic variability and agronomic management on GLS content and composition of horseradish plants.

Botanical description and origin

Horseradish has well defined botanical characteristics which clearly separate the species from other Brassicaceae. Various Latin names have been used in the course of time by several authors for this species. Taxonomically, horseradish has been placed in various genera by different botanists (Weber 1949). In 1969, Courter and Rhodes have recorded the names for classification of horseradish during three historical periods, as shown in Table 1. It was classified as *Nasturtium armoracia* Fries, *Roripa armoracia* Hitchc., *Radicula armoracia* Robinson, *Cochlearia armoracia* L., *Armoracia armoracia* Britton, and *A. rusticana* Gaertn. (Weber 1949). Specific epithets used were *rivini*, *rusticana*, *armoracia* or *lapathifolia*. Although *A. lapathifolia* is favored by some taxonomists, Lawrence (1953) and Fosberg (1965) have pointed out that *A. rusticana* should have preference (Courter and Rhodes 1969). The first valid published scientific name of horseradish which resulted from the generic name *Armoracia* was *A. rusticana* in 1800 by Gaertner, Meyer, and Scherbius: this binomial is used today (Shehata et al. 2009). Even more complex is the variety of common names used to designate this species whose polynomial is due to different languages and dialectical differentiation in different territories. Horseradish has its gene center in Eastern Europe and the south western parts of Russia (Wedelsbäck Bladh and Olsson 2011). In fact, early records indicate that horseradish is a native of the temperate regions of Eastern Europe and western Asia, where wild types are found growing from Finland and Poland to the regions around the Caspian Sea and the desert of Cumania (now Romania) and Turkey. From here, horseradish spread to Western Europe and across the Atlantic to the New World by early settlers. Today, horseradish has become naturalized in many parts of

Table 1 Classification of horseradish during three periods of history

Name	Source
Ancient	
Persicum sinapi	Dioscorides
Sinapi persicum	Dioscorides
Thlaspi cratevae	Dioscorides
Persicon napy	Pliny
Renaissance	
Thlaspi alterum	Turner
Raphanus vulgaris	Matthioli
Raphanus magna	Dodoens
Raphanus rusticanus	Gerard
Raphanus sylvestris	Bauhin J.
Linnaeus to modern	
Armoracia rivini	Ruppins
Cochlearia armoracia	Linnaeus
Cochlearia rusticana	Lamarck
Armoracia lapathifolia	Gilibert
Armoracia rusticana	Gaertner, Meyer and Scherbius
Nasturtium armoracia	Fries
Roripa rusticana	Gren. & Godr.
Roripa armoracia	Hitchoock
Radicula armoracia	Robinson
Armoracia armoracia	Britton

Table from Courter and Rhodes (1969) reproduced with permission

the world and can be found both cultivated and growing wild (De Candolle 1890; Shehata et al. 2009).

According to De Candolle (1890) the most probable place of origin of *A. rusticana* was the temperate region of Eastern Europe because of the word *chren*, common in Slavic languages which was the most primitive name for horseradish. *Chren* was introduced into German and French dialects in the forms of *kren*, *kreen*, *cran* and *cranson*. *Meerretig*, *meer-radys* and *meridi*, which literally mean sea-radish, are other words for horseradish that are common in several western European languages, but these words are less primitive than the name *chren*. The names *horseradish* in English, *raifort* in French, and *pepperrot* in Swedish are also of more recent origin than *chren* (Courter and Rhodes 1969). The first use of the term *horse radish* was made by John Gerard in his famous English herbal (1597) that contains a lengthy entry with a woodcut and clear description of the plant. Some believe that the English called the plant horseradish in reference to its propensity to rapidly spread in a 'galloping' behavior (i.e., it is difficult to control once it has been introduced in an area) (Shehata et al. 2009). It is also possible that it was called *harsh* radish because it was so bitter on the tongue (McCann 2004). The word *armoracia* has been commonly used as a generic name or specific epithet: *Armoracia* is formed from the Celtic: *ar* 'near', *mor* 'the sea', *rich* 'against', viz., a plant growing near the sea (Courter and Rhodes 1969).

Armoracia belongs to the Cardamineae, which comprise 10 genera and more than 340 species that grow in moist habitats. The three species within the genus (*A. macrocarpa*, *A. rusticana*, *A. sisymbrioides*) have an affinity for wet habitats and commonly spread through rhizomes (Al-Shehbaz et al. 2006; Sampliner and Miller 2009). Brzezinski (1909) believes that horseradish is not a natural species but a hybrid (Weber 1949). Sampliner and Miller (2009) hypothesized that *A. rusticana* is a species known only from cultivation, and that cultivated *A. rusticana* populations were derived from natural populations of either *A. macrocarpa* or *A. sisymbrioides*.

Horseradish is a large-leaved, hardy, and glabrous perennial herb that grows to a height of up to 120 cm (Fig. 1). Cauline leaves vary in shape and margin depending on the habitat in which they are growing. The leaves are long-petioled, oblong-ovate, cordate at the base, unevenly crenate, and they grow to a length of 30 cm up to 100 cm. (Sampliner and Miller 2009; Shehata et al. 2009). Moreover, cultivars with an acute leaf-base have smooth leaves, while those with a cordate leaf-base have crinkled leaves; some genotypes have intermediate leaf-types (Courter and Rhodes 1969). The leaves on stalk are smaller in size, have narrow bases and mostly sessile, are alternate, lanceolate, and unevenly serrate to entire-margined. It is interesting to note, as reported by Shehata et al. (2008), that horseradish leaf morphology varies through the season from entire (laminate) in the summer to divided (pinnate or fern leaf) in the fall; intermediate types of leaves are visible throughout the season. The same authors (Shehata et al. 2008) have shown in their work a photo with the different types of leaves. The unstable leaf morphology of horseradish is of considerable interest to botanists. In general, the change from entire to pinnate in most obvious in the fall as plants approach dormancy, suggesting environmental influence (Shehata et al. 2008).

Fig. 1 *Armoracia rusticana* plant grown in Basilicata Region (South Italy). Personal photo

The plant may or may not form a flower stalk depending on environmental conditions (Weber 1949). In natural habitats, horseradish blooms very profusely and for a long period of time, until mid-August (Winiarczyk and Bednara 2008). Shehata et al. (2009) reported that *A. rusticana* produces numerous fragrant flowers with 5–7 cm long upright pedicels borne on racemes that have four sepals, four petals, and six tetradynamous stamens: their sepals are 2.5–3 mm long, broadly ovate and have membranous white margins; petals are white, 5–7 mm long, and broadly obovate; the inner stamens are 2.5 mm long and the outer ones are 1.5 mm long; the stigma is broad, round, and gently two-lobed. Horseradish bears 4–6 mm long, globose to obovate siliques with persistent style on 20 mm long, upright-spreading stems (Shehata et al. 2009). *A. rusticana* produces few if any seeds, usually none, at most 6 seeds per pod (Sampliner and Miller 2009). The seeds are smooth and brown when mature (Shehata et al. 2009). Winiarczyk and Bednara (2008) observed that, a dozen or so days after the fall of the perianth of the inflorescence, siliques started to form (Fig. 2): most of the flowers were withered, but on every inflorescence, there were a few dozen fruits, the majority of which, however, were shriveled and deformed; in every silique, most of the ovules were aborted, some were much smaller and deformed, but sporadically there were also large, properly developed young seeds. As a matter of fact, many early botanists reported that horseradish rarely produced viable seed and, until the early part of the twentieth century, the plant was considered to be highly sterile (Courter and Rhodes 1969). Weber (1949) observed partial pairing of chromosomes and other irregularities during both microsporogenesis and megasporogenesis. Winiarczyk and Bednara (2008) noted on the stigma of *A. rusticana* morphological symptoms of the reaction of self-incompatibility, typical for some species of the Brassicacea family. This could explain the fact that most *A. rusticana* plants are incapable of producing viable seeds. In addition, the plants growing in a given area, i.e. constituting a certain population, are often

Fig. 2 Typical silique of a horseradish plant 2-year-old: **a** Fruits-silique started to form at the end of the vegetative period; **b** single silique; **c** development of seeds in silique. Images captured with stereo microscope (Carl Zeiss) at ×6.3 by a camera Olympus 8 Megapixel. The *bar* in Fig. 2b is 2 mm

genetically identical with the parent plant, as a consequence of their vegetative reproduction (Winiarczyk and Bednara 2008). In fact, horseradish is asexually propagated by planting root sections collected from the previous year's crop (Walters and Wahle 2010) through the formation of adventitious buds (Kamada et al. 1995). In particular, lateral roots are separated from the healthy taproots for next year's planting stock (Shehata et al. 2009). The root system of horseradish consists of a long, white, cylindrical or tapering main root that can grow to about 60 cm in loose soils; several thin lateral roots are formed around the main root and near the collar of the crown; undisturbed, the root system can reach a depth of 3–4 m with a lateral spread of about 1 m (Shehata et al. 2009). Horseradish roots exhibit distinct polarity with a proximal end (or point of attachment to the main root that sprouts producing new vegetation) and distal end (Walters and Wahle 2010).

According to Sampliner and Miller (2009) the clonal propagation of *A. rusticana* may have resulted in increased sterility due to (1) the accumulation of deleterious mutations in gene regions associated with seed production; (2) reproduction of sterile individuals derived from an interspecific hybridization; or (3) self-incompatibility mechanisms precluding fertilization between widespread, genetically-identical plants. Furthermore, research aimed at obtaining horseradish plants capable of sexual reproduction could result in better adaptation to various environmental conditions and pest resistance (Winiarczyk et al. 2007).

Traditional uses as a spice and as folk medicinal plant

The use of horseradish as food or folk medicinal plant has spread from the Eastern and Mediterranean areas both north- and westward during the Middle Ages (De Candolle 1890; Wedelsbäck Bladh and Olsson 2011). It is believed that horseradish became popular as a condiment in old Europe because there was no refrigeration and its sharp spiciness covered the taste of tainted meat (Shehata et al. 2009). In Bulgaria, Romania and Russia, the whole root, grated root, or piece of cut root are still used to flavour, help ferment, aerate pickling liquid, and conserve cabbage for the winter; leaves were also cut and used to conserve cut vegetables for the winter (Sampliner and Miller 2009).

Horseradish was used by European Jews as a symbolic food in the ritual meal as bitter herb of Passover because its bitterness remembered the suffering of their ancestors of the exodus from Egypt (Schaffer 1981). By the late 1600s the English people added horseradish to beef and oysters, and particularly in spring, the tender leaves frequently were used for greens mixed with other wild plants (Shehata et al. 2009); for Germans, horseradish roots were an excellent addition to meat and fish when cut in very small pieces, then crushed and mixed with salt and vinegar (Courter and Rhodes 1969). These latter authors wrote that horseradish was also mixed in catsup used to flavour ground beets or added to mustard as a seasoning. As reported by Shehata et al. (2009), horseradish was often consumed boiled as a pot herb; it was boiled, the water was drained, and it was boiled a second time to eliminate bitter or harmful substances before consumption.

The main portions of this plant are still used today to prepare traditional dishes in many countries around the world. In Poland, roots and leaves are used for seasoning or as preservatives: the roots, usually collected from the wild plants, were utilized as a condiment, with pickled cucumbers, grated with chopped boiled eggs, soups or meat dishes, often used at Easter. The leaves placed in the oven under baking bread, partly to prevent the bread from sticking and partly to flavour the bread, are still widely used (Łuczaj and Szymański, 2007). In Bulgaria, Romania and Russia, grated roots are used as a condiment for any cooked meat (e.g., lamb, pig, and chicken); they are often combined with vinegar, salt, oil, cream, or yogurt (Sampliner and Miller 2009). Particularly, in Romania, grated roots, with or without oil, are eaten with potatoes or polenta, or are grilled and eaten with cream accompanying lamb and chicken; a mix of grated horseradish, apples, salt, sugar, and vinegar is a garnish used for meals; small pieces of cut *A. rusticana* root are eaten in soups; leaves can also be put into bread dough, which is then grilled (Sampliner and Miller 2009). In South Italy there are many traditional culinary uses, particularly, in the Basilicata area this plant is typical base for preparing dishes during the carnival period (Sarli et al. 2012): horseradish is grated as condiment for pasta or home-made noodles ('ferriciedd'), and it is used in fried eggs paste to prepare a traditional dish ('rafanat') during the carnival period; pieces of roots are also used for

aromatization of hand-made sausages; it is used raw grated as condiment for cooked pork meat or for lamb and chicken where the pieces of roots are often combined with oil, salt and vinegar; the leaves are used for salad, mixed to other vegetable species (Pieroni et al. 2005; Sarli et al. 2012). Grated roots are often combined with oil and used all year round, also smeared on grilled bread (Sarli et al. 2012). The root is still popular in Europe and North America and is appreciated freshly grated, mixed with vinegar or in a sauce to form a condiment often used with meats or fish and as a flavour in other recipes in salads and soups, on sandwiches, and also in drinks such as the Bloody Mary (Wedelsbäck Bladh and Olsson 2011).

In addition to be used as food and condiment, horseradish has a long history of utilization for medicinal purposes in the traditional medicine (Shehata et al. 2009). In a recent review, Wedelsbäck Bladh and Olsson (2011) have well documented the historical uses of horseradish as a medicinal plant in different parts of the world, and have shown also a table with the different uses in various historical periods. Since the ancient Greeks horseradish was used as an aphrodisiac and as a rub to alleviate pain in the back. Material medica's of ancient physician and the herbal of early botanists record many medical virtues of horseradish (Courter and Rhodes 1969). Early records, believed to be about horseradish are found in "Naturalis Historia" by the Roman naturalist and philosopher Gajus Plinius Secundus (AD 23-79) that recommended the plant freshly grated for the digestion after a heavy meal, and in "De Materia Medica" by the Greek physician Pedanius Dioscorides (AD 40-90) that described the plant as a diuretic and very hot herb (Bostock and Riley 1856; Courter and Rhodes 1969; Wedelsbäck Bladh and Olsson 2011). Hildegard of Bingen (1098–1179), a German abbess, in her book about medicinal plants, recommended horseradish mixed with warm vine or water as a treatment for lung diseases; to cure heartache or heart diseases, dry and pulverized horseradish could be mixed with *Alpinia galanga*, a Chinese herb often used as a stimulant and drug (Wedelsbäck Bladh and Olsson 2011). In 1597, the botanist and herbalist John Gerard claimed that horseradish reduced pain from sciatica, relieved colic, increased urination, and killed worms in children (Courter and Rhodes 1969). It was stated to be an expectorant, soothing for respiratory problems, and may help to relieve rheumatism by stimulating blood flow to inflamed joints (Shehata et al. 2009). The most common use of horseradish was as a remedy for scurvy, as also recommended by herbalist William Coles (1626–1662), especially for seamen on long voyages that had not access to fresh vegetables or fruit, so the lack of vitamin C in the diet often caused scurvy outbreaks on board (Wedelsbäck Bladh and Olsson 2011). In 1880, Bentley and Trimen reported that grated horseradish root was used mixed with honey and warm water for influenza, and it could be used as poultice by adding cornstarch to fresh horseradish and applying it to the affected areas in a gauze bandage. Native Americans used horseradish to treat toothaches, as a urinary aid for gravel (kidney stones), as a diuretic, as a gastrointestinal aid to improve digestion, and as a respiratory aid to treat asthma, cough, and bronchitis (Moerman 1998; McCann 2004; Shehata et al. 2009). The tradition to use the plant for medicinal purpose is still applied in countries like Bulgaria, Romania, and some part of Russia: grated roots or leaves are put in a cloth (sometimes with alcohol or vinegar) and applied to the skin to ease pain, or a paste made from the root is applied to the throat to ease breathing problems. Mixtures with honey from *Robinia pseudoacacia* are used for coughs and bronchitis, while in combination with vinegar, salt, and sugar it is used for lowering blood pressure (Sampliner and Miller 2009). Furthermore, in an ethnobotanical study about a community of *Russlanddeutsche* (Germans from Russia) living in Künzelsau (Germany), it was reported that Russian *sauerkraut*, used as an important home medicine for treating flu and liver disease, contained among other herbs, also horseradish leaves (Pieroni and Gray 2008). In the Basilicata region (South Italy), horseradish leaves and roots have been traditionally used as a remedy for rheumatism, headaches, sinuses, coughs and bronchitis. Added to water, vinegar, salt, sugar, or aromatised with *Anethum graveolens* and *Laurus nobilis* leaves, this plant is also used as a remedy for healing drunkenness. Particularly, in Castelmezzano (Basilicata), the Ukrainian women have introduced the functional use of pickled tomatoes (with dill, bay and horseradish leaves), consumed to recover from a drunken state. Leaves, added to dog food, are used because of their antimicrobial activity (Pieroni et al. 2004; Pieroni and Quave 2005; Sarli et al. 2012).

Also, according to Wedelsbäck Bladh and Olsson (2011), the traditional knowledge of using horseradish in food preservation at home is generating new applications for the plant as a natural anti-bacterial component in food (e.g., to reduce coli-bacteria or other microbial growth) and the new knowledge of bioactive components such as GLSs and their breakdown products, combined with traditional knowledge of medicinal properties, is encouraging the use of horseradish roots and leaves in functional food and medicine to inhibit different cancer forms or gastric lesions.

Glucosinolates, the main flavour constituents of horseradish

Horseradish tissues, when unbroken, are inodorous. The intense pungent aroma and the lachrymatory odour of horseradish results from crushing, grinding, or chewing the cells (Courter and Rhodes 1969; Jiang et al. 2006) and therefore it is specially cultivated to supply a hot spicy flavour (Sultana et al. 2003). The characteristic taste and odour is due to compounds such as GLSs and their mostly volatile breakdown products (Mucete et al. 2006; Redovnikovic et al. 2008a). GLSs are hydrolysed by the enzyme commonly known as myrosinase to a variety of compounds as isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines. The most common products are isothiocyanates (Bones and Rossiter 2006). GLSs are compounds mainly found in plants belonging to the order of Capparales, with particular reference to several species of the Brassicacee family (radish, cabbage, cauliflower, broccoli, mustard, turnip, oilseed rape) including horseradish (Fahey et al. 2001; Blazevic and Mastelic 2009). These secondary metabolites are characterized by a core sulfated isothiocyanate group, which is conjugated to thioglucose, and a further R-group derived from amino acids (Clarke 2010). GLSs can be divided into three classes based on the structure of different amino acid precursors: (1) aliphatic GLSs derived from methionine, isoleucine, leucine or valine; (2) aromatic GLSs derived from phenylalanine or tyrosine; and (3) indole GLSs derived from tryptophan (Wittstock and Halkier 2002; Redovnikovic et al. 2008b). The number of reported GLSs is now approaching 200 (Clarke 2010). A number of plants contain only a single GLS, the majority contain 2–5, while 34 individual GLSs are reported in the seeds and leaves of a collection of ecotypes of *Arabidopsis thaliana* (Kliebenstein et al. 2001; Clarke 2010). GLS concentrations in plants, although highly variable, are around 1 % of dry weight in some *Brassica* vegetables (Rosa et al. 1997; Clarke 2010). The GLS content is higher in black mustard seed (*Brassica nigra*) and horseradish roots (over 10 % by dry weight) than in the other constituent parts of the Brassicaceae (Li and Kushad 2004; Mucete et al. 2006). However, very little is known about the concentration and biochemical composition of horseradish, especially the distribution of GLSs in the different organs of the plants (Li and Kushad 2004).

A first study about the identification of GLSs of horseradish roots by GC–MS (gas chromatography-mass spectrometry) was published by Grob and Matile (1980). They identified 30 GLSs (Table 2) which exceeds the number of GLSs detected before in any other species. The authors reported that a close examination of the chromatograms may indicate that the number of structures present in horseradish may be even larger, although today it is necessary to investigate and confirm all individual molecules listed by Grob and Matile (1980). Several methods have been developed to detect either intact GLSs or their desulfoglucosinolates (Lee et al. 2006; Cataldi et al. 2007).

The structural identity of GLSs is known and described only for those which can be isolated and identified by traditional methods. With increasing complexity of GLS composition and decreasing concentration of individual GLSs in the sample, more sensitive and selective analytical methods are required for their identification (Agneta et al. 2012; Lelario et al. 2012). Hence, for their accurate structural identification, in addition to traditional methods, sensitive and selective analytical methods are required, especially when GLSs are present in trace amounts only. Mevy et al. (1997) investigated the occurrence of GLSs in various differentiated horseradish tissues (Table 3) by HPLC analysis of desulphoglucosinolates. Particularly, the individual GLSs present in regenerated plantlets were compared with those of embryoids, suspension cells and calli in order to investigate whether the distribution of these compounds resulted from cellular differentiation. Moreover, the authors revealed the presence of two GLSs, i.e. 2-phenylethyl- and 2-propenyl-, which appeared to be confined to roots and leaves, respectively, while the highest

Table 2 Glucosinolates of horseradish roots as identified by their corresponding mustard oils (Table modified from Grob and Matile 1980) and odour description (reported by Sultana et al. 2003)

GLS common name	GLS systematic name	μg/g[a]	Odour description[b]
Glucoviorylin	2-Methylthioethyl-	2	
Glucoputranjivin	*Iso*-propyl-	1	Chemical, weak mustard like
Sinigrin	Allyl-	240	Strongly pungent, mustard-like, lachrymatory, bitter
Glucoarabidopsithalin	2-Hydroxypropyl-	3	
Glucoiberverin	3-Methylthiopropyl-	5	Strongly radish-like, pungent
Glucoiberin	3-Methylsulfinylpropyl-	3	
Glucocheirolin	3-Methylsulfonylpropyl-	3	
	n-butyl-	5	
	sec-butyl-	22	Chemical, weak mustard like
Glucoconringianin	*iso*-butyl-	6	Sweet, chemical
Gluconapin	3-Butenyl-	14	Green, pungent, aroma
Glucocappariflexin	3-Hydroxybutyl-	0.5	
Glucoerucin	4-Methylthiobutyl-	0.5	
Glucokohlrabiin	*n*-pentyl-	1	
	3-Methylbutyl-	8	
	2-Methylbutyl-	0.5	
Glucobrassicanapin	4-Pentenyl-	20	Green, pungent, acrid, fragrant leaf
Glucomoracialapathin	2-Hydroxypentyl-	0.1	
	3-Hydroxy-4-pentenyl-	0.8	
Glucoberteroin	5-Methylthiopentyl-	0.2	Radish-like, pickle-like
	3-Hydroxy-5-methylthiopentyl-	0.2	
	Iso-hexyl- structure unknown	0.4	
Glucowasabiamin	5-Hexenyl-	2	Green, pungent, fatty
Glucolesquerellin	6-Methylthiohexyl-	0.1	Radish-like, sweet, fatty
	Iso-heptyl- structure unknown	0.05	
Glucotropaeolin	Benzyl-	4	Strongly radish-like, pungent, strong watercress aroma, tingling sensation
Gluconasturtiin	2-Phenylethyl-	55	
Glucoarmoracialapicin	3-Phenylpropyl-	0.1	
Glucoarmoracialafolicin	4-Phenylbutyl-	0.5	
	Methoxybenzyl-	0.5	

The common names are those generally accepted (Fahey et al. 2001; Bellostas et al. 2007a; Clarke 2010)

[a] Concentration is expressed as μg/g fresh weight

[b] Odour description of the corresponding isothiocyanates

content of indole-3-methyl-GLS (glucobrassicin) was found in calli. Their data clearly demonstrated that the occurrence of indole GLSs in embryoids, suspensions cells and calli relates to their age. It has also been reported that younger and growing tissues synthesize more indole GLSs than older and senescent tissue, so their results raise the question of the role of GLSs in healthy plants (Mevy et al. 1997). Mevy et al. (1999), by comparison of immobilized and suspension cells of horseradish, showed that GLS production may be improved by cell immobilization technique. Moreover, the chromatograms obtained revealed that glucobrassicin occurred earlier than its hydroxylated form in cells, therefore their data suggest that hydroxyglucobrassicin might result directly from modification of the side chain of glucobrassicin.

Table 3 Glucosinolate contents of various horseradish tissue

Materials	Medium[a]	Glucosinolate contents (μg/g dry wt.)[b]				Total contents
		IMG	OH-IMG	PhG	PrG	
Callus (28 days)	MS	4.95 (0.30)[c]	0.32 (0.03)	–	–	5.27
Suspension cells (21 days)	MS	0.09 (0.02)	–	–	–	0.09
Suspension cells (21 days)	D-MS	0.31 (0.04)	–	–	–	0.31
Embryoids (28 days)	D-MS	0.71 (0.02)	–	–	–	0.71
Root of r. p.[d] (3 months)	H-MS	–	–	2.35 (0.40)	–	2.35
Small leaves of r. p. (3 months)	H-MS	–	–	–	16.4 (4.00)	16.4
Large leaves of r. p. (3 months)	H-MS	–	–	–	28.3 (5.00)	28.3

Table modified from Mevy et al. (1997)

[a] Murashige and Skoog basal salts with organic substances, MS; 2,4-D-free-MS, D-MS; hormone-free-MS, H-MS

[b] Indole-3-methylglucosinolte, IMG; 4-hydroxy-indole-3-methylglucosinolate, OH-IMG; 2-phenylethylglucosinolate, PhG; 2-propenylglucosinolate, PrG

[c] Mean values of three independent extractions ±(SD)

[d] *r. p.* regenerated plantlets

Redovnikovic et al. (2008a) analyzed horseradish plantlets leaves by reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of desulphoglucosinolates and showed that the aliphatic GLS 2-propenyl-GLS (sinigrin) accounted for more than 80 % of total GLSs. Lower amounts of phenylethyl-GLS (gluconasturtiin) and of the three indole compounds 3-indolylmethyl-GLS (glucobrassicin), 4-methoxy-3-indolylmethyl-GLS (4-methoxyglucobrassicin) and 4-hydroxy-3-indolylmethyl-GLS (4-hydroxyglucobrassicin) were found. Li and Kushad (2004) evaluated roots from 27 horseradish accessions and leaves from 9 accessions for their GLS content by RP-HPLC as desulphoglucosinolates. In roots, total GLS concentration ranged from 2 to 296 μmol g^{-1} of dry weight (DW), with sinigrin representing on average 83 % of the total GLSs, followed by gluconasturtiin with 11 %, and glucobrassicin with 1 %. In some of the accessions, other GLSs as progoitrin, gluconapin, 4-hydroxyglucobrassicin, and 4-methoxyglucobrassicin, were detected in minor concentrations (<1 %). According to the same authors (Li and Kushad 2004), the origin of plant did not seem to have an effect on total GLSs level; furthermore, the average total GLS concentration in most horseradish roots accessions was similar to the value for brown and oriental mustard seeds, but significantly higher than what the authors had previously reported for broccoli (13 μmol g^{-1} of DW), brussels sprouts (25 μmol g^{-1} of DW), cabbage (11 μmol g^{-1} of DW), cauliflower (15 μmol g^{-1} of DW), or kale (15 μmol g^{-1} of DW). The total GLS levels in the leaves, instead, ranged from 34 to 201 μmol g^{-1} of DW. Similar to the roots, sinigrin was the dominant GLSs in the leaves, representing on average 92 % of the total. Leaves also contained gluconasturtiin, but at a much lower concentration (0.2 μmol g^{-1} of DW) than in roots (10.3 μmol g^{-1} of DW). Conversely, leaf tissues contained neoglucobrassicin (2.5 % of total) instead of glucobrassicin.

Recently, Alnsour et al. (2012), referring to the GLS content, confirmed previous results of Redovnikovic et al. (2008a) and Mevy et al. (1997) showing that the overall GLS concentration (maximum 40 μmol g^{-1} of DW) in the in vitro cultivated *Armoracia* plants is much lower than that found by Li and Kushad (2004) in soil-grown ones (in average 100 μmol g^{-1} of DW). Surprisingly, in contrast to soil grown plants, where the GLS concentration in roots and leaves were quite similar, in the in vitro plantlets the concentration of GLS in the root was much lower than in the leaves (Alnsour et al. 2012).

The distribution of the GLSs varies among plant organs, with both quantitative and qualitative differences between roots, leaves, stems and seeds; in any case the plant age is a major determinant of the qualitative and quantitative GLS composition in the plants (Fahey et al. 2001; Velasco et al. 2007; Cleemput and Becker 2011). Li and Kushad (2004) reported differences in GLS content between young

and fully developed roots and leaves of horseradish (Table 4), noting that the major differences were observed regarding indole GLSs, in fact the younger tissue of roots and leaves tended to accumulate more glucobrassicin and neoglucobrassicin, respectively, than fully developed organs (>60-fold higher in young leaves than fully developed leaves). Due to the complexity of this vegetal matrix, further studies are required to better understand the accumulation of various GLSs during different growth stages also by comparing of different genotypes. For the model plant *Arabidopsis thaliana*, a member of the Brassicaceae, it was reported that GLS accumulation varies significantly among different organs and developmental stages, with regards to both composition and concentration (Brown et al. 2003; Yang and Quiros 2010). Particularly, dormant and germinating seeds had the highest concentration (2.5–3.3 % by dry weight); inflorescences and siliques had the next highest levels (0.6–1.2 %) followed by roots, stems and cauline leaves, and rosette leaves (0.3–1.0 %) (Fahey et al. 2001; Brown et al. 2003). The GLSs are plant defense compounds that accumulate preferentially in the reproductive organs as seeds, flowers, fruits and young sprouts (Fahey et al. 2001; Brown et al. 2003; Bellostas et al. 2007b). The low GLS concentration in the leaves and stems in comparison with the seeds could be attributed to the dilution of GLSs during plant growth (Clossais-Besnard and Larher 1991; Cleemput and Becker 2011). Recently, Agneta et al. (2012) gave a complex profile of naturally occurring intact GLSs in sprouts and roots of a horseradish accession largely diffused in southern Italy by using reversed-phase liquid chromatography coupled with electrospray ionization and a hybrid quadrupole linear ion trap and Fourier transform ion cyclotron resonance mass spectrometry (LC-ESI-FTICR MS). In sprouts 16 and in roots 11 GLSs were identified (Table 5). The authors confirmed the presence of sinigrin, 4-hydroxyglucobrassicin, glucobrassicin, gluconasturtiin, and 4-methoxyglucobrassicin and identified glucoiberin, gluconapin, glucocochlearin, glucoconringianin, glucosativin, glucoibarin, 5-hydroxyglucobrassicin, glucocapparilinearisin or glucobrassicanapin, glucotropaeolin, and glucoarabishirsutain, not previously characterized in horseradish. Particularly notable was the presence of the putative 2-methylsulfonyl-oxo-ethyl-GLS, not reported before. In addition, horseradish

Table 4 Change in total, individual and residual glucosinolates in two accession of horseradish roots and leaves as a function of growth stage

Accession	Growth stage	Sinigrin	Glucobrassicin	Gluconasturtiin	Total GLSs[a]	Residual GLSs[b]
		μmol g^{-1} of DW				
Roots						
810-A	Small	58.0b[c]	13.4b	0.2c	74.7b	3.1c
810-A	Mature	258.0a	2.8c	20.1a	295.8a	14.9a
1573	Small	77.3b	33.9a	0.2c	119.1b	7.7ab
1573	Mature	47.5b	0.7c	2.0b	55.5c	5.3bc
Accession	Growth stage	Sinigrin	Neoglucobrassicin	Gluconasturtiin	Total GLSs	Residual GLSs
		μmol g^{-1} of DW				
Leaves						
810-A	Small	77.6ab	66.2a	0.5a	150.2a	2.3a
810-A	Mature	114.8a	1.2b	0.1a	126.0a	1.1a
1573	Small	60.3b	69.7a	0.3a	129.2a	2.4a
1573	Mature	48.6c	1.1b	0.4a	52.7b	1.2a

Table modified from Li and Kushad (2004)

[a] Total glucosinolates in roots and leaves represent the sum of sinigrin, glucobrassicin, and gluconasturtiin plus residual glucosinolates and the sum of sinigrin, neoglucobrassicin and gluconasturtiin plus residual glucosinolates, respectively

[b] Residual glucosinolates is the sum of at least four additional glucosinolates

[c] Values represent the means of four replicates per accession. Mean separation within each column by Duncan's multiple-range test, $P = 0.05$

Table 5 Glucosinolates identified in different portions of horseradish plants by LC-ESI-FTICR MS: common name, retention time (t_R), molecular formulae, monoisotopic value as $[M\text{-}H]^-$ ion (*m/z*), mass error (ppm)

GLS common name	t_R (min)	Molecular formulae	Monoisotopic calculated value as $[M\text{-}H]^-$ ion (*m/z*)	Mass error (ppm)[a]
Glucoiberin	4.3	$C_{11}H_{21}NO_{10}S_3$	422.02549	−1.30
Sinigrin	4.4	$C_{10}H_{17}NO_9S_2$	358.02720	−0.59
2-Methylsulfonyl-oxo-ethyl-GLS	4.6	$C_{10}H_{17}NO_{12}S_3$	437.98402	−1.21
Gluconapin	5.5	$C_{11}H_{19}NO_9S_2$	372.04285	−1.30
Glucocochlearin	6.2	$C_{11}H_{21}NO_9S_2$	374.05850	1.00
Glucoconringianin	6.4	$C_{11}H_{21}NO_9S_2$	374.05850	−0.70
Glucosativin	6.5	$C_{11}H_{21}NO_9S_3$	406.03057	1.35
Glucoibarin	7.3	$C_{15}H_{29}NO_{10}S_3$	478.08808	−0.10
4-Hydroxyglucobrassicin	7.4	$C_{16}H_{20}N_2O_{10}S_2$	463.04866	−1.50
5-Hydroxyglucobrassicin	7.5	$C_{16}H_{20}N_2O_{10}S_2$	463.04866	−1.70
Glucocapparilinearisin or Glucobrassicanapin	7.8	$C_{12}H_{21}NO_9S_2$	386.05850	−0.20
Glucotropaeolin	8.5	$C_{14}H_{19}NO_9S_2$	408.04285	−1.10
Glucobrassicin	10.3	$C_{16}H_{20}N_2O_9S_2$	447.05375	−1.50
Gluconasturtiin	12.1	$C_{15}H_{21}NO_9S_2$	422.05850	−1.20
4-Methoxyglucobrassicin	12.7	$C_{17}H_{22}N_2O_{10}S_2$	477.06431	−1.20
Glucoarabishirsutain	16.7	$C_{15}H_{29}NO_9S_3$	462.09317	−1.20

Table modified from Agneta et al. (2012)

[a] Mass error in part per million, ppm = 10^6 × (accurate mass-exact mass)/exact mass

sprouts were found to be richer in GLSs than roots. Further studies are required to quantify the content of these molecules that are responsible for the typical flavour of this plant.

Normally, the components that cause the intense pungency are physically separated from one another (Shehata et al. 2009). GLSs are stored in the vacuole (Grob and Matile 1979; Helmlinger et al. 1983; Koroleva et al. 2000) while the myrosinase enzyme that causes their degradation (Mucete et al. 2006) is confined within specialized cells known as myrosin cells (Kissen et al. 2009; Borgen et al. 2010). Upon crushing horseradish roots or leaves allyl and 2-phenylethyl isothiocyanate are produced from sinigrin and 2-phenylethyl-GLS. Allyl isothiocyanate is reported to be a lachrymatory compound, bitter in taste with a strong, pungent smell, while 2-phenylethyl isothiocyanate had no pungency and lachrymatory role at all, like allyl isothiocyanate (Sultana et al. 2003; Shehata et al. 2009). Both isothiocyanates are largely responsible for the typical, characteristic flavour and pungency in mustard as well as in horseradish (Mithen et al. 2000; Sultana et al. 2003; Kosson and Horbowicz 2008).

Recently, eighteen compounds that contribute to the flavour of horseradish were identified in roots immediately after harvesting (Table 6) by D'Auria et al. (2004), using SPME–GC–MS (solid phase microextraction coupled with gas chromatography mass spectrometry). The main compounds were allyl isothiocyanate, 4-isothiocyanato-1-butene, and 2 phenylethyl isothiocyanate. Allyl isothiocyanate was the largest peak in the chromatogram and accounted for 81 % of the overall integrated peak area. It is noteworthy that methyl, ethyl, and isopropyl isothiocyanate were not found. By the same authors (D'Auria et al. 2004) the different volatile organic compounds listed in Table 6 were identified in cut horseradish kept at 5 °C for 12 h. The concentration of most of the volatile compounds declined rapidly and 2-phenylethyl isothiocyanate compounds became the most abundant compound. The fact that the flavour composition of cut horseradish samples changes is relevant when specifying the treatment of horseradish to obtain mustard.

To preserve the pungency and quality, the ground product should be consumed quickly or refrigerated to minimize loss of volatile flavour compounds.

Table 6 Volatile organic compounds found in the fresh horseradish root and in the cut of roots kept at 5 °C for 12 h

Compound	r.t. (min)	Fresh ppm	5 °C for 12 h ppm
Thiobismethane	1.76	0.8 ± 0.2	1.4 ± 0.1
Carbon disulphide	1.81	0.8 ± 0.2	–
3-Butenenitrile	2.38	7.8 ± 0.1	–
3-Methylbutanal	2.73	0.4 ± 0.2	0.6 ± 0.1
2-Ethylfuran	2.78	1.2 ± 0.1	5.8 ± 0.3
3-Methyl-1-butanol	3.16	–	1.4 ± 0.1
Toluene	3.69	–	0.8 ± 0.1
Hexanal	4.32	4.5 ± 0.1	3 ± 0.3
E-2-hexenal	5.42	1.6 ± 0.1	3 ± 0.2
Heptanal	6.45	–	0.3 ± 0.1
Allyl isothiocyanate	6.64	3,300 ± 2.0	175 ± 2.0
Benzaldehyde	7.78	–	0.5 ± 0.1
Isobutyl isothiocyanate	7.85	15 ± 0.3	–
4-Isothiocyanato-1-butene	8.45	63 ± 1.0	–
2-Pentylfuran	8.45	–	3 ± 0.3
Butyl isothiocyanate	8.74	13 ± 0.3	–
2-Ethyl-1-hexanol	9.24	–	1.8 ± 0.2
Phenylacetaldehyde	9.60	–	0.3 ± 0.1
3-Methylbutyl isothiocyanate	9.98	8.6 ± 0.2	–
Pentyl isothiocyanate	10.78	3.7 ± 0.2	–
Nonanal	10.85	1.6 ± 0.2	1.4 ± 0.2
4-Ethylbenzaldehyde	12.01	–	0.4 ± 0.1
4-Methylpentyl isothiocyanate	12.02	0.4 ± 0.1	–
L-(−)-Menthol	12.20	–	0.2 ± 0.1
Naphthalene	12.43	–	0.2 ± 0.1
Decanal	12.76	–	0.5 ± 0.1
Trans,trans-nona-2,4-dienal	12.95	–	0.2 ± 0.1
Benzenepropanenitrile	13.49	0.8 ± 0.1	0.2 ± 0.1
Benzyl isothiocyanate	15.67	14 ± 0.2	8.8 ± 2.0
Junipene	16.43	–	0.2 ± 0.1
Italicene	17.24	–	0.8 ± 0.1
2-Phenylethyl isothiocyanate	17.44	400 ± 1.0	432 ± 3.0

Table modified from D'Auria et al. (2004)

However, ground horseradish slowly losses its pungency, becomes dark, and develops off flavours even under refrigeration; this quality loss can be slowed by adding a fat or oil, such as cream (Courter and Rhodes 1969). Kosson and Horbowicz (2009) showed that higher storage temperature (2, 8 and 18 °C were tested) caused faster decline of isothiocyanates concentration in horseradish cream. Therefore, to maintain the human health-promoting compounds in processed horseradish, the authors suggested storing horseradish cream under cold conditions. Furthermore, also dehydrated horseradish in diced, flaked or powder form has a great potential as a flavour ingredient in sauces, dressings, and seasoning formulations (Sahasrabudhe and Mullin 1980). Sahasrabudhe and Mullin (1980) recommended that in processing horseradish to a dried product, one must process the cleaned roots immediately after dicing or crushing and maintain the temperature below 65 °C, to retain viability of the myrosinase to obtain a product with the desired flavour intensity and acceptable color. In addition, Sahasrabudhe and Mullin (1980) evaluated the odour intensity of the powders by sniffing, after adding water: they noted that freeze dried

samples and samples processed at 65 °C had the same odor intensity as fresh samples while samples processed at 90 °C lacked odor, as a consequence of deactivation of myrosinase at temperatures higher than 70 °C.

Further investigations are required to quantify the content of the molecules that are responsible for the typical flavour, which is one of the main characteristics for making a high-quality product and to achieve a high marketable yield, as requested by the agro-industrial and pharmaceutical sectors.

Biological activities of *Armoracia rusticana*: human health, antimicrobial and insecticidal effects

When plant tissue is disrupted or crushed, myrosinase hydrolyzes GLSs to variety of molecules. These products, especially isothiocyanates, are reported to have biological activities including anticarcinogenic activity (Verhoeven et al. 1997; Murillo and Mehta 2001; Balasinska et al. 2005). GLSs and their breakdown products may act as inhibitors of microbial growth (Tierens et al. 2001), deterrents for herbivores, and as attractants of specialist insects (Redovnikovic et al. 2008b); moreover, these secondary metabolites suppress soil-borne organisms such as bacteria, fungi, viruses, nematodes and weeds (Tedeschi et al. 2011).

Particularly, the activity of isothiocyanates such as sulforaphane against numerous human pathogens (e.g. *Escherichia coli*, *Salmonella typhimurium*, *Candida* sp.) could contribute to the medicinal properties ascribed to Brassicaceae vegetables, such as cabbage and mustard, which have been used as wound poultices and antitumor agents for centuries (Hartwell 1982; Fahey et al. 2001). More recent interest has been focussed on the potential anticarcinogenic activity of GLS degradation products (Mithen 2001). The reason of this increasing interest is due to the strong correlation found between the consumption of Brassicaceae vegetables and the decreased risk for pancreas, lung, stomach, prostate, and breast cancer, as recently reported by Velasco et al. (2010). Particularly, several studies have shown that allyl isothiocyanate may inhibit different kinds of human prostate cancer (Srivastava et al. 2003) or could contribute to the lower incidence of bladder cancer (Munday 2002). Horseradish contains more than 10 times as much GLSs as broccoli. The high levels of sinigrin in horseradish (Balasinska et al. 2005), easily hydrolyzed by myrosinase to allyl isothiocyanate when the roots are crushed (Matsuda et al. 2007), makes this root interesting as a possible cancer-protecting component in the diet (Talalay and Fahey 2001; Wedelsbäck Bladh and Olsson 2011). As reported by Shehata et al. (2009), some German studies have investigated the effects of horseradish on nonspecific urinary tract infections and the antibacterial action of its essential oils. A preparation with horseradish containing sinigrin or allyl isothiocyanate was patented in 1995 for topical application as a spray or applied with a swab onto the affected mucosa, for treatment of nasal and sinus dysfunction (Friedman 1995). Horseradish has been approved in Germany for the treatment of infections of the respiratory tract and as supportive treatment in urinary tract infections. In the United States, the root is the active ingredient of Rasapen ®, a urinary antiseptic drug (Shehata et al. 2009).

Recently, reported health benefits of horseradish and its constituents include plasma cholesterol reduction in mice (Balasinska et al. 2005; Hara et al. 2008), tumor cell proliferation and cyclooxygenase activity prevention (Weil et al. 2005; Hara et al. 2008), as well as an antimicrobial effect on *Vibrio parahaemolyticus* by horseradish extract (Yano et al. 2006; Hara et al. 2008). Matsuda et al. (2007) showed that allyl isothiocyanate provided significant protection against ethanol-induced gastric lesions. Majewska et al. (2004) have evaluated some antioxidant properties of leaf and root extracts and mustard oil from four different types of horseradish growing under different environmental conditions: although leaf and root extracts did not exhibit strong antioxidant properties, the different environmental conditions affected these properties significantly; also, volatile oil obtained from horseradish roots revealed stronger antioxidant properties than pure allyl isothiocyanate.

According to Mari et al. (2003) allyl isothiocyanate, a naturally occurring flavour compound in mustard and horseradish, has a well-documented antimicrobial activity, therefore this volatile substance can be employed successfully in modified atmosphere packaging or as a gaseous treatment before storage. Also, horseradish distillate is a potentially useful antimicrobial adjunct in packaged, refrigerated precooked meats, in fact, essential oils of Brassicaceae plants, including horseradish, have long been known to possess antibacterial activity versus pathogenic

bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Serratia grimesii* (Ward et al. 1998). Khomvilai et al. (2006) observed the fungicidal activities of allyl isothiocyanate against *Saprolegnia parasitica*, a fish-pathogen oomycete, and determined both minimum inhibitory concentration for mycelia growth (68 mg/l with 60 min exposure) and zoospore germination (42.5 mg/l with 5 min exposure). Recently, first results of antifungal tests demonstrated that horseradish ethanol extracts also present a fungistatic activity against *Sclerotium rolfsii* Sacc., *Fusarium oxysporum* Schlecht and *Fusarium culmorum* (Wm. G. Sm.) Sacc. (Tedeschi et al. 2011).

Moreover, Park et al. (2006) have tested essential oils from 40 plant species for their insecticidal activities against larvae of *Lycoriella ingenue* (Dufour) using a fumigation bioassay: horseradish showed the most potent insecticidal activity among the plant essential oils (only 1.25 µl/l caused 100 % mortality) the most toxic compound to *L. ingenue* was allyl isothiocyanate. In addition, also the results obtained by Wu et al. (2009) suggest good insecticidal efficacy of allyl isothiocyanate obtained from *A. rusticana* as alternative fumigant against four major pest species of stored products, maize weevil *Sitophilus zeamais* (Motsch.), lesser grain borer *Rhizopertha dominica* (F.), *Tribolium ferrugineum* (F.) and book louse *Liposcelis entomophila* (Enderlein). Furthermore, interesting and significant insecticidal activity against larvae of *Aedes albopictus* (Skuse) (LC_{50} value of 2.34 g/l) was obtained by using horseradish extract prepared from fresh plants in a solution of ethanol 80 % (Tedeschi et al. 2011). Recently, Chen et al. (2011) have assessed the fumigant toxicity of essential oil from horseradish against *Plodia interpunctella* (Hübner) and *Sitophilus zeamais* Motschulsky. The essential oil of *A. rusticana* showed fumigant bioactivity against all stages of *P. interpunctella* and adults of *S. zeamais*. It caused high mortality of all stages of *P. interpunctella* when exposed to 32 µl/l gas vapour of *A. rusticana*. The oil appeared not to have much impact on the adults of *S. zeamais* when the gas vapour was lower than 16 µl/l, however, at 32 µl/l, the percentage mortality was 100 %. GC–MS showed that the main component of the essential oil from *A. rusticana* was allyl isothiocyanate (98 %).

Armoracia rusticana oil or its major constituents could be efficient fumigants and also could be integrated with other pest management procedures (Chen et al. 2011). For the practical use of horseradish oil and their constituents as novel fumigants, further study is necessary on the safety of these materials to humans and on development of formulation to improve efficacy and stability, and to reduce cost (Park et al. 2006). Although higher concentrations of these natural anti-fungal and insecticidal products should be used, their intrinsically low toxicity towards humans makes them an interesting alternative to current chemicals. All these researches demonstrated that horseradish extracts could be promising alternatives to synthetic agrochemical products although more work is needed (Tedeschi et al. 2011).

Another suggested use for horseradish includes weed suppression via the allelopathic response of GLSs in soil. GLSs and their byproducts can act as biofumigants when used as green manure, thus showing potential for weed control in organic production (Shehata et al. 2009).

Genetic variability and influence of agronomic management on glucosinolate content

The GLS content and composition *in planta* can be strongly influenced by genotype and environment (Rosa et al. 1997, 2005; Sarikamiş et al. 2009; Kabouw et al. 2010).

The genetic variability may play a primary role in determining the amount of functional metabolites, such as GLSs (Brown et al. 2002; De Pascale et al. 2007). Only few studies have investigated GLS variation within different horseradish genotypes. Among these, Li and Kushad (2004) have evaluated the GLS levels in root and leaf samples of horseradish accessions of a germplasm collection of the Vegetable Research Farm, University of Illinois, Urbana-Champaign, IL. They found that GLS content varied widely among all accessions. According to Faltusová et al. (2011), genotypes with high GLS contents will be further utilized as a potential genetic source for breeding. To date, information on genetic diversity between different accessions of horseradish, are scarce. Although molecular marker analyses are successfully used for characterization of genetic resources of many species, bio-agronomic characterization is the first step for the description and classification of germplasm (Lotti et al. 2008; Sarli et al. 2012).

Rhodes et al. (1969) have classified 20 genotypes of horseradish, grown at the University of Illinois Horticultural Farm, chosen from a gene pool containing over 400 genotypes cultivars to represent the extreme and intermediate forms of morphological variability found in horseradish. Two methods of classification were compared. One classification was based on 2 highly diagnostic characters (basal angle and rugose index) that showed the extreme and intermediate limits of the germplasm in form of a scatter plot. The other classification was based on 40 characters. For this last classification, methods of numerical taxonomy were used to show the germplasm diversity by scatter diagrams and phenograms. The two classifications appeared to be equal in defining the extreme limits of the genetic variability, although minor differences were found among the relative positions of some genotypes. Sarli et al. (2012) have recorded and statistically analyzed data regarding the variation of 26 morphological descriptors by using modified UPOV (International Union for the Protection of New Varieties of Plants) guidelines for performing tests for distinctness, uniformity and stability for horseradish. In this study morphological traits of 30 horseradish accessions from Basilicata region (South Italy) were characterized and compared to two European genotypes: the resulting cluster displayed four major groups of genotypes, on the base of the characteristics of rhizome and leaves. The results showed a significant degree of variability among accessions. Also, the results of univariate and multivariate analysis showed that the investigated accessions cannot be clearly separated according to their place of origin, assuming that a distribution of an accession is facilitated by territorial continuity and similarity. According to Sarli et al. (2012), this preliminary screening of a horseradish germplasm collection is quite useful to perform future programs of conservation *in situ* and *ex situ*, in order to expand and improve the cultivation of *A. rusticana*. In any case, there is a lack of basic biological and genetic information about horseradish (Shehata et al. 2009). The first studies, as reported above, could be a good starting point to develop methods to measure genetic diversity in different accessions of horseradish. The genetic relationships among cultivars, breeding lines, and wild accessions need to be determined, so that the greatest possible gain can be made in specific crosses (Shehata et al. 2009). Until today, only few studies were performed on the characterization of genetic diversity of horseradish. Various molecular marker systems applied in plants belonging to the Brassicaceae family (Margalé et al. 1995; Farnham 1996; Rabbani et al. 1998; Das et al. 1999, Negi et al. 2004; Faltusová et al. 2011) may be useful for a first detection of polymorphisms in this species. However, the morphological and genetic variation that seems to exist among different horseradish accessions could be compared with the GLS content of various horseradish accessions for developing improved cultivars. In fact, there is a growing interest in the metabolic engineering of plants for the production of high-value bioactive compounds, because the availability of phytochemicals in their natural sources is often limited (Møldrup et al. 2011). Therefore the information on genetic diversity could be utilized in order to identify genotypes with desirable traits for future breeding programs.

There are even less studies on the effects of environment and cultivation techniques on yield and GLS content of this plant. Some researchers have demonstrated that GLS profile can be manipulated by cultural management (Rosen et al. 2005). About the crop management, fertilization have been shown to significantly affect GLS concentration in plant, particularly sulphur (S) and nitrogen (N) (Kim et al. 2002; Rosen et al. 2005), but some controversy still exists on such effects (Aires et al. 2006). Horseradish is a crop that needs a lot of nutrients. Its powerful root system drains large amounts of soil moisture and nutrient reserves (Perlaki and Djurovka 2009). However, until today, there is little information about the GLS content in relation to S and N fertilization, with particular regard to sprouts, young leaves and roots. S and N are necessary for the synthesis of amino acids, proteins, and various other cellular components, including thiol compounds and the so-called secondary sulphur compounds, which have a significant bearing on protection of plants against stress and pests (Anjum et al. 2012). GLS content is highly correlated with the S content (Koroleva et al. 2000), therefore, there is a strong potential for modifying GLS accumulation in crop plants by altering S nutrition (Falk et al. 2007). Several reports indicated that S fertilization may affect the GLS level more than N fertilization (Rosen et al. 2005; De Pascale et al. 2007). In fact, each GLS molecule contains at least two sulfur atoms. One is part of the sulfate group originating from 3′-phosphoadenosine

5′-phosphosulfate (PAPS), while the other, originating from cysteine, is part of an S-linked glucose residue. In addition, many GLSs are synthezised from methionine and so may have a third sulfur atom in their structures (Falk et al. 2007). S fertilization leads to increases in GLS content in most cases. In some cases an over 10-fold increase was observed, as reported by Falk et al. (2007) in their review about the effect of S supply on GLS content.

Alnsour et al. (2012) showed recently that in vitro grown horseradish plantlets contained increasing GLS concentrations when the culture media was supplemented with additional sulfate. They concluded that GLS concentrations in the stalks and leaves of *Armoracia* in vitro plants could be modulated 20-fold by varying the sulfate concentration in the medium, while the increase of sulfate concentration had nearly no impact on the GLS concentration in the roots.

The GLS content of a plant is dependent not only on S nutrition, but also on the N level of the plant. Particularly, through handling of fertility management, the experimental results of Rosen et al. (2005) clearly demonstrate that GLSs in cabbage were maximized at low N and high S application rates. As the ratio of N to S increases, GLS content declines, probably because vegetative growth outpaces GLS biosynthesis, diluting the content of these metabolites (Kim et al. 2002; Rosen et al. 2005; Falk et al. 2007). To quantify the effects of supply and combination of these nutrients on GLS composition, Li et al. (2007) showed as the total GLS concentration in turnip roots was strongly influenced by N and S supply: with low S supply (10 and 20 kg ha^{-1}), total GLS concentrations were 0.8–2.3 times higher at 80 kg N ha^{-1} compared to treatments with 160 and 240 kg N ha^{-1}; however, at the high S level (60 kg ha^{-1}), increasing N supply (160, 240, and 320 N ha^{-1}) did not affect total GLS concentration. In broccoli, Omirou et al. (2009) found that the GLS concentration was low in the 50 kg ha^{-1} N treatments in all plant organs, but it did not respond to N application above 250 kg ha^{-1}, while total GLS concentrations, clearly responded to increasing S applications within the whole wide range of S applications (from 10 to 150 kg ha^{-1}), indicating that S is main determinant of the concentration of total GLSs in the plant organs.

All these studies suggest that manipulating the N and S supply might be one means of altering GLS level in plants, thereby providing an opportunity to enhance organoleptic properties and health benefits of horseradish, or its value as biofumigant (Falk et al. 2007; Li et al. 2007).

To maintain proper nutrition, fertilizer is applied based on results from chemical soil analysis, soil type, cultivar, and cropping history (Shehata et al. 2009). As reported in the review of Walters and Wahle (2010), in *A. rusticana* a typical fertilizer program includes about 168 kg ha^{-1} nitrogen (N), with phosphorus (P) and potassium (K) rates based on soil type or soil test results, with applications generally ranging from 56 to 280 kg ha^{-1}. In addition, about 1–2.8 kg ha^{-1} boron and 17–28 kg ha^{-1} sulfur is made with the initial N–P–K broadcast application. Excessive nitrogen rates should be avoided, as this can result in excessive foliar growth and highly branched, irregular root formation (Shehata et al. 2009). For the best response to fertilizers, a pH range of 6–7.5 is ideal. The horseradish plant thrives in deep loam or sandy soil types, well-drained; organic matter is often added to maintain a good soil structure, while shallow soils or those with hard pans are not suitable as they compromise strong root development and thus may curtail yield (Shehata et al. 2009; Walters and Wahle 2010). Fields are prepared for planting with ridgers to create raised beds that increases yield of high-quality roots by ensuring that the soil is loose so that large roots can develop. Planting is done either by hand or using modified transplanters that place sets (about 10 cm deep for hand planting) in the beds at a 45-degree angle; the spacing adopted is 40–60 cm between plants and 75–90 cm between rows, giving a plant population of 20,000–25,000 plants ha^{-1} (Shehata et al. 2009). Irrigation during drought conditions, especially during late summer and fall, is sometimes done to improve marketable yields because most root-sizing occurs during this period (Walters and Wahle 2010). Usually, horseradish is harvested in late October–November once the foliage has been killed by frost, continuing through the winter and early spring months (Shehata et al. 2009; Walters and Wahle 2010).

Finally, to preserve the traditional knowledge related to crop management, it is important to underline that in regions like Bavaria (Germany) or Hajdúság (Hungary), some cultivation practices (e.g. removing sprouts after planting to obtain a root with a single crown), even today, take place mainly by hand.

More studies are required for a better understanding particularly of the effects of irrigation, fertilization

and time of harvest on yield and quality of horseradish to optimize crop management.

Conclusion

Over recent years, the GLSs, a large group of nitrogen- and sulfur-containing secondary metabolites, particularly abundant in plants belonging to the Brassicaceae and related families, have attracted the attention of the scientific community for their fungicidal, bactericidal, nematicidal, allelopathic and anticancerogenic properties. Among the Brassicaceae vegetables, horseradish is particularly interesting because it is an exceptional rich source of GLSs which could be employed for agro-industrial and pharmaceutical purposes.

Considering this interest, more studies are needed on qualitative and quantitative characterization of GLSs, especially in relation to the variability of the currently available horseradish germplasm, growing conditions and crop management, plant phenological phases and harvest dates.

In addition, it is important to stress that recently the interest in natural substances has increased and numerous studies on the biocidal activity of a wide range of secondary metabolites have been reported, as for example for the allyl isothiocyanate produced from sinigrin with a well-documented antimicrobial activity. As reported by Mari et al. (2003), in Japan, synthetic allyl isothiocyanate is registered as a food additive and preliminary data showed that food preserved with this compound contains only very low residue of allyl isothiocyanate (Isshiki et al. 1992). Therefore, natural extracts of horseradish could be used as alternative to synthetic molecules.

For the great heritage about the gastronomic and folk medical uses of horseradish, the species should be valorized to prevent extinction of genotypes of high nutritional value and specific taste and flavour and farmers should continue to cultivate landraces in traditional cultivation areas, maintaining the links with territory and its history. In fact, the clonal propagation of *A. rusticana* endangers its genetic diversity, so efforts are required to establish well-documented collections and to analyze genetic diversity in all accessions available. In addition, legal protection of geographic identity of horseradish landraces could contribute to conservation of genetic resources, and at same time, could increase the viability of little farms. For example, by following a centuries-old tradition, horseradish is today grown by farmers in Bavaria and Hajdúság regions as an PGI (Protected Geographical Indication) and PDO (Protected Designation of Origin), respectively, to preserve the historical link with the territory. For small scale growers, horseradish has potential marketing outlets through fresh sales to local restaurants, grocery and specialty food stores, and to specialized food processing companies. They can also make sales at farmers markets and roadside stands. For large scale production, it is common for condiment processors to work on a contract basis with growers. These markets can be competitive and difficult to break into (Bratsch 2009).

Today, further investigations on the chemical, biological and molecular aspects of the GLSs and the composition and partitioning in the plant are needed to achieve the goal of efficient biodiversity conservation to prevent the genetic erosion of local genotypes of horseradish and the risk to lose traits that can be useful in future breeding programs.

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