Journal of Applied Botany and Food Quality 91, 232 - 236 (2018), DOI:10.5073/JABFQ.2018.091.031

Friedrich-Alexander-Universität Erlangen-Nürnberg, Lehrstuhl für Pharmazeutische Biologie, Erlangen, Germany

Development and validation of a quick assay for the total glucosinolate content in horseradish (*Armoracia rusticana*) using glucose strips and a blood glucose meter

Nadine Meitinger, Wolfgang Kreis*

(Submitted: August 28, 2018; Accepted: October 7, 2018)

Summary

A quick assay to determine the total glucosinolate content of fresh horseradish roots in less than 10 minutes is described. The method involves the following steps: 1. Maceration of horseradish root with 4% phosphoric acid to avoid enzymatic degradation of endogenous glucosinolates, 2. neutralization of the extract and determination of free glucose using a commercial blood glucose meter, 3. enzymatic hydrolysis of the glucosinolates by exogenous myrosinase, 4. detection of released glucose, again using a blood glucose meter, and 5. calculation of the glucosinolate content on the basis of the difference between the two glucose values determined. The newly developed assay ('ITC quick test') was compared with a standard high-performance liquid chromatographic method for glucosinolate analysis.

Keywords: *Armoracia rusticana,* Glucosinolates, Allyl isothiocyanate, Sinigrin, Glucose

Introduction

The root of *Armoracia rusticana* P. Gaertn., B. Mey. & Scherb. (horseradish) is extensively used as a spice, usually grated fresh as a condiment or as a food additive for its pungent flavor. Both taste and quality of horseradish as well as of other cruciferous vegetables are significantly determined by the glucosinolate content. Glucosinolates are anionic and sulfur-rich natural products, which primarily occur in plants of the Brassicaceae family. The characteristic flavor mainly originates from the breakdown of water-soluble glucosinolates, stored in the plant vacuole, into volatile isothiocyanates (Sampliner and Miller, 2009; Agneta et al., 2012) Another feature of these breakdown products is their antimicrobial, antifungal and chemopreventive action (Glenn et al., 1988; Brabban and Edwards, 1995; Talalay and Zhang, 1996). Disruption of glucosinolate-containing plant material by herbivore attack, chopping or chewing not only results in the release of isothiocyanates, thiocyanates and nitriles but also of glucose and sulfate in amounts equimolar to the amount of glucosinolate precursor present. The conversion of the glucosinolates is initiated by the action of a thioglucosidase termed myrosinase.

Several methods for glucosinolate analysis determining the total glucosinolate content as well as individual glucosinolates and their breakdown products are already available (Moreno et al., 2006). The method most commonly used for glucosinolate determination is the quantitative analysis of desulfoglucosinolates by reversed-phase HPLC (EN ISO 9167). This method, which was developed by Fenwick et al. (1982), uses an on-column enzymatic desulfation treatment of plant extracts followed by HPLC detection of the resulting desulfoglucosinolates. Many of the other described methods resulted from various attempts that have been made to simplify and accelerate the glucosinolate quantification. Still, most of the available methods include time-consuming sample preparation – especially those involving a column purification step (Smith and Dacombe, 1987; Heaney et al., 1988; Doorn et al. 1999; Gallaher et al., 2012). Not in all cases validation of the described methods have been reported and, frequently, rapeseed samples were analyzed. There is thus still a demand for rapid and reliable methods for glucosinolate quantification. Such a fast and simple method could be applied in the field or used for rapid screening of incoming or stored plant material and goods. Tholen et al. (1993) proposed the use of glucose test strips to test for glucose released by myrosinase action to estimate glucosinolates. These attempts were also restricted to rapeseed analysis. Interestingly, this very simple and rapid method has been ignored in recent reports and reviews and has not been developed further.

We here adopted the idea of Tholen et al. (1993) and combined a quick and simple extraction procedure with the determination of the total glucosinolate content by measuring the glucose released following glucosinolate hydrolysis with exogenous myrosinase. The released glucose was then determined with a commercial blood glucose meter and the appropriate test strips. We present a simple, fast and reliable method for estimating the total glucosinolate content in horseradish roots. This method may turn out an alternative to more sophisticated, costly and rather time-consuming HPLC, GC and/or spectrophotometric methods (Fig. 1).

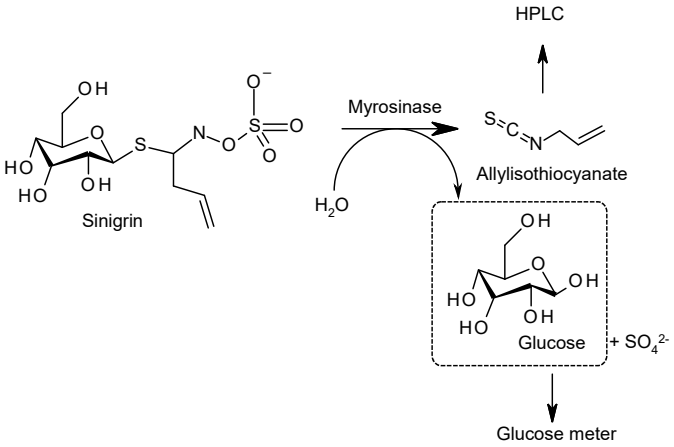

Fig. 1: Rapid assay ('ITC quick test') for estimating the glucosinolate content of horseradish. Exogenous myrosinase is added to allow for the complete degradation of endogenous glucosinolates. Equimolar amounts of glucose, sulfate and isothiocyanate are released. Glucose is determined using a commercial glucose strip/meter system.

Materials and methods

Materials

Sinigrin monohydrate was purchased from Phytoplan Diehm & Neuberger GmbH (Heidelberg, Germany). Fresh horseradish roots were provided by Schamel Meerrettich GmbH & Co.KG (Baiersdorf, Germany). *Sinapis alba* L. seeds were purchased in a local store. All other chemicals were used in *p.a.* quality and purchased from Carl Roth GmbH & Co.KG (Karlsruhe, Germany).

* Corresponding author

Glucosinolate extraction and determination using the glucose strip/glucose meter method ('ITC quick test')

A 1 cm thick slice was cut from a horseradish root. From this, two pieces were carved with a cylindrical plunge borer with an inner diameter of 8 mm. The horseradish pieces were homogenized with 1 mL of 4% v/v phosphoric acid using a mortar and a pestle. The slurry was diluted with 4 mL of distilled water and filtered through a disposable syringe filled with cotton wool. The extract was neutralized by adding 165 µL of 5 N KOH.

To determine the glucosinolate content together with the endogenous glucose content, 50 µL of the extract were mixed with 100 µL of exogenous myrosinase solution (minimum activity 10 U/mL) on a piece of Parafilm M®. To determine the endogenous glucose content only, 50 µL of the extract were mixed with 100 µL of distilled water as a blank control. After 5 min the glucose content of the sample and the blank was measured with an Accu-check Aviva® blood glucose meter (Roche Diagnostics, Mannheim, Germany). The glucosinolate content of the sample was calculated with the formula:

$$\text{GLS (mM)} = 0.079 \times (\text{sample [mg/dl]} - \text{blank [mg/dl]} \times 0.82)$$

The unit mg/dl (which is US standard) was used here since this value can directly be read from the blood glucose meter. The standard curve was prepared by dissolving sinigrin in horseradish extract. The concentrations of the prepared standard solutions ranged from 0.5 mM to 12 mM. Standard solutions were measured as described above. Analyzing the pure horseradish extract (without additional sinigrin) served to eliminate the glucosinolate and endogenous glucose concentration present in the extract.

The factor which takes into account that the blank consists of a mixture of extract and water while in the sample extract is mixed with myrosinase solution was determined by comparing blanks prepared with 100 µL of double distilled water to blanks prepared with 100 µL of heat-denatured myrosinase solution. Additionally, extracts spiked with glucose in three different concentrations were analyzed with water and heat-denatured myrosinase addition.

HPLC and GC-MS

HPLC: 1 mL of the horseradish extract was mixed with 150 µL of 0.5 M barium acetate. Subsequent desulfation of the glucosinolates was performed following the protocol of Li et al. (2004). A 20 µL fraction of the desulfoglucosinolate-fraction (water eluate) was injected onto a HPLC system (Binary HPLC Pump 1525, Dual λ Absorbance Detector 2487, Autosampler 717plus; Waters GmbH, Eschborn, Germany) equipped with a ReproSil Fluosil 100 PFP column (250 × 4.6 mm; 5 µm) (Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany). The following gradient of acetonitrile (B) and water (A) was used at a flow rate of 1 mL/min: 0 - 5 min: 0% B; 5 - 30 min: 30% B, 30 - 32 min: 40% B, 32 - 36 min: 40% B; 36 - 40 min: 100% B; 40 - 45 min: 100% B. Desulfoglucosinolates were identified with a UV detector at 229 nm. Sinigrin concentration was estimated by comparison of HPLC retention time with that of an authentic sinigrin standard after on-column desulfation as described above.

GC-MS: Isothiocyanates (ITC) in the extracts were analyzed by GC-MS. 1 mL of the horseradish solution was extracted with 1 mL of dichloromethane by vortexing for 30 s. The mixture was centrifuged for 5 min at 13000 × *g* (Heraeus Fresco 17, Heraeus Deutschland GmbH & Co., Hanau Germany) before the organic phase was separated. GC-MS analysis of the organic phase was performed using GC-2010 equipped with the mass spectrometer QP-2010S (Shimadzu Deutschland GmbH, Duisburg, Germany) using the following conditions: DB-5ms (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) column (30 m × 0.25 mm, film 0.25 µm); column oven temperature: 60 °C; injector temperature: 230 °C; injection volume: 1 µL; split ratio: 1:100; ion source temperature: 200 °C; interface temperature: 210 °C; MS in scan mode: 35 m/z - 200 m/z. The flowing temperature gradient with helium as gas carrier was implemented at a flow rate of 46.3 cm/s. The temperature was kept constant for three min at 60 °C before it was increased to 210 °C at a rate of 10 °C per min.

Isolation of myrosinase and myrosinase assay

Myrosinase was extracted from *Sinapsis alba* (mustard) seeds. 10 g seeds were ground in a commercial electric coffee grinder and homogenized with double distilled water in a 1:10 ratio (w/v) for 30 min at 4 °C. The homogenate was filtered through 3 layers of Miracloth® and centrifuged for 20 min at 20000 × *g* for 4 °C (SS34 rotor, Sorvall RC5B Plus; Kendro Laboratory Products GmbH, Langenselbold, Germany). $(NH_4)_2SO_4$ was slowly added to the extract as a fine powder while stirring for 30 min to achieve a 50% salt saturation. After centrifugation at 20000 × *g* for 20 min the pellet was discarded. The $(NH_4)_2SO_4$ concentration in the supernatant was increased to 100% before the solution was treated as above. The harvested pellet was dissolved in 10 mL of double distilled water and centrifuged again. The protein solution was desalted and concentrated to an activity of at least 10 U/mL using Amicon Ultra-15 (10 kDa NMGG) centrifugation filters (Merck Millipore, Darmstadt, Germany).

Myrosinase activity was measured following the protocol of Li et al. (2004) with minor modification. 900 µL of sodium phosphate buffer (20 mM, pH 6.5) and 100 µL of a sinigrin solution (1 mM in water) were mixed in a quartz cuvette and pre-incubated at 37 °C for 1 min. The reaction was started by adding 100 µL of protein extract previously diluted 1:40 with water and the decline as result of sinigrin hydrolysis was measured at 227 nm for 180 s using a UV-vis spectrophotometer.

Method validation

For validation we compared the 'ITC quick test' with an established HPLC method (Li et al., 2004).

To determine recovery, sinigrin was dissolved in 4% phosphoric acid to yield concentrations between 1.0 - 10 mM sinigrin. These spiked phosphoric acid solutions were used to prepare the horseradish extracts as described above. The glucosinolate content of the prepared extracts was determined as outlined above and compared with the corresponding non-spiked extracts.

Results and discussion

Developing a rapid method for glucosinolate determination ('ITC quick test')

Hydrolysis of total glucosinolates with myrosinase yields equimolar amounts of glucose. Therefore, glucosinolates were here quantified by measuring the amount of glucose released from them after complete enzymatic hydrolysis. Glucose was determined using a commercial blood glucose meter and the appropriate analytic strips. As mentioned before, Tholen at al. (1993) already used commercial glucose test strips typically used for the semi-quantitive determination of glucose in urine. Others (Smith and Dacombe, 1987; Heaney et al., 1988) describe the use of a glucose/peroxidase assay for glucose quantification as a surrogate for glucosinolates present in a given extract. Whenever glucosinolates are quantified based on enzymatically released glucose it has to be taken into account that plant extracts also contain free glucose and glucosides others than glucosinolates. Therefore, an on-column purification step to remove endogenous glucose (Heaney et al., 1988) or the removal of endogenous glucose from the extract by using glucose oxidase before glucosinolates were hydrolyzed by exogenous myrosinase (Doorn

et al., 1999) is described. The protocol of SMITH and DACOMBE (1986) involves the parallel preparation of two extracts, one with water as extractant to achieve glucosinolate hydrolysis utilizing endogenous myrosinase, the other with acidified methanol (40%) to inhibit endogenous myrosinase and thus to determine the amount of endogenous glucose present in the extract. The method of THOLEN et al. (1993) hydrolyzes glucosinolates at pH 9.0 which was supposed to prevent the liberation of glucose from sources other than glucosinolates. This approach was justified by the fact that myrosinase is quite active at this pH whereas most other glucosidases are inactive at pH values higher than pH 8.0. However, the amount of endogenous glucose was not directly considered in their method and can therefore not be used for plant materials others than rapeseed which contain negligible amounts of free glucose only. We here aimed at avoiding the time-consuming removal of endogenous glucose from the extract. At the same time the method should allow for the estimation of endogenous free glucose in a given plant material.

Glucosinolates were extracted from ca. 1 g of a fresh horseradish root using 1 mL of phosphoric acid. Sampling was standardized by withdrawing two pieces of horseradish with a cylindrical plunge borer with an inner diameter of 8 mm (1.04 ± 0.01 g; n = 10). Before use the horseradish extract was diluted with 4 mL of water and filtered through a syringe partly filled with cotton wool to remove any residual plant tissue. Glucosinolate extraction from the horseradish roots was a first critical step because glucosinolates are immediately hydrolyzed upon homogenization of the plant material by endogenous myrosinase. Extraction with hot water or methanol is therefore used in many protocols in order to avoid enzymatic degradation (GALLAHER et al., 2012; HEANEY et al., 1988; LI and KUSHAD, 2004). The use of boiling solvents is, however, not suitable for a quick glucosinolate assay, that can be used in the field. If organic solvents are used to inhibit the endogenous myrosinase during extraction, the solvent would have to be removed before the glucosinolates can be hydrolyzed by the addition of myrosinase solution. Glucosinolate extraction using 4% phosphoric acid according to DOORN et al. (1998) at room temperature turned out to be a fast and reliable method. No myrosinase activity was detected in horseradish root extracts under these conditions. The activity was also not detectable after the extracts had been neutralized with KOH which indicated that the myrosinase was irreversibly inhibited. In contrast myrosinase activities of 0.17 ± 0.04 U/mL were found in horseradish preparations derived from extraction of horseradish roots with double-distilled water. Each of the different horseradish extracts was also analyzed for the presence of isothiocyanates by GC/MS (Fig. 2). In the extracts prepared with phosphoric acid, no isothiocyanates were detected, not even after the extracts were neutralized with KOH showing that glucosinolates were not hydrolyzed during extraction. When water was used for horseradish extraction, AITC (allyl isothiocyanate) as well as PEITC (phenethyl isothiocyanate) were present demonstrating the glucosinolate breakdown. Sinigrin, the major glucosinolate of horseradish (about 80%), is stable in the acidic extraction medium (DOORN et al., 1998). After neutralization of the extract the glucosinolates were hydrolyzed by the addition of exogenous mustard myrosinase which was prepared from *Sinapsis alba* seeds (see Material and methods). Neutralization of the extract was necessary to optimize the conditions for the addition of the exogenous mustard myrosinase. The addition of 165 µL 5 N KOH neutralized the extract (pH 7.01 ± 0.01; n = 4). Endogenous free glucose was determined after adding water instead of myrosinase solution to the extract. The difference of sample and blank then corresponded to the amount of glucosinolates present in the extract.

The myrosinase solution applied for hydrolysis had to be desalted before use since it was found that high salt concentrations resulted in an error reading of the glucose meter. The desalted enzyme solution could be stored at -20 °C for several weeks without a significant loss of activity.

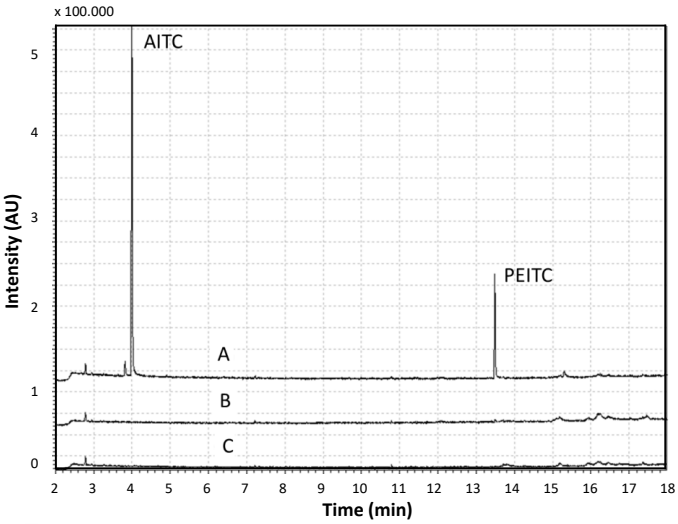

Fig. 2: GC-MS analysis of horseradish extracts prepared with different extracting agents. **A** – Aqueous extract; **B** – 4% Phosphoric acid extract; **C** – 4% Phosphoric acid extract after neutralization. AITC – allyl isothiocyanate; PEITC – phenethyl isothiocyanate)

Complete glucosinolate hydrolysis was achieved after 5 min of incbubation when 1 U/mL of myrosinase was added to 50 µL of horseradish extract. This assay was linear up to 16 mM sinigrin.

Influence of the sample matrix on glucose determination

We used a commercial blood glucose meter for quantifying the glucose content in horseradish extracts. This technique allows glucose quantification within seconds. Blood glucose meters are used to determine the glucose content of blood samples. Therefore, we had to verify its accuracy for measuring glucose in horseradish root extracts. A linear relationship between the actual glucose concentration and the values measured with the glucose meter was proven using three different matrices (Fig. 3). The measured glucose values strongly depended on the sample matrix. When glucose was dissolved in pure water the measured glucose values displayed on the glucose meter were twice as high as predicted by the glucose meter. On the other hand, when glucose was dissolved in a horseradish extract/myrosinase solution the measured glucose concentration matched the amount of glucose present in the assay.

To allow for this matrix effect a standard curve was generated with different amounts of sinigrin dissolved in horseradish extract. The glucose release was then measured after addition of myrosinase and incubation using the blood glucose meter. The values were each corrected by subtracting the glucose content of the crude extract (Fig. 4). Linearity was provided up to 15 mM sinigrin. The factor derived from the slope of the line was used for calculating the glucosinolate content of the extract. The slope of the curve was 1.4 times higher when sinigrin was dissolved in water instead of horseradish extract. This fact corresponds well the matrix effect mentioned above. We also accounted for matrix effects for other constellations (Stop solutions, controls) and finally calculated the total glucosinolate content using the following formula:

$$\mathbf{GLS\ (mM) = 0.079 \times (sample\ [mg/dl] - blank\ [mg/dl] \times 0.82)}$$

where 'GLS' is glucosinolates, 'sample' is the read of the sample after myrosinase action and 'blank' is the read when water instead of myrosinase solution was added.

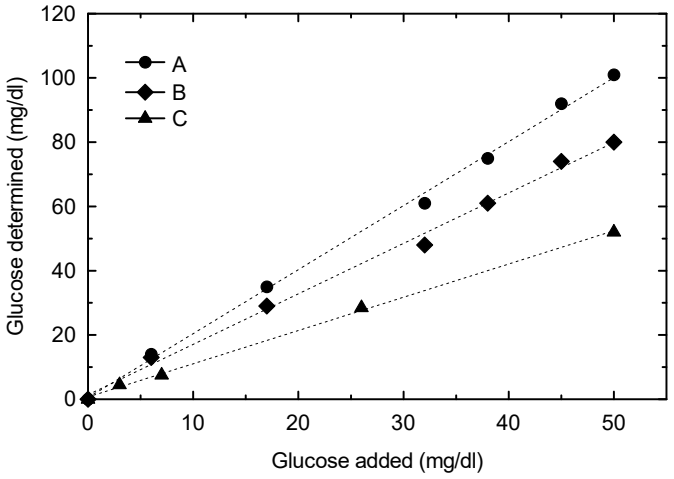

Fig. 3: Influence of the sample matrix on the glucose concentration values measured with a blood glucose meter. **A** – Glucose dissolved in pure water (y = 2.0 x; r^2 = 0.99); **B** – Glucose dissolved in water and treated with myrosinase solution (y = 1.6 x; r^2 = 0.99); **C** – Glucose dissolved in horseradish extract and treated with myrosinase solution. The glucose concentration originating from the extract is eliminated by subtraction. (y = 1.1 x; r^2 = 0.99).

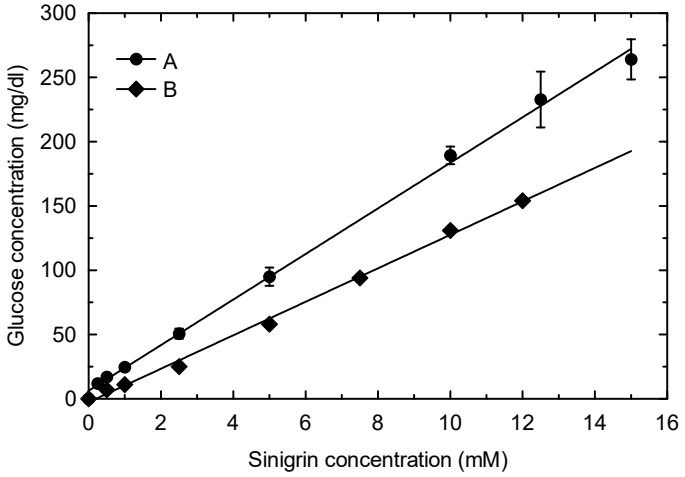

Fig. 4: Calibration curves prepared with sinigrin as standard. **A** – Calibration curve prepared with sinigrin dissolved in extract (y = 12.7 x; r^2 = 0.99). This fact takes the "matrix effect" into account. The glucose concentration originating from the extract is eliminated by subtraction. **B** – Calibration curve prepared with aqueous sinigrin solutions (y = 18.3 x; r^2 = 0.99).

Method validation

To check the recovery in the 'ITC quick test', horseradish roots were extracted with 4% phosphoric acid spiked with sinigrin in the concentration range between 1.0 – 10.0 mM sinigrin (n = 10). The recovery assays were compared with non-spiked extracts. The recovery rate was 90 ± 7%.

In order to challenge accuracy and reproducibility of the developed quick test the glucosinolate contents of four different horseradish roots were analyzed in quadruplicate. All extracts were analyzed using the 'ITC quick test' as well as by HPLC (Li and Kushad, 2004) and the results were compared (Fig. 4). For the calculations it has to be considered that sinigrin represents about 80% of the total glucosinolate content (Li and Kushad, 2004; Uematsu et al., 2002; Kübler, 2010; own spot samples; Fig. 5). The 'ITC quick test' therefore shows values about 20% higher than the respective HPLC values for sinigrin. Comparison of the 'ITC quick test' results with those obtained by HPLC showed that there was reasonable good agreement within the two methods. Both methods showed similar standard deviations, which justified the use of the quick assay using commercial glucose strips.

The 'ITC quick test' has been developed for the estimation of glucosinolates in fresh horseradish roots but it is also applicable for estimating glucosinolates in other cruciferous vegetables. For example, we also estimated the glucosinolate content in garden cress (2.3 mM), radish (0.3 mM) and broccoli (0.5 mM) using this method. It is advised, however, to calibrate the method for each type of vegetable to address the matrix effects (see above).

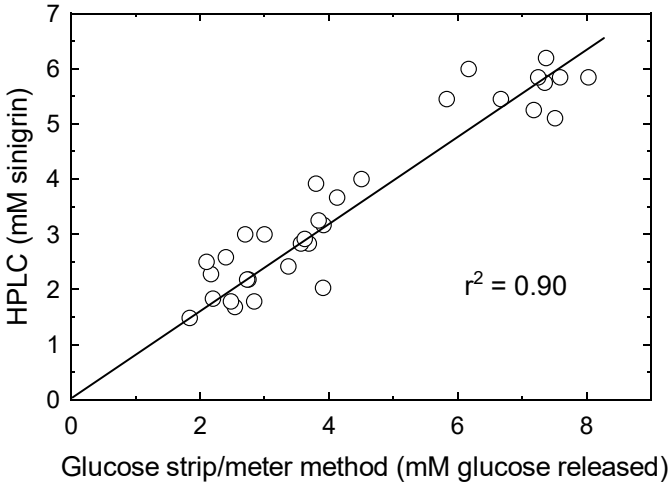

Fig. 5: Correlation between the amount of sinigrin determined by HPLC and glucose released from the same samples using the 'ITC quick test' developed here.

Conclusion

We here have picked up the idea of Tholen at al. (1993) to use commercial glucose test strips to quantify glucosinolates. We have developed a very simple, fast, reliable and robust technique to determine the glucosinolate content in fresh horseradish roots. Our assay can be carried out in less than 10 min and requires very simple and small equipment. Therefore, this method suits well for the screening of horseradish and other glucosinolate-containing plants, either in the field or in quality control of incoming and stored goods.

Acknowledgements

We thank Barbara White for linguistic advice. We also thank the students Steffen Baier, Melike Yazici and Marie Krenauer for technical assistance. We thank Schamel Meerrettich GmbH & Co. KG for their financial support.

References

Agneta, R., Rivelli, A.R., Ventrella, E., Lelario, F., Sarli, G., Bufo, S.A., 2012: Investigation of glucosinolate profile and qualitative aspects in sprouts and roots of horseradish (*Armoracia rusticana*) using LC-ESI-hybrid linear ion trap with fourier transform ion cyclotron resonance mass spectrometry and infrared multiphoton dissociation. J. Agric. Food Chem. 60, 7474-7482. DOI: 10.1021/jf301294h

Brabban, A.D., Edwards, C., 1995: The effects of glucosinolates and their hydrolysis products on microbial growth. J. Appl. Bacteriol. 79, 171-177.

Doorn, H.E., van der Kruk, G.C., van Holst, G.J., 1999: Large scale determination of glucosinolates in Brussels sprouts samples after de-

gradation of endogenous glucose. J. Agric. Food Chem. 47, 1029-1034. DOI: 10.1021/jf9808170

Doorn, H.E., van Holst, G.J., van der Kruk, G.C., Raaijmakers-Ruijs, N.C., Postma, E., 1998: Quantitative determination of the glucosinolates sinigrin and progoitrin by specific antibody ELISA assays in Brussels sprouts. J. Agric. Food Chem. 46, 793-800. DOI: 10.1021/jf970523z

Fenwick, G.R., Heaney, R.K., Mullin, W.J., Van Etten, C.H., 1982: Glucosinolates and their breakdown products in food and food plants. Food Sci. Nutr. 18, 123-201. DOI: 10.1080/10408398209527361

Gallaher, C.M., Gallaher, D.D., Peterson, S., 2012: Development and validation of a spectrophotometric method for quantification of total glucosinolates in cruciferous vegetables. J. Agric. Food Chem. 60, 1358-1362. DOI: 10.1021/jf2041142

Glenn, M.G., Chew, F.S., Williams, P.H., 1988: Influence of glucosinolate content of *Brassica* (Cruciferae) roots on growth of vesicular-arbuscular mycorrhizal fungi. New Phytol. 110, 217-225.

Heaney, R.K., Spinks, E.A., Fenwick, G.R., 1988: Improved method for the determination of the total glucosinolate content of rapeseed by determination of enzymically released glucose. Analyst 113, 1515-1518. DOI: 10.1039/AN9881301515

Li, X., Kushad, M.M., 2004: Correlation of glucosinolate content to myrosinase activity in horseradish (*Armoracia rusticana*). J. Agric. Food Chem. 52, 6950-6955. DOI: 10.1021/jf0401827

Kübler, K., 2010: Analyse von Glucosinolaten und Isothiocyanaten mittels Flüssigkeitschromatographie- bzw. Gaschromatographie-Massenspektrometrie, Dissertation, Justus-Liebig-University, Gießen.

Moreno, D.A., Carvajal, M., López-Berenguer, C., García-Viguera, C., 2006: Chemical and biological characterization of nutraceutical compounds of broccoli. J. Phar. Biomed. Anal. 41, 1508-1522. DOI: 10.1016/j.jpba.2006.04.003

Sampliner, D., Miller, A., 2009: Ethnobotany of horseradish (*Armoracia rusticana*, Brassicaceae) and its wild relatives (*Armoracia* spp.): reproductive biology and local uses in their native ranges. Econ. Bot. 63, 303-313. DOI: 10.1007/s12231-009-9088-1

Smith, C.A., Dacombe, C., 1987: Rapid method for determining total glucosinolates in rapeseed by measurement of enzymatically released glucose. J. Sci. Food Agric. 38, 141-150. DOI: 10.1002/jsfa.2740380206

Talalay, P., Zhang, Y., 1996: Chemoprotection against cancer by isothiocyanates and glucosinolates. Biochem. Soc. Trans. 24, 806-810. DOI: 10.1042/bst0240806

Tholen, J.T., Buzza, G., McGregor, D.I., Truscoir, R.J.W., 1993: Measurement of the glucosinolate content in rapeseed using the trubluglu meter. Plant breed. 110, 137-143. DOI: 10.1111/j.1439-0523.1993.tb01225.x

Uematsu, Y., Hirata, K., Suzuki, K., Iida, K., Ueta, T., Kamata, K., 2002: Determination of isothiocyanates and related compounds in mustard extract and horseradish extract used as natural food additives. Shokuhin Eiseigaku Zasshi. 43, 10-17. DOI: 10.3358/shokueishi.43.10

Address of the corresponding authors:
E-mail: wolfgang.kreis@fau.de