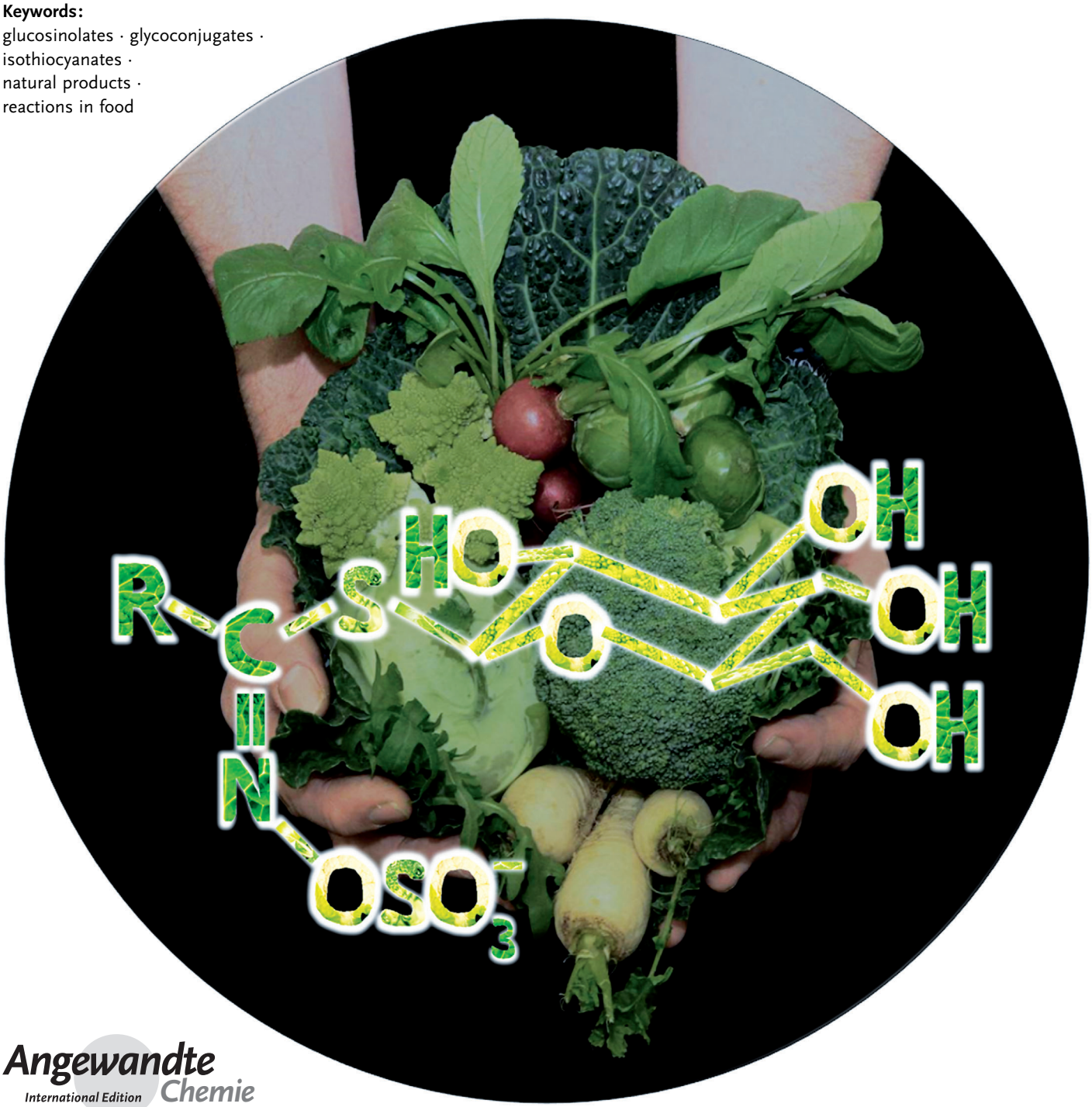
DOI: 10.1002/anie.201402639

Natural Products

Reactivity and Stability of Glucosinolates and Their Breakdown Products in Foods

Franziska S. Hanschen, Evelyn Lamy, Monika Schreiner, and Sascha Rohn*

Keywords:
glucosinolates · glycoconjugates · isothiocyanates · natural products · reactions in food



© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

The chemistry of glucosinolates and their behavior during food processing is very complex. Their instability leads to the formation of a bunch of breakdown and reaction products that are very often reactive themselves. Although excessive consumption of cabbage varieties has been thought for long time to have adverse, especially goitrogenic effects, nowadays, epidemiologic studies provide data that there might be beneficial health effects as well. Especially Brassica *vegetables, such as broccoli, radish, or cabbage, are rich in these interesting plant metabolites. However, information on the bioactivity of glucosinolates is only valuable when one knows which compounds are formed during processing and subsequent consumption. This review provides a comprehensive, in-depth overview on the chemical reactivity of different glucosinolates and breakdown products thereof during food preparation.*

From the Contents

1. Introduction

1.1. History

Many vegetables that are a fundamental part of traditional diets are members of the order *Brassicales*.[1] Plants that belong to this order characteristically contain glucosinolates, which are sulfur-containing secondary plant metabolites, a fact that was established almost 200 years ago. The first glucosinolates were discovered in the early 19th century: the widely distributed prop-2-enyl glucosinolate (sinigrin, **1**) was isolated from black mustard (*Brassica nigra*) seed[2] and 4-hydroxybenzyl glucosinolate (sinalbin, **2**) from white mustard (*Sinapis alba*) seed.[3] Soon after, it was discovered that these substances decompose under the influence of a constituent called "myrosin", now known as myrosinase. Myrosinase was also isolated from black mustard seeds and is able to produce the volatile mustard oils.[4] In 1897, Gadamer proposed a general common structure for the class of glucosinolates.[5] However, the structure could not explain the occurrence of nitriles that were by-products of the "myrosin"-induced hydrolysis to mustard oils, which were primarily identified as isothiocyanates. In 1956, Ettlinger and Lundeen revised this general structure and proposed a modified structure for the "prototype of mustard oil glucosides", namely the "myronate ion…sinigrin", which comprises a β-D-thioglucose group, a side chain (R), and a sulfonated oxim moiety (Figure 1b).

This structure was confirmed by synthesis in 1957.[6–7] Finally, X-ray crystallographic studies showed the C=N bond to have an *anti* (*Z*) configuration.[8] Therefore, glucosinolates are also called β-D-thioglucoside-*N*-hydroxysulfates, (*Z*)-(or *cis*)-*N*-hydroximinosulfateesters, or *S*-glucopyranosylthio hydroximates. The side chain R is structurally multifaceted and a variety of glucosinolates have been described so far.[1,9]

Over the past 50 years, many reviews have summarized studies of glucosinolate biochemistry and their physiological effects[2,10] and provided information on glucosinolate distribution among the plants.[1,9,10i] However, to date, only a limited number of studies have addressed the reactivity of this interesting group of secondary plant metabolites,[11] which are central to the regulation of the ecophysiological plant–environment interaction and the induction of dietary-mediated adverse or positive physiological effects.

1.2. Structural Classification of Glucosinolates

To date, more than 200 glucosinolates have been identified in plants belonging predominantly to the order *Brassicales*.[9] Glucosinolates are usually classified into aliphatic (**1**, **3**–**15**), aromatic (**2**, **16**, **17**), and indole glucosinolates (**18**–**21**; Figure 2). This classification is based on the variable side chain of the basic chemical structure (Figure 1b) that results from the amino acid precursor. About 50% of the glucosi-

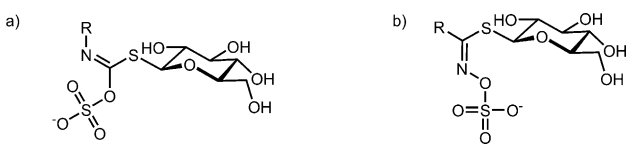


Figure 1. Glucosinolate structure according to a) Gadamer[5] and b) Ettlinger und Lundeen.[6] R^1 = side chain.

[*] Dr. F. S. Hanschen, Prof. Dr. M. Schreiner
Department of Quality, Leibniz-Institute of Vegetable and Ornamental Crops Großbeeren and Erfurt e.V.
Theodor-Echtermeyer-Weg 1, 14979 Großbeeren (Germany)
E-mail: hanschen@igzev.de
Homepage: http://www.igzev.de
Dr. E. Lamy
Department of Environmental Health Sciences
Freiburg University Medical Centre
Breisacher Str. 115 b, 79106 Freiburg i.Br. (Germany)
Prof. Dr. S. Rohn
Hamburg School of Food Science
Institute of Food Chemistry, University of Hamburg
Grindelallee 117, 20146 Hamburg (Germany)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201402639.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

nolates identified so far are aliphatic glucosinolates with highly variable side chain structures. This class of glucosinolates can be subdivided into straight- or branched-chain alk(en)yl glucosinolates without or with a hydroxy group (**1**, **3**–**6**), and into a large group of those that contain an additional sulfur atom in the side chain. Depending on the oxidation state of this sulfur atom, the latter group can be further subdivided into methylsulfanylalkyl- (S^{II}) (**7**, **8**, **12**), methylsulfinylalkyl- (S^{IV}) (**9**–**11**, **13**), or methylsulfonylalkyl glucosinolates (S^{VI}). The major side-chain structures of glucosinolates from the *Brassicales* species are shown in Figure 2.

The structure of the different glucosinolate side chains can be very complex. Moreover, functionalization at the C2′ or C6′ position of the β-D-thioglucose moiety by cinnamoyl-, sinapoyl-, and benzoic acid salts or esters have also been reported.[12] Other "exotic" aromatic glucosinolates contain *O*-glycosidically linked sugars, such as L-rhamnose, L-arabinose, or D-apiose, at the aromatic ring of the side chain.[13] Finally, there are further sulfur-containing aliphatic glucosinolates, such as dimeric 4-mercaptobutyl- **15**, 4-(glucodisulfanyl)butyl- **14**, or 4-(cysteine-*S*-yl)butyl glucosinolate.[14]

1.3. Glucosinolates in Plants: Biosynthesis, Hydrolysis, and Function

In plants, glucosinolates are involved in the response to biotic stress. Induced by herbivores[15] or fungal penetration,[16] enzymatically formed breakdown products of glucosinolates activate the defense system of the plant and act as deterrents. Depending on insect species and plant genotype,[17] a herbivorous attack leads to a jasmonic acid mediated increase in glucosinolate concentration. This can also be affected by UVB pretreatment[18] or a lack of water.[19] As glucosinolates can contribute to the natural plant resistance by reducing phytopathogen attacks, plants rich in glucosinolates have some potential to be used as biofumigants in agriculture by incorporating crushed plants into the soil.[20]

Amino acids are precursors for the biosynthesis of glucosinolates. Aliphatic glucosinolates principally derive from methionine, indole glucosinolates from tryptophan, and aromatic glucosinolates mostly derive from phenylalanine. The biosynthesis proceeds through three separate biosynthetic steps: 1) the chain elongation of selected precursor amino acids (relevant for methionine- and phenylalanine-derived glucosinolates), 2) the formation of the glucosinolate core structure, and 3) modifications of the side chain.[10c,21] Glucosinolate biosynthesis starts with the conversion of the precursor amino acids to the corresponding aldoxime by cytochrome P450 monooxygenases of the CYP79 family. The aldoxime enters the glucosinolate core biosynthesis pathway and is oxidized by CYP83s and conjugated to the S donor, probably through a glutathione-*S*-transferase-like enzyme.

Thiohydroximic acid is then formed by cleavage of the thiohydroximate catalyzed by a *C*-*S* lyase. Finally, glucose is coupled by an *S*-glucosyltranferase to the sulfur atom and the resulting desulfoglucosinolate is then sulfonated by a sulfotransferase to form the final glucosinolate. Secondary modifications of aliphatic glucosinolates include oxygenation, hydroxylation, alkenylation, and benzoylation, while indole and aromatic glucosinolates can undergo hydroxylation and methoxylation.[22] Although glucosinolates are present in all plant organs,[23] their concentrations and composition can vary enormously within the plant and ontogenetic changes can lead to fluctuating glucosinolate profiles that depend on the stage of plant development. Seeds usually have the highest glucosinolate contents, which comprise especially aliphatic or

Franziska Hanschen studied food chemistry at the Technische Universität Berlin and obtained her PhD in 2012 for investigations of the thermal degradation of glucosinolates. Since then, she has been working as a postdoctoral researcher at the Leibniz-Institute for Ornamental and Vegetable Crops in Großbeeren. Her research focusses on degradation pathways of glucosinolates as well as on bioactive effects of the resulting degradation products.

Monika Schreiner obtained her Master of International Agricultural Development and her PhD at the Technische Universität Berlin. She worked as a Postdoctoral Scientist at the Leibniz Institute of Agricultural Engineering in Bornim before becoming the head of the Department of Quality at the Leibniz-Institute of Vegetable and Ornamental Crops in Großbeeren/Erfurt and Professor at the Leibniz Universität Hannover. Her major research interests focus on the study of secondary plant metabolites, particularly glucosinolates, and their functions.

Evelyn Lamy, born 1979, obtained her PhD in human biology at the Medical Faculty of the University of Giessen in 2008. She is a Junior Group Leader at the Institute of Environmental Health Sciences, Freiburg University Medical Center. Her major research interests focus on the anticancer effects of plant compounds, especially on signaling of selective cell aging and telomerase regulation.

Sascha Rohn, born 1973, is a full professor for Food Chemistry at the Hamburg School of Food Science, University of Hamburg. His group deals with the analysis of secondary plant metabolites, their antioxidant activity, but especially with the reactivity and stability of these bioactive compounds with the aim to identify degradation products that serve as process markers during food/feed processing or as biomarkers in nutritional physiology.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

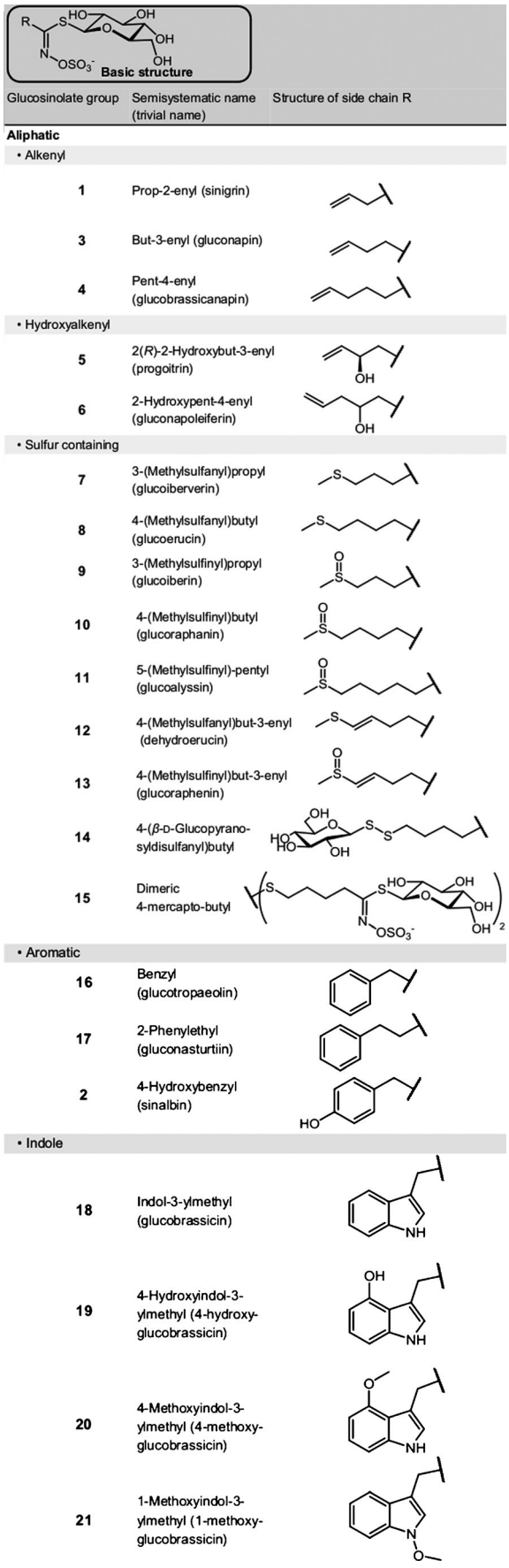


Figure 2. Major glucosinolate side-chain structures of *Brassica* species.

aromatic glucosinolates.[23,24] After germination, total glucosinolate contents decline but can later increase again, and indole glucosinolates are formed that dominate especially in root tissues.[23–25] Glucosinolates finally accumulate in the inflorescences and fruits containing the unripe seeds.[23,24]

In addition to the (onto)genetic background, glucosinolate synthesis is strongly regulated by ecophysiological factors such as plant nutrition and water availability. Fertilization with sulfur increases aliphatic, methionine-derived glucosinolates when the nitrogen level is moderate, whereas nitrogen supply can enhance indole glucosinolate levels and even decrease aliphatic glucosinolates when sulfur supply is low.[26] Both effects are based on amino acid metabolism and glucosinolate biosynthesis, as the reduction of sulfur-containing cysteine to methionine is nitrogen dependent[27] and indole glucosinolate biosynthesis from L-tryptophan is limited by the sulfur donor for the thiohydroximate.[28] Furthermore, the water supply greatly affects glucosinolate contents and water-limited conditions can increase especially aliphatic glucosinolates.[19b,29] The effect is influenced by ontogeny, genotype, and the growing season.[29] Topsoil drying was shown to be a modern technique to enhance aliphatic glucosinolates without a reduction in biomass.[30] Temperature or radiation affect glucosinolates as well.[31] For example, in field-grown broccoli the content of aliphatic methylsulfinyl glucosinolates increased with decreasing temperature and increasing radiation. Broccoli grown at daily mean temperatures of less than 12 °C combined with increasing radiation (up to 100 mol $m^{-2} d^{-1}$) produced high contents of alkyl glucosinolates.[31c]

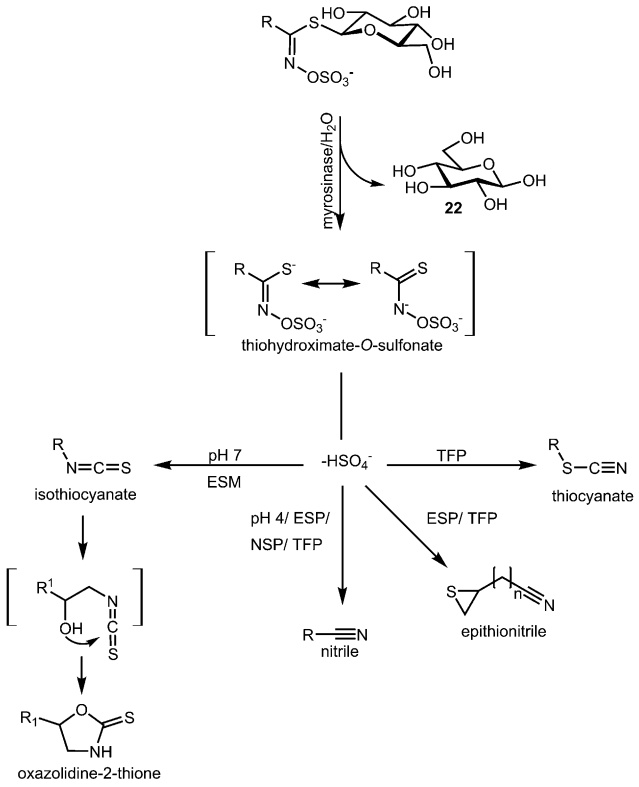
The radiation-induced effects are based on light-dependent flavin monooxygenases that are involved in the biosynthesis of methylsulfinyl glucosinolates.[32] Additionally, high-energy UVB radiation is able to induce the formation of glucosinolates in ecologically relevant doses.[18,33] This effect is mediated by the UVR8 receptor, which initiates signaling and plant response,[34] thus triggering glucosinolate biosynthesis through signaling pathways involving jasmonic and salicylic acid.[18,34b,35] Therefore, external application of these signaling molecules leads to a distinct increase of certain glucosinolates as well.[35a]

1.3.1. *Enzymatic Degradation*

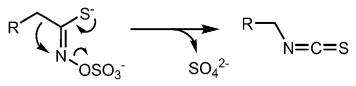
Glucosinolates are stored in the plant vacuole, separately from the endogenous glucosinolate-hydrolyzing enzyme myrosinase (a β-D-thioglucosidase).[36] Upon cell rupture, myrosinase can come into contact with the glucosinolates and the enzymatic degradation (Scheme 1) is initiated, resulting in a variety of breakdown products.

In a first step, the enzyme cleaves the glucosinolate to give β-D-glucose **22** and an unstable thiohydroximate-*O*-sulfonate. The aglucon is then transformed to the isothiocyanate in the so-called Lossen rearrangement, or decomposes to the corresponding nitrile and molecular sulfur (Scheme 2). As low pH values inhibit the Lossen rearrangement, isothiocyanates are usually formed under more neutral conditions.[37] However, some plants modulate this transformation by specific proteins, such as the epithiospecifier protein

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



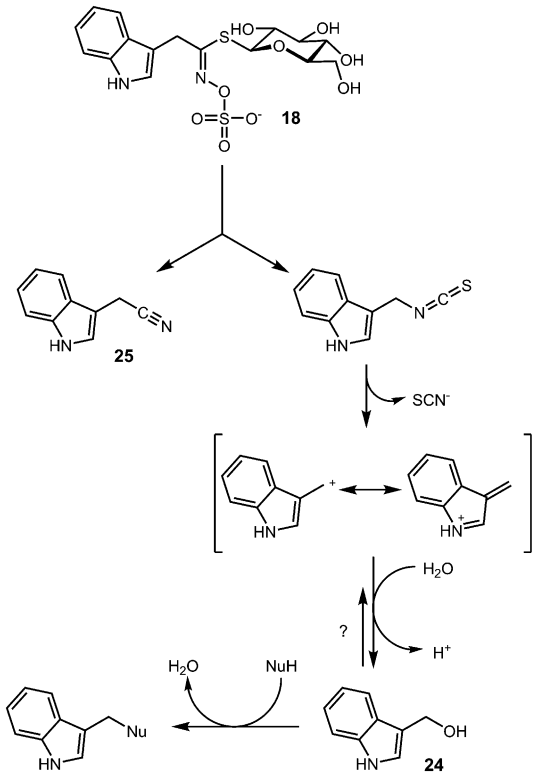
Scheme 1. Formation of enzymatic-breakdown products of glucosinolates. ESP: epithiospecifier protein; TFP: thiocyanate-forming protein; ESM: epithiospecifier modifier protein; NSP: nitrile-specifier proteins. R: variable side chain; R^1: alkenyl side chain.



Scheme 2. Lossen rearrangement of the thiohydroximate-*O*-sulfonate to the isothiocyanate. R: variable side chain

(ESP),[38] the nitrile specifier proteins (NSPs),[39] or the thiocyanate-forming protein (TFP),[40] which can interact with the unstable aglucon and favor the formation of nitriles. Iron(II) influences the activity of ESP and NSPs and can induce the formation of nitriles by itself.[37,39a,41] Additionally, the ESP protein promotes the formation of epithionitriles from unsaturated aliphatic glucosinolates such as **1.**[38a,41,42] The presence of TFP can generate thiocyanates from **1**, benzyl glucosinolate (**16**), and 4-methylsulfanylbutyl glucosinolate (**8**) or favor the formation of the nitrile from other glucosinolates.[43] On the other hand, the epithiospecifier modifier protein (ESM) blocks the formation of epithionitrile and nitrile in favor of the isothiocyanate.[44] For detailed information about the enzymatic hydrolysis of glucosinolates and the function of the specifier proteins, the reader is referred to reviews from Burow and Wittstock.[45]

Some glucosinolates form unstable isothiocyanates. While 2-hydroxyalkenyl isothiocyanates cyclize to oxazolidine-2-thiones (Scheme 1), the 4-hydroxybenzyl isothiocyanate **23** and the very reactive indole isothiocyanates decompose, probably via an indol-3-ylmethyl carbocation, to their corresponding alcohols, thereby releasing the thiocyanate ion.[46] For example, indole-3-carbinol **24** can then, probably again through the formation of the carbocation, readily react with water, further molecules of **24** or other nucleophiles to form a variety of oligomers (Scheme 3).[46c,47]



Scheme 3. Decomposition of indole-type glucosinolates and reactions of breakdown products. NuH: nucleophilic compound, **25**: indol-3-acetonitril. Modified following Ref. [46c].

From the hydrolysis products, isothiocyanates are especially responsible for the pungent flavor of several *Brassica* vegetables, and for their antimicrobial,[48] antifungal,[49] and antiherbivorous properties.[50] In addition, there is increasing evidence that these glucosinolate breakdown products might have several beneficial health effects in humans, for example through cancer prevention or anti-inflammatory effects.[10g,51]

1.3.2. Metabolism of Glucosinolates

During chewing of raw *Brassica* vegetables or later in the stomach, glucosinolates are partly hydrolyzed as a result of plant tissue disruption, and subsequent liberation and absorption was shown to be dependent on the food matrix.[52] Because of the apolarity of the formed isothiocyanates, they can be absorbed passively through the membrane of the enterocyte, after which they are rapidly transformed. They are conjugated with glutathione and accumulate in the enterocytes.[53] The resulting dithiocarbamates are able to re-release the isothiocyanates which can bind to other thiols or proteins[10f,52,54] or will be excreted by the multidrug-resistance-associated protein 1 into the blood or back into the gut lumen.[53a,55] The glutathione adducts are further metabolized

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

through the mercapturic acid pathway and excreted as *N*-acetyl-L-cysteine conjugate in the urine.[56] The bioavailability of free isothiocyanates is comparatively high, and concentrations of metabolites in human plasma reach a maximum between one and six hours after vegetable consumption.[56b,57] Concentrations of single metabolites in the plasma can reach 16 μM, however in most studies metabolite concentrations range between 1–3 μM.[56b,57] Additionally, up to 85% of ingested glucosinolate/isothiocyanate were shown to be excreted with the urine.[57]

The fate of intact, nonhydrolyzed glucosinolates is still discussed controversially. The prerequisite for the hydrolysis not to take place is the inactivation of the myrosinase, which can be achieved by thermal treatment, for example, blanching. However, a certain percentage of the glucosinolates seem to be hydrolyzed in the stomach,[58] or absorbed in the stomach by passive transport (protonated form) or by facilitated uptake (diffusion) in the small intestine.[59] Finally, in the colon, several bacteria strains are able to hydrolyze glucosinolates to form isothiocyanates, amines, or nitriles depending on the type of bacterial myrosinase-like activity involved.[60] Furthermore, a recent study in gnotobiotic (sterilely kept) mice showed that intact glucosinolates can also be excreted through feces.[56c] If hydrolyzed, the isothiocyanates will then be absorbed, metabolized, and excreted through the urine.[61]

To date, information about the uptake, metabolism, and excretion of nitriles and epithionitriles is even more limited. Because of their structure and molecular size, it is hypothesized that they are absorbed passively.[10f,62] Similar to isothiocyanates, epithionitriles are known to be conjugated with glutathione and excreted as their corresponding mercapturic acids in urine.[62,63] In contrast, aliphatic nitriles are metabolized in the liver, resulting in the release of an aldehyde and cyanide ion. Organic thiocyanates are also metabolized by gluthathione-*S*-transferases resulting in the cyanide ion.[64] The latter can be metabolized and excreted as thiocyanate ion through the urine.[65]

1.3.3. Physiological Effects of Glucosinolates and Implications for Health Prevention

In the past, glucosinolates and their breakdown products were mainly considered to be toxic and goitrogenic (goiter-producing) compounds.[65a,66] Especially enzymatically derived oxazolidine-2-thiones were shown to be potent goitrogens in animal studies.[66f,67] The thyroid hormone axis is also significantly inhibited by a competitive effect of thiocyanate ions and iodine, especially in situations when iodine malnutrition is manifested.[68] However, with regard to the chemical structure, only a few glucosinolates are able to preferably form oxazolidine-2-thiones (glucosinolates **5** and **6**) and thiocyanate ions (glucosinolates **2**, **18**–**21**). As these goitrogenic compounds are all products from chemically unstable isothiocyanates (Scheme 1 and **3**), the occurrence of these substances is dependent on enzymatic degradation, protein cofactors, and processing conditions (see also chapters 1.3.1 and 2). Therefore, as most vegetables rich in 2-hydroxyalkenyl glucosinolates, such as Brussels sprouts, are cooked prior to consumption, goitrogenic effects should be of no concern in an average human diet.[69] Adverse effects however may still play a role in some parts of the world where low iodine supply is still of a problem. Additionally, goiter was induced by carry-over of oxazolidine-2-thiones into cow milk, and this way of exposition should still be considered and monitored.[70] Furthermore, goitrogenic effects played an important role in animal nutrition when feeding rapeseeds rich in glucosinolate **5**. However, by reducing the glucosinolate content and supplementation of iodine, malnutritional effects can be avoided.[67b,68b,71]

Although some glucosinolates provide goitrogenic potential, epidemiological data and preliminary research indicated that glucosinolates might also have beneficial health effects.[72] Since then, adverse and beneficial health effects of glucosinolate have been the topic of a controversial scientific debate.[73]

Several epidemiological studies reported an inverse correlation between the intake of *Brassica* vegetables and the risk for several types of cancer.[74] In 1992, Zhang et al. identified 4(*R*)-4-methylsulfinylbutyl isothiocyanate (sulforaphane) **26**, a breakdown product of 4-(methylsulfinyl)butyl glucosinolate (**10**), as a principle cause of the cancer preventive effects.[72] Since then, such effects of this and other isothiocyanates were exhaustively investigated. It was shown that isothiocyanates inhibit the development of cancer in several organs in rats and mice, including bladder, breast, colon, lung, stomach, and liver.[75] Also, short-term intervention studies using xenobiotic enzyme modulation and cytogenetic endpoints, such as micronuclei and DNA damage induction, provide first indications on the chemopreventive effects of isothiocyanate-containing foods in humans.[76] Meanwhile, the first clinical phase I studies have been accomplished to establish the safety and tolerance of glucosinolates and isothiocyanates of broccoli sprouts,[77] and based on promising preclinical findings, clinical trials with isothiocyanate **26** and broccoli sprouts are running for prevention or treatment of lung, breast, and prostate cancer.[78]

A large number of studies have been conducted to identify the factors determining the bioactivity of isothiocyanates and shed light on the behaviour of their structures in cellular compartments and organisms.[57] Basically, isothiocyanates react with electrophilic substitutes such as thiol, amine, and hydroxy groups, for example, the phenolic group of tyrosine.[79] The reaction with thiol groups is 10^3 to 10^4 times faster than with amine or hydroxy groups.[80] While the conjugation of isothiocyanates with small thiol moieties is reversible, isothiocyanates are thought to form irreversible thiourea derivatives with amino groups. Glutathione is the most abundant thiol-containing antioxidant molecule within the cell and its coupling reaction and consequent efflux out of the cell presents the first step of the inactivation of isothiocyanates. This in turn leads to changes in the redox-equilibrium state of the cell, thus demanding for compensatory mechanisms such as new synthesis of glutathione. However, at increasing isothiocyanate concentrations, glutathione is depleted and coupling with other cellular proteins dominates the reaction, which then results in controlled or finally uncontrolled cell death.[57] A recent proteomic analysis

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

led to the identification of more than 30 proteins of the cytoskeleton, redox regulation, proteasome, and apoptosis/cell survival signaling as targets for intracellular isothiocyanate interaction.[81] The intensity of isothiocyanate reactivity with thiol moieties and consequently their bioactivity greatly varies and depends on the carbonyl chain length, substituents, molecular geometry, and chemical stability.[57]

The process of carcinogenesis can be subdivided into three phases: initiation, promotion, and progression (Figure 3). A cell can be initiated by carcinogen binding and irreversible

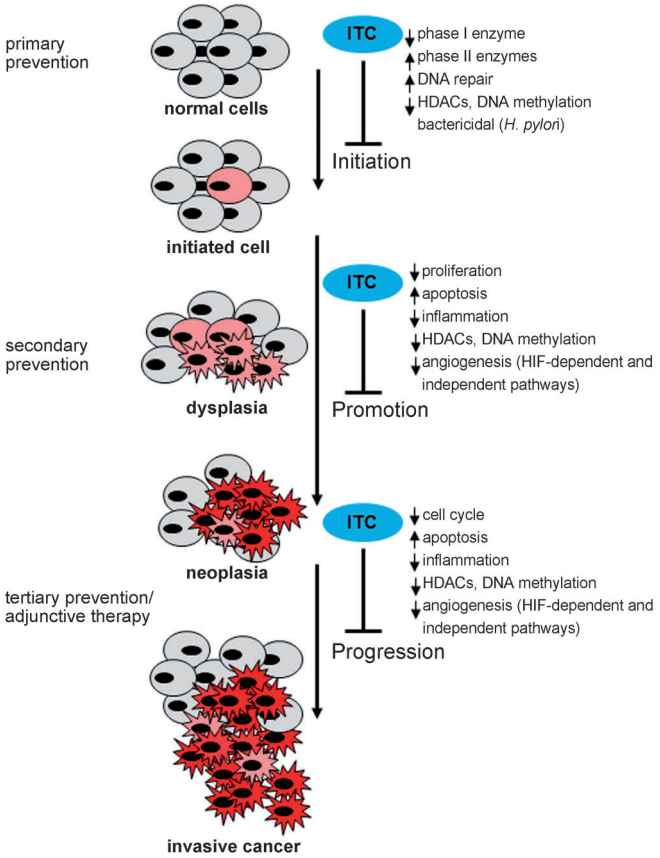


Figure 3. Modes of action of isothiocyanates-mediated cancer prevention.

damage to the DNA. In the promotion phase, clones of initiated cells expand as a result of functional loss of regulatory proteins and cellular checkpoints that are important for proliferation and apoptosis. Progression defines the stage in which the tumor cells have irreversibly altered phenotypically and genotypically and demonstrate increased growth speed and invasiveness. In this model, isothiocyanates are identified as pleiotropic acting biochemicals that interfere with all three steps of chemoprevention. The best investigated mechanisms of action for blocking initiation include the tandem and cooperating inhibitory effects of isothiocyanates on phase-I cytochrome P450 metabolizing enzymes. This effect is probably mediated by a combination of enzyme suppression and direct inhibition of their catalytic activities.[82] Thereby, the levels of ultimately formed carcinogens within the cell are lowered, and the chances of mutagenic events that lead to cell initiation are reduced. The activation of phase-II enzyme expression through the KEAP1/Nrf2/ARE pathway is another important mechanism that protects the cell from initiation as a result of elevated detoxification of carcinogenic compounds. For compound **26**, evidence for phase-II enzyme induction is available from in vitro studies in animals and humans.[83] Mechanisms for preventing promotion and progression involve the inhibition of events that are controlled by cascades of cellular signal-transduction molecules. Besides hormones or growth factors, inflammations are regarded as important tumor-promoting stimuli and anti-inflammatory effects through the inhibition of the NF-κB pathway have been clearly identified for isothiocyanates.[84] Recent findings suggest that *Brassica*-vegetable-derived isothiocyanates are also regulators of epigenetic mechanisms; they may inhibit histone deacetylase transferases (HDAC) and aberrant CpG island methylation of various genes, but also short noncoding microRNAs.[85]

Isothiocyanates prevent the growth and survival of cells that are predetermined to become malignant. Thus, the treatment with isothiocyanates results in an increased sensitivity of cancer cells to growth arrest at the G1, S, or G2M phase,[86] and apoptosis (intrinsic and extrinsic) is triggered, even in chemoresistant cancer cells that express high levels of anti-apoptotic bcl-2 family members.[87] Moreover, isothiocyanates, and in particular isothiocyanate **26**, were shown as inhibitors of anti-apoptotic telomerase enzyme in different cancer cells.[88] Furthermore, hypoxia inducible factors (HIF) may be key targets mediating the anti-angiogenic activity of isothiocyanates, that is, the inhibition of the formation of new blood vessel in preneoplastic tissue or neoplasia, but HIF-independent pathways have also been described.[89] These include other transcription factors, such as NF-κB, AP1, MYC, and tubulin. The critical question is whether a normal dietary intake of *Brassica* vegetables might deliver sufficient amounts of isothiocyanates to transfer in vitro/in vivo results to the human situation, or if pharmacological application of purified isothiocyanates will be necessary. As indicated above, some cancer-preventive mechanisms have already been verified in humans following *Brassica*-vegetable consumption. However, further investigations are needed to determine minimum necessary consumption levels to achieve such protective effects, but should also investigate upper dosing limits. Because of their reactivity, isothiocyanates are in principle able to act genotoxic, form DNA adducts, and induce DNA mutations. Some studies even report on carcinogenic effects.[90]

Metabolites derived from indole glucosinolates show different modes of action. In an aqueous environment and in acidic gastric juice, carbinol **24**, which derives from glucosinolate **18**, is converted to numerous condensation products, of which the dimer 3,3-diindolylmethane is the most active and effective metabolite.[91] Moreover, studies in humans implicate that **24** serves as the prodrug for the therapeutically active dimer.[92] To date, both compounds have been demonstrated to possess cancer-preventive properties in numerous epidemiological and preclinical studies. The modulation of multiple signaling pathways that control DNA repair, inflammation, cell growth, apoptosis, and angiogenesis are made responsible for these effects.[91a,93] However, the

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

basis for its potency against hormone-dependent cancers, such as breast, cervical, and prostate cancer, is thought to be the ability to modulate the estrogen metabolism. This effect, observed in vitro and in vivo, has been confirmed in human clinical trials.[94] Both compounds negatively regulate estrogen receptor α (ERα) signaling and alter the cytochrome P450 mediated estrogen metabolism.[95] Ligand-independent activation of ERβ has also been shown for the 3,3-diindolylmethane.[96] Currently available clinical data on these compounds indicate a good safety profile and only minor adverse effects.[97] However, the promotion of cancer was found in rat liver after treatment with carbinol **24**.[98] Moreover, breakdown products of glucosinolate **21** were shown to exert genotoxic and mutagenic effects in bacterial and mammalian cells.[99] The extremely unstable isothiocyanate can interact with the DNA by forming a resonance-stabilized carbocation that readily reacts with nucleophilic structures[99b,100] (Scheme 3). Additionally, the corresponding 1-methoxyindole-3-carbinol can be activated by human sulfotransferase hSULT1A1 to the sulphate, which can also decompose to the reactive carbocation.[99b,101] However, whether glucosinolate **21** exerts a carcinogenic risk to humans is still unknown. Clearly, genotoxic effects of isothiocyanates have hampered the clinical development of purified isothiocyanates as chemopreventive agents. However, at dietary consumption levels, such toxicities from stable isothiocyanates such as isothiocyanate **26** are unlikely to occur in humans. With regard to the chronic consumption of dietary supplements or botanical products that are highly enriched with isothiocyanates, a more thorough toxicological evaluation is critical to meet safety requirements and promote acceptance. In contrast to isothiocyanates and **24**, knowledge about possible beneficial health effects of nitriles and epithionitriles is limited. According to some studies, they play only a minor role in *Brassica*-vegetable-based cancer prevention,[102] whereas other studies have reported a moderate induction of phase-II enzymes, for example, by the nitrile of 2(*R*)-2-hydroxybut-3-enyl glucosinolate (**5**).[102c,103] These studies have led to the assumption that nitriles show pronounced differences in their functionality depending on the chemical structure of their side chain.

Therefore, the metabolism of glucosinolates is strongly dependent on either the occurrence of myrosinase or related bacterial enzymes or further chemical transformations prior to consumption. However, most *Brassica* vegetables are consumed after domestic processing, such as cooking, a process that can inactivate myrosinase. Therefore, it is important to ascertain the fate of glucosinolates during food preparation and determine the factors that influence the glucosinolate breakdown and formation of breakdown products and the stability of these compounds themselves. Without this knowledge, it seems to be impossible to gain any deeper insight into mechanisms of how (a mixture) of glucosinolates and their degradation products might act physiologically with a corresponding risk–benefit evaluation of *Brassica*-vegetable-based food.

Here, we provide details on the reactivity of glucosinolates and their breakdown products in foods with special reference to the food matrix.

2. Glucosinolates in Food Plants: Transformations during Food Processing

With a worldwide production of 100 million tons in 2011, *Brassica* species, and hence glucosinolates, are an integral part of human nutrition.[104] Distribution and concentrations of glucosinolates in important, highly consumed vegetables of the *Brassica* family are provided in the Supporting Information. The average daily intake of glucosinolates in the German population was estimated to be 14.2 mg for men and 14.8 mg for women, approximately 10% of which is glucosinolate **10**. Broccoli, Brussels sprouts, cauliflower, and radish contributed the most to the total intake of glucosinolates in Germany.[105] Broccoli is rich in glucosinolate **10** (11–34 mg/100 g fresh weight (FW)) and **18** (7–17 mg/100 g FW).[105,106] Brussels sprouts on the other hand contain mainly glucosinolates **1** (22–45 mg/100 g FW), **5** (7–14 mg/100 g FW), **9** (6–14 mg/100 g FW), and **18** (25–44 mg/100 g FW).[31a,105] Cauliflower also contains mainly glucosinolates **1** (1–6 mg/100 g FW), **9** (0–6 mg/100 g FW), and **18** (5–19 mg/100 g FW).[31a,105,106] Contrarily, radish is very rich in glucosinolate **12** (50–119 mg/100 g FW).[31a,105] Some of those vegetables are ingested raw, for example, radish, rocket salad, sprouts, cress, mustard, or cabbage salads, but usually they are consumed after a preparation procedure. Such food processing can have a pronounced impact on the concentration of glucosinolates and their corresponding breakdown products.

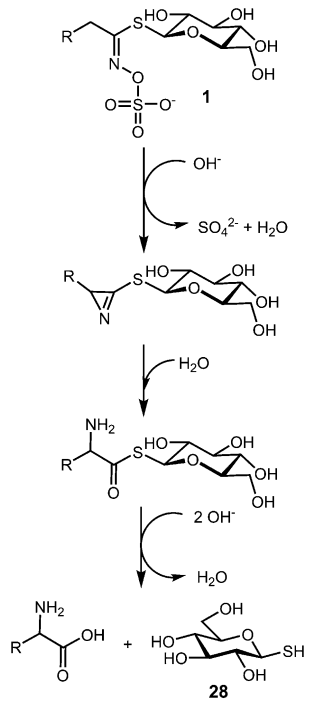
As pre- and postharvest influences and changes on glucosinolate levels induced by processing, and the genetic influence of processing procedures on vegetable quality have been reviewed recently,[107] this present review focuses on the chemical changes of glucosinolates and mechanisms along with the reactivity of glucosinolate breakdown products in food.

Storage usually decreases glucosinolate levels,[108] and freezing of raw *Brassica* vegetables causes enzymatic degradation of glucosinolates induced by freeze–thaw fractures of the plant cells.[109] However, glucosinolates of blanched vegetables are not affected when stored in a freezer.[110] Furthermore, cutting results in the same enzymatic degradation and thus in severe loss of glucosinolates. However, the cutting of the vegetables into bigger slices and their storage for 48 h can even increase indole glucosinolate levels.[111] Fermentation, as is common for white cabbage in Germany ("Sauerkraut"), includes two different ways of degradation: 1) enzymatic degradation by myrosinase, resulting from softening or disruption of the cell wall, and 2) microbial degradation when microorganism possess myrosinase-like enzymes.[60d,f,112] The major breakdown products in fermented white cabbage are aliphatic isothiocyanates, and ascorbigen, derived from **24** and nucleophilic ascorbic acid (compare Scheme 3, ascorbic acid = NuH).[112] In addition, heat treatments, such as blanching, cooking, or canning, will modulate the enzymatic degradation mechanism differently, and influence glucosinolate content and the formation of breakdown products. For example, while maintaining myrosinase activity, mild heat treatment inactivates the ESP protein, thus leading to an increase in the formation of isothiocyanates.[113] Longer heat treatment will also inactivate myrosinase and therefore

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

inhibit enzymatic breakdown more or less completely.[114] Moreover, gentle microwave cooking and steaming were shown to preserve most of the glucosinolates,[109,113c,114a,115] while blanching and domestic cooking can result in a glucosinolate loss of more than 50%, a process mainly caused by leaching.[109,115,116]

However, the inactivation of myrosinase by the cooking process does not necessarily mean that the breakdown of glucosinolates is stopped. Glucosinolates are not inert substances, but are degraded depending on several other conditions such as strong acids or bases or different types of metal salts.[6,11,71a,117] Proton-catalyzed hydrolysis will lead to the formation of carbonic acids and sugar **22**,[6,118] whereas bases hydrolyze the glucosinolate through a Neber-type rearrangement and form the alkyl amino acid and 1-β-D-thioglucose **28** (Scheme 4).[117b] Therefore, in addition to leaching and enzymatic degradation, a thermally induced chemical degradation might also occur during food preparation, especially with longer cooking times and higher temperatures as during canning (e.g. "broccoli soup"), baking (e.g. "broccoli gratin"), frying (e.g. "stir-fried broccoli"), or deep-frying (e.g. "broccoli tempura").



Scheme 4. Base-catalyzed Neber-type rearrangement of glucosinolate **1** to an amino acid and sugar **28**. Modified following Ref. [117b], R: $CH_2{=}CH_2$.

2.1. Thermal Stability of Individual Glucosinolates and Formation of Thermally Induced Degradation Products

From most studies of cooked *Brassica* vegetables, contribution of enzymatic breakdown, leaching effects, or thermally induced degradation cannot be separated sharply. Therefore, in this section, only studies are discussed that excluded leaching or enzymatic breakdown, so that thermal degradation is clearly the main influence. With such a diversity of structurally different glucosinolates present in *Brassica* species, it is not unexpected that glucosinolates differ in their susceptibility toward heat. It is now widely accepted that during heat treatment of *Brassica* vegetables, indole glucosinolates are thermally less stable than aliphatic ones.[119] Since indole glucosinolates are able to form resonance-stabilized products, their reactivities differ significantly from aliphatic glucosinolates. 4-Hydroxyindol-3-ylmethyl glucosinolate (**19**) was least stable when *Brassica* crops were heated under conditions that excluded losses as a result of leaching and enzymatic degradation.[119a,d,e] On the other hand, 1-methoxyindol-3-ylmethyl glucosinolate (**21**) and 3-indolylmethyl glucosinolate (**18**) were less susceptible,[119a,d] as was predicted by McDanell et al. in 1988.[120] In 1989, Slominski and Campbell studied the thermally induced degradation of indole glucosinolates in *Brassica* species and identified the corresponding indole-3-acetonitriles as the main products of thermal breakdown.[121] In contrast, Chevolleau et al. (1997, 2002) studied the thermal stability of the pure glucosinolate **18** and observed only 10% degradation when heated in water for 60 min. Furthermore, this group did not detect nitrile **25**, but 2-(indolyl-3′-methyl)indolyl-3-methyl glucosinolate as the only reaction product.[122]

Aliphatic glucosinolates are more stable in comparison to indole glucosinolates. Hanschen et al. (2012) observed that the structure of the glucosinolates determines the degradation rate of aliphatic glucosinolates during the heating of powdered broccoli sprouts. Within the group of sulfur-containing aliphatic glucosinolates, the methylsulfinylalkyl glucosinolates were more stable than the methylsulfanylalkyl glucosinolates.[119d,123] In these studies, 3-(methylsulfanyl)propyl glucosinolate **7** was identified as the most unstable aliphatic glucosinolate and 59% were degraded after one hour of cooking at 100 °C at the natural pH value of 5.3.[123] The unsaturated 4-(methylsulfanyl)but-3-enyl glucosinolate (**12**) is very labile as well. Within 15 min of steaming of fresh kaiware daikon radish at 121 °C in an autoclave, 22% of this glucosinolate were converted from the naturally occurring *E* isomer to the *Z* isomer, and 18% were completely degraded. After 90 min of this treatment, 89% were degraded completely.[124]

With regard to structure–activity relationships, it was generally observed that glucosinolates with a hydroxy function in the side chain, for example, glucosinolates **5** or **19**, are more labile compared to their corresponding nonhydroxylated relatives, for example, but-3-enyl glucosinolate **3** and **18**.[119d,123] The study of Oerlemans et al. (2006) gave comparable results with regard to the order of stability (below 110 °C). However, using kinetic modeling, this group showed that when starting at temperatures above 110 °C in aqueous media, the degradation kinetics of aliphatic and indole glucosinolates will equalize.[119a] Therefore, the temperature can affect the thermal degradation kinetic as well. On the other hand, during a dry heat treatment (roasting), aliphatic and indole glucosinolates in broccoli sprouts showed different thermal stabilities, both at 100 °C and at 130 °C.[119d]

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

When undergoing a thermally induced degradation process, aliphatic glucosinolates form isothiocyanates and nitriles. In 1970, MacLeod and MacLeod proposed nitriles to be the predominant thermal degradation products of glucosinolates,[125] and later proved their theory by heating the pure glucosinolates **1**, **16**, and 2-phenylethyl glucosinolate (**17**) on a gas chromatography column. The glucosinolates were predominantly decomposed to nitriles. Isothiocyanates appeared at higher temperatures ($\geq 150\,^{\circ}C$).[126] Moreover, when the natural plant matrix was treated directly, nitriles were the major thermally induced degradation products,[123,127] and isothiocyanates were only detected at a comparatively "low" temperature of 100 °C, but not at all at higher temperatures.[123]

2.2. Other Factors that Influence Thermally Induced Degradation

The formation of chemical breakdown products of glucosinolates is determined by the chemical structure of the glucosinolate, the thermal treatment conditions, and additional factors, such as the surrounding matrix, water content, iron concentration, and the pH value.

2.2.1. Influence of the pH Value

When *Brassica* vegetables were cooked at the natural pH value of the plant of 5.3, glucosinolates were comparatively stable. Contrarily, at higher pH values ($pH > 8.0$), aliphatic and indole glucosinolates are less stable.[119d,123] The recovery of the nitriles, the main thermally induced degradation products under such conditions, was lower compared to the slightly acidic environment, suggesting a thermal instability of the nitriles themselves.[123] However, pure glucosinolates are more stable in comparison,[123] and the percentage of isothiocyanates as breakdown products is higher.[123,128] Gronowitz et al. (1978) reported a higher thermal stability of the pure glucosinolate **5** at a pH value of 8 compared to that at pH 5.[129] These different results clearly point to a "plant matrix effect" that influences glucosinolate stability. Furthermore, a severe influence of buffer solutions on the thermal model experiments was also reported. For example, sodium tetraborate buffer was shown to degrade glucosinolates very effectively, even at room temperature.[129]

2.2.2. Influence of the Food Matrix

Dekker et al. (2009) demonstrated the "plant matrix effect" by cooking five different *Brassica* vegetables. They observed that the thermal susceptibility of glucosinolates differed within the vegetables, but that the order of stability remained the same (**3** > **18** > **20**).[119b] Lately, a similar study with four *Brassica* vegetables also described different thermal stabilities in the cooking water by kinetic modeling.[130] Moreover, Hennig et al. (2012) reported that glucosinolates in different *Brassica* vegetables grown at the same site and during the same season show the same thermal stability; however, stabilities differed between vegetables grown in different seasons.[131] This finding implies that the thermal degradation is influenced by growing conditions rather than by the genotype of the vegetable. Further studies from the same group showed that the thermal stability of glucosinolates seems to be also regulated genetically.[132] However, to date, the specific genes involved in this regulation are unknown.

2.2.3. Influence of the Water Content of the Plant Material

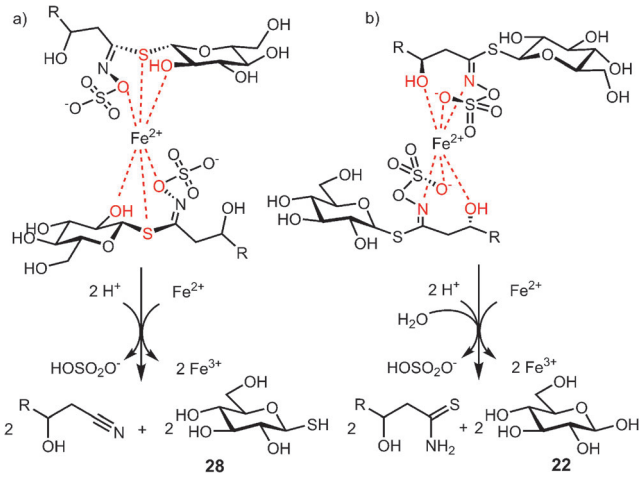
The water content of the plant material also affects the thermally induced degradation of glucosinolates. Glucosinolates in plant samples with low water content were most labile at 120 °C, compared to those with higher water content, whereas at 100 °C, the degradation was lower in samples with low residual moisture.[123,128,133] With regard to the chemical structure, the chain length of methylsulfanylalkyl and methylsulfinylalkyl glucosinolates did not affect the thermal stability of wet broccoli sprout samples, but did when heating dry ones.[123]

2.2.4. Influence of Iron Concentration

Another factor that influences thermally induced degradation is the concentration of iron ions in the samples. It is known that Fe^{II} can degrade glucosinolates in a non-enzymatic pathway. For example, in 1967, Youngs and Perlin demonstrated that glucosinolate **1** was degraded by Fe^{II}. As degradation products, they detected but-3-enenitrile (**29**) and bis(β-D-glucopyranosyl)disulfide and proposed that prior to the degradation, a glucosinolate–metal complex is formed, consisting of two glucosinolate molecules and the Fe^{II} ion.[134] This complex was proposed based on a description of a stable 1:2 complex of Ni^{II} and methylthiohydroxamic acid,[135] which has similar structural features compared to the glucosinolate. On the other hand, hydroxyalkenyl glucosinolates are affected by Fe^{II} in a different way, and next to the nitrile, the thionamide can be found as a major breakdown product.[136] Heat increases this degradation reaction, a phenomenon that is also observed for indole glucosinolates.[127,134,137] At equimolar Fe^{II} concentrations, 14 min at 95 °C were sufficient to completely degrade (*S*)-2-hydroxybut-3-enyl glucosinolate (epiprogoitrin).[136a] Based on the complex postulated by Youngs and Perlin (1967),[134] Bellostas et al. (2008) proposed a mechanism for this non-enzymatic degradation pathway (Scheme 5).[138]

The effect of low, but plant-relevant, Fe^{II} concentrations on the thermal degradation of glucosinolates was studied by adding the 1.3-fold concentration of soluble Fe or the 1.3-fold of the total Fe concentration present in broccoli sprouts as Fe^{II} ions. A reduced stability of the glucosinolates and a two- and even three-fold increase in nitrile formation was observed.[123] The influence of Fe^{II} or Fe^{III} as well as the broccoli sprout matrix or vitamin C addition on the thermally induced degradation of pure glucosinolate **1** was studied with model experiments.[128] Fe^{III} has no effect on the thermally induced degradation process at all, whereas Fe^{II}, even at very low concentrations (0.008 mol equivalents of Fe^{II}), decreased the thermal stability of the glucosinolate and accelerated the formation of the nitrile, which correlated well with the

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 5. Mechanisms of Fe^{II}-catalyzed non-enzymatic degradation of glucosinolates. A) Degradation of a 2-hydroxyalkenyl glucosinolate to nitrile and **28.** B) Degradation of 2-hydroxyalkenyl glucosinolates to thionamides and **22**. Based on the mechanism proposed in Ref. [138].

formation of sugar **28** instead of the release of **22.**[128] Vitamin C can promote the Fe^{II}-catalyzed degradation as a result of redoxcycling.[128] Moreover, other antioxidant compounds, such as kaempferol or quercetin derivatives, were recently shown to be linked with thermal stability, and it was suggested that they also act through redoxcycling of Fe^{III} to Fe^{II}.[132b]

Thus, in addition to temperature, the plant matrix and therefore the presence of Fe^{II} ions also have a strong influence on the stability of glucosinolates during thermal food preparation of *Brassica* vegetables. Unravelling the underlying mechanisms behind these influencing factors would therefore be a key step to advance understanding of (thermal) glucosinolate stability and in predicting outcomes.

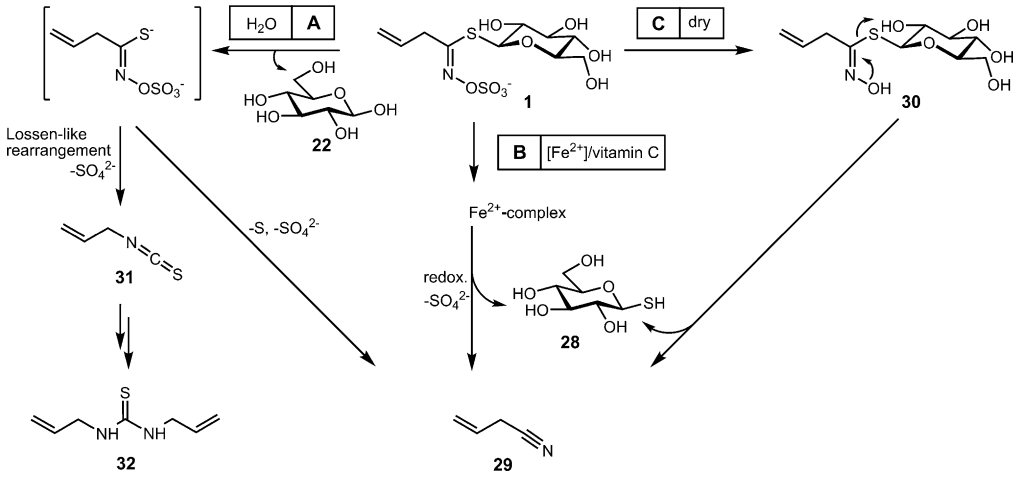
2.3. Thermally Induced Degradation Pathways

A study of the thermally induced degradation of glucosinolate **1** resulted in the identification of further degradation products.[128] Hanschen et al. (2012) identified the desulfoprop-2-enyl glucosinolate **30** as one of the major breakdown products after dry-heat treatment of **1**.[128] Consequently, the authors proposed that **30** acts as an important intermediate during the thermally induced degradation pathway and proved their hypothesis by degrading desulfoglucosinolates further to the corresponding nitriles. The formation of a desulfoglucosinolate as a result of chemical degradation was suggested by Shahidi and Gabon (1990), who treated mustard seeds rich in glucosinolate **1** with a solvent mixture of methanol/ammonia/water-hexane (at ambient temperatures) to remove the glucosinolate, and detected minor amounts of desulfoglucosinolate **30**. The latter was suggested to be an intermediate during this base-catalyzed degradation.[117c]

Three independent pathways were proposed to be of importance for the thermally induced degradation of glucosinolates (Scheme 6). Pathway **A** is similar to that of enzymatic degradation and occurs under aqueous conditions. Sugar **22** is cleaved and nitrile **29** or the thermally labile allyl isothiocyanate **31** is formed. The latter can further degrade and form *N*,*N*′-1,3-diallylthiourea **32**. Pathway **B** contains steps from Fe^{II}-catalyzed degradation and is based on the mechanism proposed by Bellostas et al. (2008),[138] in which the nitrile and sugar **28** are formed. Pathway **C** can occur during dry-heat treatment. Primarily by desulfatation, compound **30** is formed and then degrades further to sugar **28** and the nitrile **29**.[128]

The results for the thermal stability of glucosinolates and the influencing factors lead to valuable suggestions for chemical mechanisms, explaining the formation of the thermally induced breakdown products. An interpretation of the mechanisms is provided in the Supporting Information.

The thermally induced degradation of glucosinolates during processing is therefore strongly influenced by the



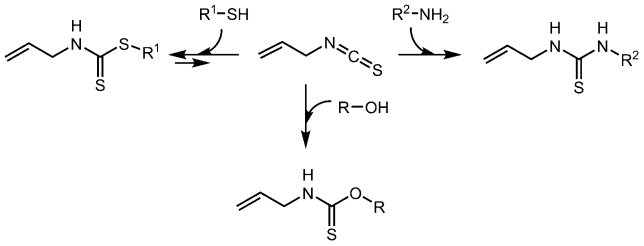
Scheme 6. Thermal degradation pathways (**A**–**C**) of aliphatic glucosinolates, presented for alkenyl glucosinolate **1** [according to Hanschen et al. (2012)].[128] A) Thermally induced degradation under aqueous conditions. B) Fe^{II}-catalyzed degradation of glucosinolates. C) Thermally induced degradation under dry conditions via the desulfoglucosinolate **30**.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

chemical structure, water content, and the pH value, as well as the content of Fe^{II} and vitamin C in the plant tissue.

3. Reactivity of Glucosinolate Breakdown Products

Glucosinolates are usually degraded to nitriles and isothiocyanates. Nitriles are comparatively stable substances.[139] Even during heat treatments at temperatures higher than 100 °C, they were shown to hardly degrade any further.[123] Therefore, losses during cooking will predominantly occur because of their volatility. On the other hand, isothiocyanates contain a very electrophilic carbon atom and readily react with nucleophiles such as hydroxy, amino, or thiol groups, thereby forming *O*-thiocarbamates, thiourea derivatives, or dithiocarbamates, respectively[80,140] (Scheme 7). A reaction with sulfite ions can lead to alkylaminothiocarbonylsulphonates as shown for mustard paste.[141]



Scheme 7. Reactions of isothiocyanates with nucleophiles.

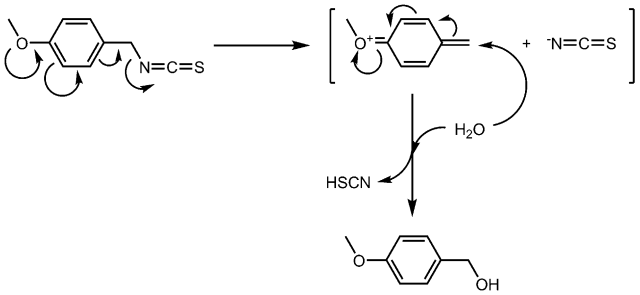
Especially in plant-based foods, reactions with water, amino acids, and proteins, and other thermally induced degradation products of isothiocyanates might also be of interest in better understanding glucosinolate-based bioactivity and quality of foods.

3.1. Reactions of Isothiocyanates under Aqueous Conditions

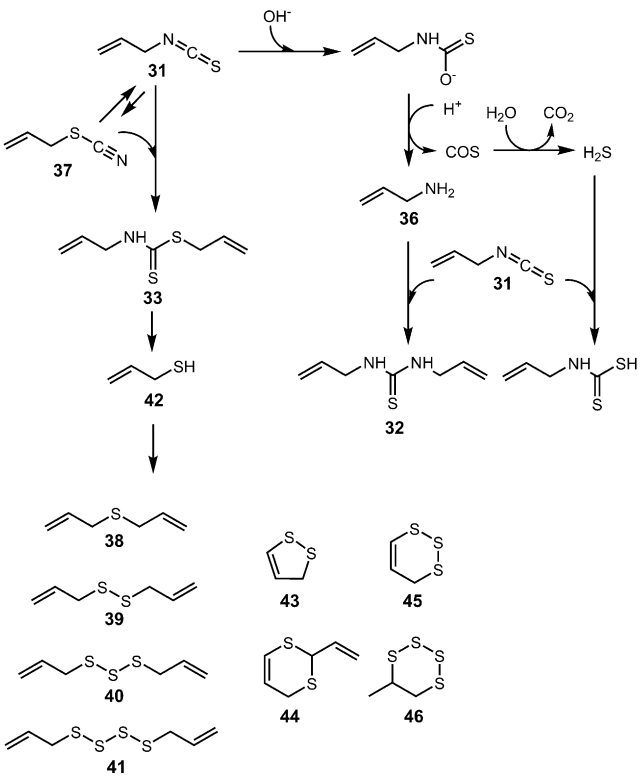
As *Brassica*-vegetable-derived isothiocyanates are usually formed under aqueous conditions, the reaction with water is one of the most important ones, especially under thermal processing conditions. Indole isothiocyanates are very labile and decompose immediately in water to produce an alcohol and a thiocyanate ion.[46e] It is postulated that indole isothiocyanate decomposition proceeds via a resonance-stabilized carbocation that is very reactive.[99,100,142] For example, the carbocation of glucosinolate **21** reacts with selected DNA bases, resulting in the formation of DNA adducts (compare Scheme 3 in chapter 1.3.).[99,100,142]

Some *ortho*- or *para*-hydroxylated (or methoxylated) benzyl isothiocyanates also form an alcohol and a thiocyanate as degradation products.[143] The presence of an electron-donating group in the *ortho* or *para* position seems to facilitate the formation of a resonance-stabilized carbocation that has been postulated for this reaction pathway (Scheme 8).[143] Other aromatic isothiocyanates, such as benzyl- or 3-methoxybenzyl isothiocyanate and aliphatic isothiocyanates, follow a different pathway. The latter form an unstable *O*-thiocarbamic acid by attacking hydroxy ions or water. The *O*-thiocarbamic acid decomposes quickly to the amine and carbonyl sulfide, which is further degraded to carbon dioxide and hydrogen sulfide. The amine might react with aromatic isothiocyanates that are still present to the corresponding *N*,*N*′-diaryl thioureas (see also Scheme 9).[80,144] In the presence of methanol, the formation of isothiocyanate-derived methyl-*O*-carbamates was also reported in aqueous media.[145]

Kawakishi and Namiki (1969) studied the aqueous degradation of isothiocyanate **31**, which results from the enzymatic breakdown of glucosinolate **1**.[146] Isothiocyanate **31** was stored at 37 °C for 10 days at pH 5.2. After this time, 75 % of the isothiocyanate **31** was degraded and a garlic-like



Scheme 8. Degradation of unstable aromatic isothiocyanates to an alcohol and a thiocyanate ion according to Ref. [143].



Scheme 9. Thermal degradation of isothiocyanate **31** according to Ref. [146, 147b, 148].

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

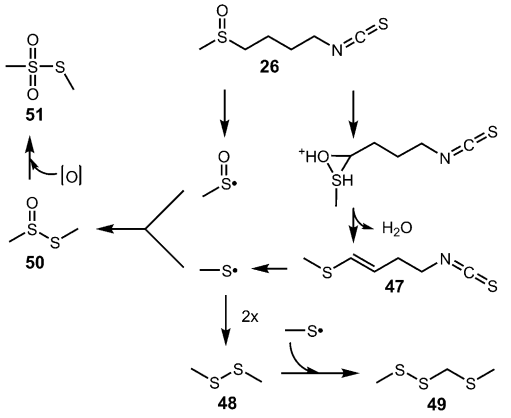
odor was observed. Diallyldithiocarbamate (**33**), its degradation products diallyl tetrasulfide (**34**) and diallyl pentasulfide (**35**), and molecular sulfur were also detected, as was compound **32**, deriving from the reaction of allyl amine (**36**) and isothiocyanate **31**.[146]

Similar to the chemical degradation of glucosinolates, the degradation of isothiocyanates in aqueous media is promoted by higher pH values and temperatures.[147] Therefore, heat treatment such as cooking quickly degrades isothiocyanates, and a variety of degradation products are formed (Scheme 9). Furthermore, the thermal stability of isothiocyanates is different. For example, isothiocyanate **31** was relatively labile. Within 1 h at 100 °C, the degradation of this substance was 57 % and 71 % with a pH value of 5.3 and 7–7.3, respectively.[145c,148] In contrast, isothiocyanate **26** is more stable, as only 21 % (pH 5.3) or 39 % (pH 7.3) were degraded under the same conditions.[145c] Moreover, at higher temperatures, an isomerization reaction of isothiocyanates occurs and the corresponding thiocyanate is formed. Consequently, this aliphatic thiocyanate can react with isothiocyanates to dithiocarbamates.[148,149] Until now, several breakdown products have been identified and their levels were also shown to depend on pH values during the reaction.

The decomposition of isothiocyanate **31** at 80 °C and a pH value of 4 resulted in amine **36**, carbon disulfide, and allyl thiocyanate (**37**) as the main degradation products. At 80 °C and pH 8, even more amine, as well as dithiocarbamate **33** and compound **32** were present.[147b] However, Chen and Ho (1998) could not detect dithiocarbamate **33** after heating isothiocyanate **31** for 1 h at 100 °C and at various pH values.[148] Instead, they detected diallylsulfide (**38**), diallyldisulfide (**39**), and diallyltrisulfide (**40**) among the mayor volatile breakdown products, and traces of diallyltetrasulfide (**41**). Interestingly, compound **32** was the mayor nonvolatile breakdown product. In contrast to Pecháček et al. (1997), Chen and Ho (1998) could not directly detect allyl mercaptan (**42**), but they were able to find traces of its cyclization products: 3*H*-1,2-dithiolene (**43**), 2-vinyl-4*H*-1,3-dithiin (**44**), 4*H*-1,2,3-trithiin (**45**), and 5-methyl-1,2,3,4-tetrathiane (**46**; Scheme 9).[147b,148] Compounds such as **44** and the various allyl sulfides also occur as breakdown products from alliin (*S*-2-propenyl-L-cysteine sulfoxide), the precursor substance of the aroma of garlic.[150] The occurrence of these substances, especially of sulfide **39**,[150b,c] therefore explains the observed garlic-like odor when heating isothiocyanate **31** in aqueous medium as reported for mustard paste.[151]

The thermal degradation of the methylsulfinyl isothiocyanate **26** partially follows a different thermal degradation pathway compared to isothiocyanate **31**. As **31**, most of compound **26** is also hydrolyzed to the amine, which results in the *N*,*N*′-di-(methylsulfinyl)butyl thiourea.[145c,152] However, the proposed mechanism for the formed volatile breakdown products differs significantly from the one proposed for isothiocyanate **31**, for which Jin et al. (1999) suggested a mechanism involving radicals.[152] The proposed cleavage of the methylsulfinyl radical could also explain the observed formation of but-3-enyl isothiocyanate, deriving from isothiocyanate **26** in the injection port of a gas chromatograph.[153]

Additionally, Jin et al. (1999) suggested that the oxygen atom of the methylsulfinyl group could form, via an epoxide, a 4-methylsulfanyl-4-hydroxybutyl isothiocyanate that reacts after the release of water to 4-methylsulfanyl-3-butenyl isothiocyanate (**47**). The latter compound could release the methylsulfanyl radical that, in addition to the methylsulfinyl radical, could form the remaining degradation products that were observed, namely dimethyldisulfide (**48**), 1,2,4-trithiolane, methyl(methylsulfanyl)methyl disulfide (**49**), or *S*-methyl methylsulfanylsulfinate (**50**) and the corresponding sulfonate **51** (Scheme 10). However, isothiocyanate **47**, which

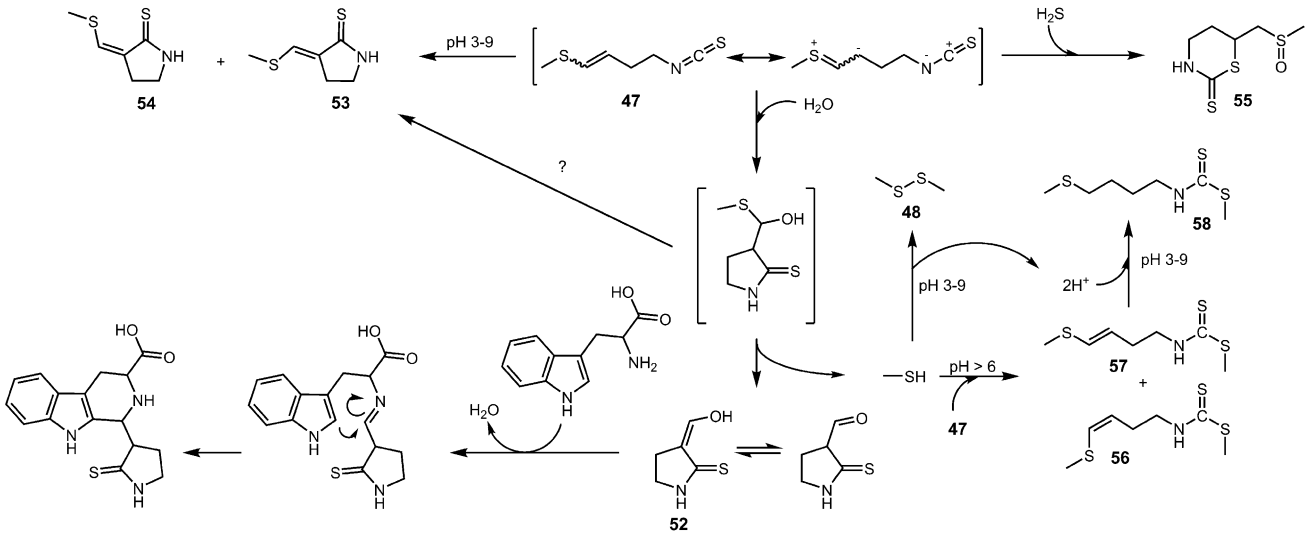


Scheme 10. Pathways for the formation of volatile thermal degradation products of isothiocyanate **26** according to Ref. [152].

is the major isothiocyanate derived from radish (*Raphanus sativus*)[31a] as well as its oxidized sulfinyl analogue, are very labile, even at room temperature.[145a,b,154] The reactivity of **47** and analogues is clearly based on the double bond in α position to the sulfanyl group, as this enables mesomerism and the nucleophilic C atom in the β position can attack the electrophilic isothiocyanate group. Therefore, several cyclic products deriving from **47**, such as 3-(hydroxy)methylene-2-thioxopyrrolidine (**52**), (*Z*)-3-(methylsulfanyl)-methylene-2-thioxopyrrolidine (**53**), its (*E*)-isomer **54**, as well as 6-[(methylsulfinyl)methyl]-1,3-thiazinan-2-thione (**55**) from the 4-(methylsulfinyl)but-3-enyl isothiocyanate, have been observed (Scheme 11). The formation of the latter is initiated by the addition of a hydrosulfide ion. Thus, the stability of unsaturated sulfinyl isothiocyanates can be enhanced by metal ions.[154] Furthermore, one of the cyclic products of **47**, compound **52**, was shown to readily react with tryptophan, ascorbic acid, and several dihydroxyphenolic compounds to form the yellow pigments that account for the yellowing of processed radish.[155] During the formation of compound **52**, methanethiol could be released and oxidized to the ill-smelling sulfide **48** or react with the isothiocyanate **47** to form the detected methyl (*Z*)-4-methylsulfanylbut-3-enyldithiocarbamate (**56**) and its (*E*)-isomer **57**, or methyl 4-methylsulfanylbutyldithiocarbamate (**58**).[145a,b,154]

As there is a great diversity in the structure of *Brassica*-vegetable-derived isothiocyanates and the degradation pathways of only some of them have been studied, it is very likely

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 11. Reactions of isothiocyanate **47** and analogues under aqueous conditions, modified according to Ref. [145b, 154–156]. Compound **55** derives from 4-(methylsulfinyl)but-3-enyl isothiocyanate, all other compounds from **47**.

that more possibilities for the formation of isothiocyanate degradation products in water exist, all of them with the potential to contribute to flavor, color, and even responsible for physiological processes.

3.2. Reactivity of Isothiocyanates with Other Food Nucleophiles

In addition to the thermally induced hydrolysis, isothiocyanates also react with other nucleophiles that are present in most foods in the form of amino acids, peptides, and proteins. These contain amino, thiol, or hydroxy groups that can act as electron donors. Several studies dealing with the kinetics of the reactions of isothiocyanates with amines and thiols showed that isothiocyanates react with the dissociated form of amino, thiol, or hydroxy groups (free bases). Therefore, a high pH value favors such nucleophilic addition reactions for which the order of reactivity is as follows: thiol group $\gg$ amino group $>$ hydroxy group.[80,157] The kinetics of the reaction depends on 1) the pH value, as it influences the first-order reaction of the amino or thiol group with the free base, 2) the isothiocyanate structure, and 3) the nucleophilic substituent/reaction partner.

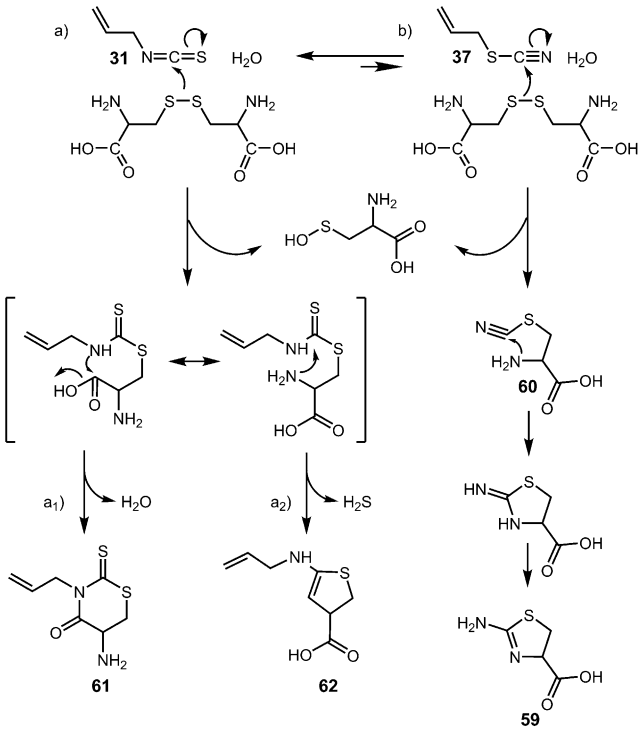
The reactivity of amino or thiol compounds toward isothiocyanates is higher with increasing basicity of these nucleophiles.[157a,158] Moreover, it is dependent on the side-chain structure of the isothiocyanates: their reactivity accelerates with smaller size and increasing electrophilic character of the side chain. This means that a lower electron density at the reacting carbon atom of the isothiocyanate function promotes reactivity, as demonstrated by Drobnica and Augustin (1965). They studied the reactivity of different arylisothiocyanates with thioglycolate and glycine and found that electron-withdrawing substituents improve the reactivity remarkably.[157b] As such addition reactions were shown to be of second order, their kinetics also depend on the concentration of the reactants.[157a,b]

In foods, isothiocyanates react with free amino acids, peptides, and proteins at even food relevant pH values (pH 4–8). While the formation of thiourea resulting from the reaction with amino groups of amino acids is slow and needs at least neutral conditions to be of relevance,[145c] the formation of dithiocarbamate with cysteine or glutathione is comparatively fast in acidic media.[145c,157a] Moreover, isothiocyanates not only react with free thiols, but can also cleave disulfide bonds by oxidative scission, as shown for cystine and oxidized glutathione.[159] As a result, the dithiocarbamate and a sulfeno-compound are released (Scheme 12).

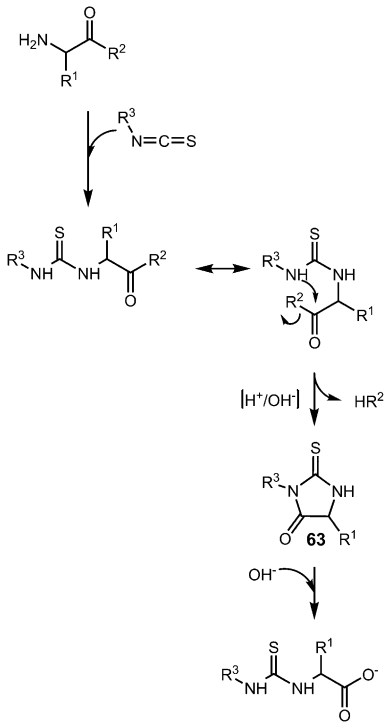
Furthermore, thiocyanate **37**, formed by isomerization of the corresponding isothiocyanate **31**, was also shown to be able to cleave the disulfide bond (Scheme 12b). The detection of 2-amino-2-thiazoline-4-carboxylic acid (**59**) led to the assumption that it is derived from cyclization and isomerization of the cleavage product β-thiocyanoalanine (**60**).[159c] The dithiocarbamates resulting from a reaction with cysteine are able to cyclize to the 3(3*H*)-allyl-5,6-dihydro-5-amino-2(2*H*)-thioxo-1,3-thiazin-4-one derivative **61** by releasing water (Scheme 12, a_1) or to the 2-allylamino-2-thiazoline-4-carboxylic acid derivative **62** by releasing hydrogen sulfide (Scheme 12, a_2).[159a,c]

However, as the nucleophilic addition is reversible, dithiocarbamates can also decompose again to isothiocyanates and amino acids by base catalysis.[160] This back reaction also takes place under physiological conditions (pH 7.3, 37 °C) and cysteine and glutathione dithiocarbamates are described to be transporting agents for isothiocyanates in vivo.[161] The stability of these dithiocarbamates was studied in detail by Conaway et al. (2001), who identified the cysteine adducts of various isothiocyanates as being less stable compared to glutathione adducts.[54] Furthermore, it was also demonstrated that the concentration of the dithiocarbamates rapidly declines when competitive thiols that form more stable dithiocarbamates or amino compounds are present.[160,161] Nakamura et al. (2009) studied the exchange

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim



Scheme 12. Proposed reaction mechanism of cystine and isothiocyanate **31** according to Ref. [159a,c]. A) Cleavage induced by the isothiocyanate. The dithiocarbamate then cyclizes through the release of a_1) water or a_2) hydrogen sulfide. B) Thiocyanate-induced cleavage of the disulfide bond.



Scheme 13. Reaction of isothiocyanates and amino acids or peptides under aqueous conditions to 2-thiohydanthoins **63** according to Ref. [163]. R^1 = amino acid residue; R^2 = *N*-bound peptide (amino acid), O^-; R^3 = alkyl residue.

reaction between the **31**-N^{α}-acetyl-L-cysteine adduct and synthetic N^{ε}-benzoyl-glycyl-L-lysine and observed that the exchange reaction from the thiol to the amino group was nearly 100 % after 24 h. As they performed the reaction under more or less physiological conditions (pH 7.4, 37 °C),[160] these reactions could also be relevant in foods. Although the amino groups of amino acids react more slowly, the reactions result in stable adducts that can also be used as markers for an isothiocyanate (glucosinolate) exposition in human plasma.[162]

Comparable with the above-mentioned dithiocarbamates, the thiourea derivatives that derived from the reaction of isothiocyanates with the amino group of peptides, are able to cyclize and can form 2-thiohydantoins **63** with water or by peptide-chain cleavage at the N-terminal end (Scheme 13).[159b,163] Formation of **63** is favored at higher pH values (pH 8), but also occurs at a low level under acidic conditions (pH 6).[159b,163] The formation of **63** by cleavage of the peptide chain is also commonly known in amino acid sequencing of proteins (during the so-called Edman degradation).[164] The reactivity of the amino groups of amino acids is quite low and Hanschen et al. (2012) showed that using synthetic lysine derivatives at a pH value of 7.3 and room temperature the α-amino group of lysine was more reactive toward isothiocyanates, such as isothiocyanate **31** or **26**, than the ε-amino group.[145c] Therefore, at first glance, the ε-amino groups of lysine in food proteins seem to be a comparatively non-attractive goal for isothiocyanate attack. However, as the ε-thioureas are more stable than α-thioureas,[145c] lysine side chains in proteins are modified with isothiocyanates nonetheless.[79,165]

As nucleophilic compounds in foods, proteins may also be a target for an isothiocyanate attack. Therefore, several investigations were performed on protein modifications caused by *Brassica*-vegetable-released isothiocyanates. However, most of these studies used isolated proteins or dealt with protein fractions, with only a few dealing with protein-rich foods.[79,165,166] However, as proteins are modified by isothiocyanate attack in vitro[167] and even in vivo,[162,168] these reactions are very likely to take place in foods and are worth to be considered. The proof of the presence of certain amino acid adducts or protein modifications in complex food or physiological matrices is still challenging, but the unsuccessful approaches so far do not relativize the relevance of such reactions. Moreover, they give evidence for the difficulties of analyzing such complex reaction mixtures with several hundreds of heterogeneous degradation and reactions products. As obvious from the literature, most studies have been performed as model approaches to overcome the difficulties "step-by-step". Further work is needed to evaluate the relevance for individual *Brassica* food and the influence of these addition reactions on food quality.

Björkman (1973) studied the interactions between serum albumin and rapeseed proteins with several ^{35}S-labeled glucosinolates after the addition of myrosinase and at different pH values. He observed that radioactivity increased rapidly with the addition of isothiocyanates at pH values higher than 6, and that the isothiocyanates mainly reacted with the low-molecular-weight basic protein fraction.[169]

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

The interaction of isothiocyanate **31** with the high-molecular-weight protein fraction 12S (which is low in cysteine) of mustard was studied by Murthy and Rao (1986). At pH values higher than 7, they observed a reduced content of available lysine as a result of the modification of ε-amino residue to thiourea-like derivatives. A second effect was a reduced tryptic degradability, and the authors hypothesized that some of the OH functions of tyrosine may be modified.[79] The reduced tryptic digestibility of proteins caused by isothiocyanate modification at pH values between 7.5 and 9 was also reported by other groups and has been attributed to a modification of lysine and probably arginine side chains.[166b,170] Additionally, the nitrogen atom of the secondary amine tryptophan can also be a target for modification by isothiocyanates. This has been shown for myoglobin with only amino groups, but no thiol groups.[165,171] In this study, MALDI-TOF mass spectrometry enabled the identification of up to 16 molecules of benzyl isothiocyanate being added to the amino groups of one molecule of myoglobin.[165]

Compared to the reactivity of amino groups, thiol groups of cysteine protein side chains can be even more susceptible to modification by isothiocyanates and react even in acidic medium.[170b,172] Rade-Kukic et al. (2011) demonstrated that in a first step isothiocyanates react with the thiols that are present, and amino groups are only modified in excess concentrations of the isothiocyanates.[172] The release of carbon disulfide from those proteins functions as a marker for the formation of dithiocarbamate (and its further degradation), as shown for egg white proteins and the enzyme bromelain from pineapple (*Ananas comosus*).[166a,170a] Furthermore, Kawakishi and Kaneko (1987) studied the reactions of isothiocyanate **31** with several food proteins at pH values of 6–7.5. They found that especially bovine serum albumin, which is comparatively rich in disulfide bonds, quickly reacted with the isothiocyanate.[170b]

The cleavage of disulfide bonds caused by isothiocyanate attack can also lead to protein polymerization.[170b] As a consequence of this isothiocyanate-mediated protein modification, a shift in the isoelectric range to lower pH values, decreased solubility, and an unfolding and loosening of the global protein structure as a result of reduced electrostatic attraction and increased hydrophobicity were also reported.[166,170,172] This will lead to modified technofunctional properties or bioactivity of the "derivatized" proteins. For example, heat aggregation behavior, foaming, and emulsifying properties of β-lactoglobulin–isothiocyanate adducts have been modified.[172] Moreover, the proteolytic enzyme bromelain lost its enzymatic activity.[170a] With regard to nutritional aspects, an in vivo rat-feeding study showed a loss in available lysine, resulting in a decreased bioutility of nitrogen, and total protein digestibility was also found to be lower.[173] To relate this result to the human diet, a simple recipe can be taken into account: In some parts of the world, eggs are consumed with mustard. When eating one egg (60 g, 12.5 % protein) with a usual portion of mustard (10 g, 126.8 μmol of isothiocyanates[76g]), 16.9 μmol isothiocyanate are potential reactants per gram of protein. Kroll et al. (1994) demonstrated that a similar isothiocyanate concentration (15.2 μmol benzyl isothiocyanate per gram of protein) reduced the available lysine content of the egg white by 6.6 %.[166b] Therefore, these reactions will occur in sulfur- or lysine-rich foods in a combination with ingredients/side dishes from *Brassica* plants and for example meat or eggs, and it is expected that modified proteins will be of lower nutritional quality. In contrast, the reactions with thiol groups of enzymes and proteins might be responsible for the potential health-preventive effects of isothiocyanates in vivo, as shown for the induction of phase-II enzymes, induction of apoptosis, or antithrombotic effects.[81b,167,174]

Almost all of the experiments dealing with the reactivity between isothiocyanates and food nuleophiles were carried out in model systems and most of them under basic conditions. However, foods usually have low pH values (pH 4–6), and only very few, for example egg white, are basic (pH up to 9.5[173]). Thiol groups react with isothiocyanates even under acidic conditions,[145c] sulfur-rich proteins and thiols, such as cysteine or glutathione, are a target for isothiocyanates in foods and will be easily modified.

4. Summary and Outlook

The concentration of glucosinolates and isothiocyanates in *Brassica*-vegetable-based food is by far more variable than previously thought. Both compound classes were shown to be subject to a variety of changes during food processing. Among other factors, thermal degradation leads to the transformation of glucosinolates predominantly to nitriles through several chemical mechanisms. Besides the chemical structure of the glucosinolate, several conditions, such as pH value, plant matrix, Fe^{II} ions, vitamin C, other antioxidants, or the water content, strongly affect the susceptibility and the pathway of the reaction. Isothiocyanates, which are thought to provide beneficial health effects, can be severely affected by thermal treatment and decompose further to a variety of volatile and nonvolatile compounds. Therefore, they will also have an indirect impact on the flavor of cooked foods.

As these compounds react with amino acids and proteins under food-relevant conditions, it is very likely that they affect the nutritional quality and the technofunctional properties of proteins. In the human diet, lysine is the most limiting amino acid and found in much lower concentrations in most plant proteins compared to animal proteins.[175] In developing countries or more or less also in vegetarian diets, amino acid intake often does not meet the daily recommendations of the WHO.[175a,176] Additionally, high intake of *Brassica* vegetables might decrease amino acid intake because of decreased quality and digestibility of proteins. Further research is needed to evaluate the impact of such reactions on the overall quality of food. As the analysis of degradation and reaction products is quite challenging, analytical strategies and methodologies have to be improved or developed. With regard to reaction products of isothiocyanates with proteins, even traditional proteomics approaches showed only limited success. Thus, innovative *neoproteomics* strategies with chromatographic and mass spectrometric techniques are required.

Therefore, proofing the health-preventive effects of these compounds is a challenge and the outlook for nutritional

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

science seems to be still disillusioning, as knowledge is currently still insufficient. The high number of heterogenic degradation and reaction products that might contribute to the bioactivity complicates the risk–benefit evaluation. Thus, future research approaches should focus on the chemical mechanisms for solving the above-mentioned challenges. Isothiocyanate and nitrile chemistry need to be reactivated and integrated into glucosinolate research.

Please note: Changes have been made to this manuscript since its publication in *Angewandte Chemie* Early View. The Editor.

We kindly thank Dr. Ronald Maul for his help.

Received: February 21, 2014
Published online: August 27, 2014

[1] J. W. Fahey, A. T. Zalcmann, P. Talalay, *Phytochemistry* **2001**, *56*, 5 – 51.
[2] G. R. Fenwick, R. K. Heaney, W. J. Mullin, C. H. VanEtten, *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *18*, 123 – 201.
[3] F. Boutron, P. J. Robiquet, *J. Pharm. Sci. Accessoires* **1831**, *17*, 279 – 298.
[4] A. Bussy, *Justus Liebigs Ann. Chem.* **1840**, *34*, 223 – 230.
[5] J. Gadamer, *Ber. Dtsch. Chem. Ges.* **1897**, *30*, 2322 – 2327.
[6] M. G. Ettlinger, A. J. Lundeen, *J. Am. Chem. Soc.* **1956**, *78*, 4172 – 4173.
[7] M. G. Ettlinger, A. J. Lundeen, *J. Am. Chem. Soc.* **1957**, *79*, 1764 – 1765.
[8] R. E. Marsh, J. Waser, *Acta Crystallogr. Sect. B* **1970**, *26*, 1030 – 1037.
[9] D. B. Clarke, *Anal. Methods* **2010**, *2*, 310 – 325.
[10] a) A. Kjaer, *Fortschr. Chem. Org. Naturst.* **1960**, *18*, 122 – 176; b) H. L. Tookey, C. H. Van Etten, M. E. Daxenbichler in *Toxic Constituents of Plant Foodstuffs*, 2ednd ed(Ed.: I. E. Liener), Academic Press, New York, **1980**, pp. 103 – 142; c) B. A. Halkier, J. Gershenzon, *Annu. Rev. Plant Biol.* **2006**, *57*, 303 – 333; d) A. Kjaer, P. Olesen Larsen, *Biosynthesis* **1973**, *2*, 71 – 105; e) A. Kjaer, P. Olesen Larsen, *Biosynthesis* **1976**, *5*, 120 – 135; f) B. Holst, G. Williamson, *Nat. Prod. Rep.* **2004**, *21*, 425 – 447; g) M. Traka, R. Mithen, *Phytochem. Rev.* **2009**, *8*, 269 – 282; h) R. F. Mithen, M. Dekker, R. Verkerk, S. Rabot, I. T. Johnson, *J. Sci. Food Agric.* **2000**, *80*, 967 – 984; i) E. A. S. Rosa, R. K. Heaney, G. R. Fenwick, C. A. M. Portas in *Horticultural Reviews*, Wiley, New York, **1997**, pp. 99 – 215.
[11] A. M. Bones, J. T. Rossiter, *Phytochemistry* **2006**, *67*, 1053 – 1067.
[12] a) M. Linscheid, D. Wendisch, D. Strack, *Z. Naturforsch. C* **1980**, *35*, 907 – 914; b) A. A. M. Andersson, A. Merker, P. Nilsson, H. Sørensen, P. Åman, *J. Sci. Food Agric.* **1999**, *79*, 179 – 186; c) M. Reichelt, P. D. Brown, B. Schneider, N. J. Oldham, E. Stauber, J. Tokuhisa, D. J. Kliebenstein, T. Mitchell-Olds, J. Gershenzon, *Phytochemistry* **2002**, *59*, 663 – 671.
[13] a) R. N. Bennett, F. A. Mellon, P. A. Kroon, *J. Agric. Food Chem.* **2004**, *52*, 428 – 438; b) L. M. Larsen, J. K. Nielsen, H. Sørensen, *Entomol. Exp. Appl.* **1992**, *64*, 49 – 55; c) R. N. Bennett, F. A. Mellon, N. Foidl, J. H. Pratt, M. S. Dupont, L. Perkins, P. A. Kroon, *J. Agric. Food Chem.* **2003**, *51*, 3546 – 3553; d) N. Bellostas, A. D. Sørensen, J. C. Sørensen, H. Sørensen in *Advances in Botanical Research*, *Vol. 45* (Eds.: M. D. Surinder Kumar Gupta, J. C. Kader), Academic Press, New York, **2007**, pp. 369 – 415.
[14] a) S.-J. Kim, S. Jin, G. Ishii, *Biosci. Biotechnol. Biochem.* **2004**, *68*, 2444 – 2450; b) S.-J. Kim, C. Kawaharada, S. Jin, M. Hashimoto, G. Ishii, H. Yamauchi, *Biosci. Biotechnol. Biochem.* **2007**, *71*, 114 – 121; c) R. N. Bennett, F. A. Mellon, N. P. Botting, J. Eagles, E. A. S. Rosa, G. Williamson, *Phytochemistry* **2002**, *61*, 25 – 30.
[15] S. Textor, J. Gershenzon, *Phytochem. Rev.* **2009**, *8*, 149 – 170.
[16] A. Matsumura, S. Horii, T. Ishii, *J. Jpn. Soc. Hortic. Sci.* **2007**, *76*, 224 – 229.
[17] a) F. Rohr, C. Ulrichs, M. Schreiner, R. Zrenner, I. Mewis, *Plant Physiol. Biochem.* **2012**, *55*, 52 – 59; b) I. Mewis, J. G. Tokuhisa, J. C. Schultz, H. M. Appel, C. Ulrichs, J. Gershenzon, *Phytochemistry* **2006**, *67*, 2450 – 2462.
[18] I. Mewis, M. Schreiner, C. N. Nguyen, A. Krumbein, C. Ulrichs, M. Lohse, R. Zrenner, *Plant Cell Physiol.* **2012**, *53*, 1546 – 1560.
[19] a) M. A. M. Khan, C. Ulrichs, I. Mewis, *Chemoecology* **2011**, *21*, 235 – 242; b) I. Mewis, M. A. M. Khan, E. Glawischnig, M. Schreiner, C. Ulrichs, *PLoS ONE* **2012**, *7*, e48661.
[20] a) A. Gimsing, J. Kirkegaard, *Phytochem. Rev.* **2009**, *8*, 299 – 310; b) P. D. Brown, M. J. Morra, *J. Agric. Food Chem.* **1995**, *43*, 3070 – 3074; c) J. N. Matthiessen, J. A. Kirkegaard, *Crit. Rev. Plant Sci.* **2006**, *25*, 235 – 265.
[21] a) U. Wittstock, B. A. Halkier, *Trends Plant Sci.* **2002**, *7*, 263 – 270; b) I. E. Sønderby, M. Burow, H. C. Rowe, D. J. Kliebenstein, B. A. Halkier, *Plant Physiol.* **2010**, *153*, 348 – 363.
[22] C. D. Grubb, S. Abel, *Trends Plant Sci.* **2006**, *11*, 89 – 100.
[23] P. D. Brown, J. G. Tokuhisa, M. Reichelt, J. Gershenzon, *Phytochemistry* **2003**, *62*, 471 – 481.
[24] a) N. Bellostas, P. Kachlicki, J. C. Sørensen, H. Sørensen, *Sci. Hortic.* **2007**, *114*, 234 – 242; b) S. Pérez-Balibrea, D. A. Moreno, C. García-Viguera, *J. Food Sci.* **2010**, *75*, C673 – C677.
[25] M. Wiesner, R. Zrenner, A. Krumbein, H. Glatt, M. Schreiner, *J. Agric. Food Chem.* **2013**, *61*, 1943 – 1953.
[26] a) S. Li, I. Schonhof, A. Krumbein, L. Li, H. Stützel, M. Schreiner, *J. Agric. Food Chem.* **2007**, *55*, 8452 – 8457; b) E. Stavridou, H. L. Kristensen, A. Krumbein, M. Schreiner, K. Thorup-Kristensen, *J. Agric. Food Chem.* **2012**, *60*, 6268 – 6278; c) I. Schonhof, D. Blankenburg, S. Müller, A. Krumbein, *J. Plant Nutr. Soil Sci.* **2007**, *170*, 65 – 72; d) A. Krumbein, I. Schonhof, I. Smetanska, E. T. Scheuner, J. Rühlmann, M. Schreiner, *Acta Hortic.* **2010**, *856*, 37 – 48; e) M. D. Omirou, K. K. Papadopoulou, I. Papastylianou, M. Constantinou, D. G. Karpouzas, I. Asimakopoulos, C. Ehaliotis, *J. Agric. Food Chem.* **2009**, *57*, 9408 – 9417.
[27] A. Koprivova, M. Suter, R. O. den Camp, C. Brunold, S. Kopriva, *Plant Physiol.* **2000**, *122*, 737 – 746.
[28] V. Nikiforova, J. Freitag, S. Kempa, M. Adamik, H. Hesse, R. Hoefgen, *Plant J.* **2003**, *33*, 633 – 650.
[29] a) H. Zhang, I. Schonhof, A. Krumbein, B. Gutezeit, L. Li, H. Stützel, M. Schreiner, *J. Plant Nutr. Soil Sci.* **2008**, *171*, 255 – 265; b) M. Schreiner, B. Beyene, A. Krumbein, H. Stützel, *J. Agric. Food Chem.* **2009**, *57*, 7259 – 7263.
[30] Y. Tong, E. Gabriel-Neumann, B. Ngwene, A. Krumbein, E. George, S. Platz, S. Rohn, M. Schreiner, *Food Chem.* **2014**, *152*, 190 – 196.
[31] a) E. Ciska, B. Martyniak-Przybyszewska, H. Kozłowska, *J. Agric. Food Chem.* **2000**, *48*, 2862 – 2867; b) F. M. V. Pereira, E. Rosa, J. W. Fahey, K. K. Stephenson, R. Carvalho, A. Aires, *J. Agric. Food Chem.* **2002**, *50*, 6239 – 6244; c) I. Schonhof, H. P. Kläring, A. Krumbein, W. Claußen, M. Schreiner, *Agric. Ecosyst. Environ.* **2007**, *119*, 103 – 111.
[32] R. M. Wallsgrove, R. N. Bennett in *Amino Acids and their Derivates in Higher Plants (Society for Experimental Biology Seminar Series)* (Ed.: R. M. Wallsgrove), University Press, Cambridge, **1995**, pp. 243 – 259.
[33] a) M. Schreiner, A. Krumbein, I. Mewis, C. Ulrichs, S. Huyskens-Keil, *Innovative Food Sci. Emerging Technol.* **2009**, *10*, 93 – 96; b) M. S. C. Pedras, Q.-A. Zheng, *Phytochemistry* **2010**, *71*, 581 – 589.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[34] a) K. Tilbrook, A. B. Arongaus, M. Binkert, M. Heijde, R. Yin, R. Ulm, *The Arabidopsis Book* **2013**, *11*, e0164; b) M. Schreiner, I. Mewis, S. Huyskens-Keil, M. A. K. Jansen, R. Zrenner, J. B. Winkler, N. O'Brien, A. Krumbein, *Crit. Rev. Plant Sci.* **2012**, *31*, 229 – 240.

[35] a) M. Wiesner, F. S. Hanschen, M. Schreiner, H. Glatt, R. Zrenner, *Int. J. Mol. Sci.* **2013**, *14*, 14996 – 15016; b) G. I. Jenkins, *Annu. Rev. Plant Biol.* **2009**, *60*, 407 – 431.

[36] a) P. J. Kelly, A. Bones, J. T. Rossiter, *Planta* **1998**, *206*, 370 – 377; b) R. Kissen, J. Rossiter, A. Bones, *Phytochem. Rev.* **2009**, *8*, 69 – 86; c) A. M. Bones, O. P. Thangstad, O. A. Haugen, T. Espevik, *J. Exp. Bot.* **1991**, *42*, 1541 – 1550.

[37] Y. Uda, T. Kurata, N. Arakawa, *Agric. Biol. Chem.* **1986**, *50*, 2735 – 2740.

[38] a) H. L. Foo, L. M. Grønning, L. Goodenough, A. M. Bones, B.-E. Danielsen, D. A. Whiting, J. T. Rossiter, *FEBS Lett.* **2000**, *468*, 243 – 246; b) R. Bernardi, A. Negri, S. Ronchi, S. Palmieri, *FEBS Lett.* **2000**, *467*, 296 – 298.

[39] a) M. Burow, A. Losansky, R. Müller, A. Plock, D. J. Kliebenstein, U. Wittstock, *Plant Physiol.* **2009**, *149*, 561 – 574; b) M. Burow, J. Markert, J. Gershenzon, U. Wittstock, *FEBS J.* **2006**, *273*, 2432 – 2446.

[40] M. Burow, A. Bergner, J. Gershenzon, U. Wittstock, *Plant Mol. Biol.* **2007**, *63*, 49 – 61.

[41] D. J. Williams, C. Critchley, S. Pun, M. Chaliha, T. J. O'Hare, *J. Agric. Food Chem.* **2010**, *58*, 8512 – 8521.

[42] J. Lüthy, M. Benn, *Phytochemistry* **1979**, *18*, 2028 – 2029.

[43] a) J. Lüthy, M. H. Benn, *Can. J. Biochem.* **1977**, *55*, 1028 – 1031; b) X. Hasapis, A. J. MacLeod, *Phytochemistry* **1982**, *21*, 1009 – 1013.

[44] a) A. M. Wentzell, D. J. Kliebenstein, *Plant Physiol.* **2008**, *147*, 415 – 428; b) Z. Zhang, J. A. Ober, D. J. Kliebenstein, *The Plant Cell Online* **2006**, *18*, 1524 – 1536.

[45] a) M. Burow, U. Wittstock, *Phytochem. Rev.* **2009**, *8*, 87 – 99; b) U. Wittstock, M. Burow, *The Arabidopsis Book* **2010**, *8*, e0134.

[46] a) S. Kawakishi, K. Muramatsu, *Agric. Biol. Chem.* **1966**, *30*, 688 – 692; b) S. Kawakishi, M. Namiki, H. Watanabe, K. Muramatsu, *Agric. Biol. Chem.* **1967**, *31*, 823 – 830; c) N. Agerbirk, M. De Vos, J. Kim, G. Jander, *Phytochem. Rev.* **2009**, *8*, 101 – 120; d) V. Borek, M. J. Morra, *J. Agric. Food Chem.* **2005**, *53*, 8650 – 8654; e) A. Bryan Hanley, K. R. Parsley, *Phytochemistry* **1990**, *29*, 769 – 771.

[47] N. Agerbirk, C. Bjergegaard, C. E. Olsen, H. Sørensen, *J. Chromatogr. A* **1996**, *745*, 239 – 248.

[48] a) G. D. Bending, S. D. Lincoln, *Soil Biol. Biochem.* **2000**, *32*, 1261 – 1269; b) J. W. Fahey, X. Haristoy, P. M. Dolan, T. W. Kensler, I. Scholtus, K. K. Stephenson, P. Talalay, A. Lozniewski, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 7610 – 7615.

[49] a) L. M. Manici, L. Lazzeri, G. Baruzzi, O. Leoni, S. Galletti, S. Palmieri, *Pest Manage. Sci.* **2000**, *56*, 921 – 926; b) U. Smolinska, M. J. Morra, G. R. Knudsen, P. D. Brown, *Phytopathology* **1997**, *87*, 77 – 82; c) P. V. Nielsen, R. Rios, *Int. J. Food Microbiol.* **2000**, *60*, 219 – 229.

[50] a) V. Borek, L. R. Elberson, J. P. McCaffrey, M. J. Morra, *J. Agric. Food Chem.* **1998**, *46*, 5318 – 5323; b) F. Rohr, C. Ulrichs, M. Schreiner, C. Nguyen, I. Mewis in *Chemoecology, Vol. 21*, Birkhäuser, Basel, **2011**, pp. 171 – 180.

[51] E. Jeffery, M. Araya, *Phytochem. Rev.* **2009**, *8*, 283 – 298.

[52] C. C. Conaway, S. M. Getahun, L. L. Liebes, D. J. Pusateri, D. K. W. Topham, M. a. Botero-Omary, F.-L. Chung, *Nutr. Cancer* **2000**, *38*, 168 – 178.

[53] a) Y. Zhang, E. C. Callaway, *Biochem. J.* **2002**, *364*, 301 – 307; b) Y. Zhang, *Carcinogenesis* **2000**, *21*, 1175 – 1182.

[54] C. C. Conaway, J. Krzeminski, S. Amin, F.-L. Chung, *Chem. Res. Toxicol.* **2001**, *14*, 1170 – 1176.

[55] N. Petri, C. Tannergren, B. Holst, F. A. Mellon, Y. Bao, G. W. Plumb, J. Bacon, K. A. O'Leary, P. A. Kroon, L. Knutson, P. Forsell, T. Eriksson, H. Lennernas, G. Williamson, *Drug Metab. Dispos.* **2003**, *31*, 805 – 813.

[56] a) T. A. Shapiro, J. W. Fahey, K. L. Wade, K. K. Stephenson, P. Talalay, *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7*, 1091 – 1100; b) S. Platz, C. Kühn, S. Schiess, M. Schreiner, I. Mewis, M. Kemper, A. Pfeiffer, S. Rohn, *Anal. Bioanal. Chem.* **2013**, 1 – 10; c) J. Budnowski, F. S. Hanschen, C. Lehmann, M. Haack, R. Brigelius-Flohé, L. W. Kroh, M. Blaut, S. Rohn, L. Hanske, *Anal. Biochem.* **2013**, *441*, 199 – 207.

[57] E. Lamy, C. Scholtes, C. Herz, V. Mersch-Sundermann, *Drug Metab. Rev.* **2011**, *43*, 387 – 407.

[58] a) H. G. M. Tiedink, C. E. Malingre, L. W. Van Broekhoven, W. M. F. Jongen, J. Lewis, G. R. Fenwick, *J. Agric. Food Chem.* **1991**, *39*, 922 – 926; b) I. Maskell, R. Smithard, *Br. J. Nutr.* **1994**, *72*, 455 – 466.

[59] a) S. Michaelsen, J. Otte, L.-O. Simonsen, H. Sørensen, *Acta Agric. Scand. Sect. A* **1994**, *44*, 25 – 37; b) A. H. Freig, L. D. Campbell, N. E. Stanger, B. Slominski, *Can. J. Anim. Sci.* **1986**, *66*, 331.

[60] a) C. Krul, C. L. Humblot, C. Philippe, M. Vermeulen, M. van Nuenen, R. Havenaar, S. Rabot, *Carcinogenesis* **2002**, *23*, 1009 – 1016; b) L. Elfoul, S. Rabot, N. Khelifa, A. Quinsac, A. Duguay, A. Rimbault, *FEMS Microbiol. Lett.* **2001**, *197*, 99 – 103; c) D.-L. Cheng, K. Hashimoto, Y. Uda, *Food Chem. Toxicol.* **2004**, *42*, 351 – 357; d) M. L. Palop, J. P. Smiths, B. ten Brink, *Int. J. Food Microbiol.* **1995**, *26*, 219 – 229; e) B. Combourieu, L. Elfoul, A.-M. Delort, S. Rabot, *Drug Metab. Dispos.* **2001**, *29*, 1440 – 1445; f) J. A. Mullaney, W. J. Kelly, T. K. McGhie, J. Ansell, J. A. Heyes, *J. Agric. Food Chem.* **2013**, *61*, 3039 – 3046.

[61] G. B. Martínez-Hernández, P. A. Gómez, N. V. García-Talavera, F. Artés-Hernández, T. Monedero-Saiz, C. Sánchez-Álvarez, F. Artés, *Food Res. Int.* **2013**, *53*, 403 – 408.

[62] E. R. Brocker, M. H. Benn, J. Lüthy, A. von Däniken, *Food Chem. Toxicol.* **1984**, *22*, 227 – 232.

[63] J. L. Vansteenhouse, J. S. Prescott, S. A. Barker, *J. Appl. Toxicol.* **2000**, *20*, 1 – 10.

[64] a) H. Ohkawa, R. Ohkawa, I. Yamamoto, J. E. Casida, *Pestic. Biochem. Physiol.* **1972**, *2*, 95 – 112; b) W. H. Habig, J. H. Keen, W. B. Jakoby, *Biochem. Biophys. Res. Commun.* **1975**, *64*, 501 – 506; c) R. Lange, R. Baumgrass, M. Diedrich, K. P. Henschel, M. Kujawa, *Ernaehr.-Umsch.* **1992**, *39*, 292 – 296.

[65] a) M. A. Wallig, D. H. Gould, M. J. Fettman, C. C. Willhite, *Food Chem. Toxicol.* **1988**, *26*, 149 – 157; b) A. E. Ahmed, M. Y. H. Farooqui, *Toxicol. Lett.* **1982**, *12*, 157 – 163; c) H. Tanii, K. Hashimoto, A. Harada, *Environ. Res.* **1993**, *61*, 140 – 149; d) P. Boadas-Vaello, E. Jover, S. Saldaña-Ruíz, C. Soler-Martín, C. Chabbert, J. M. Bayona, J. Llorens, *Toxicol. Sci.* **2009**, *107*, 461 – 472.

[66] a) D. H. Gould, M. R. Gumbmann, M. E. Daxenbichler, *Food Cosmet. Toxicol.* **1980**, *18*, 619 – 625; b) M. A. Wallig, D. H. Gould, M. J. Fettman, *Food Chem. Toxicol.* **1988**, *26*, 137 – 147; c) B. Eggum, O. Olsen, H. Sørensen in *Advances in the Production and Utilization of Cruciferous Crops* (Ed.: H. Sørensen), Springer, Dordrecht, **1985**, pp. 50 – 60; d) T. Yamaguchi, *Agric. Biol. Chem.* **1980**, *44*, 3017 – 3018; e) J. Lüthy, B. Carden, U. Friederich, M. Bachmann, *Experientia* **1984**, *40*, 452 – 453; f) E. B. Astwood, M. A. Greer, M. G. Ettlinger, *J. Biol. Chem.* **1949**, *181*, 121 – 130.

[67] a) G. R. Fenwick, N. M. Griffiths, *Z. Lebensm.-Unters. -Forsch. A* **1981**, *172*, 90 – 92; b) M. K. Tripathi, A. S. Mishra, *Anim. Feed Sci. Technol.* **2007**, *132*, 1 – 27.

[68] a) R. Lakshmy, P. S. Rao, B. Sesikeran, P. Suryaprakash, *Horm. Metab. Res.* **1995**, *27*, 450 – 454; b) F. Schöne, B. Groppel, A.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Hennig, G. Jahreis, R. Lange, *J. Sci. Food Agric.* **1997**, *74*, 69 – 80.

[69] a) M. McMillan, E. A. Spinks, G. R. Fenwick, *Hum. Exp. Toxicol.* **1986**, *5*, 15 – 19; b) K. Renko, I. Mewis, M. Schreiner, L. Schomburg, J. Köhrle in *13th European Congress of Endocrinology, Endocrine Abstracts*, *Vol. 26*, Rotterdam, The Netherlands, **2011**, p. P51.

[70] E. Gaitan, *Annu. Rev. Nutr.* **1990**, *10*, 21 – 37.

[71] a) G. R. Fenwick, E. A. Spinks, A. P. Wilkinson, R. K. Heaney, M. A. Legoy, *J. Sci. Food Agric.* **1986**, *37*, 735 – 741; b) G. R. Fenwick, R. F. Curtis, *Anim. Feed Sci. Technol.* **1980**, *5*, 255 – 298.

[72] Y. Zhang, P. Talalay, C. G. Cho, G. H. Posner, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2399 – 2403.

[73] R. Lange, R. Baumgrass, M. Diedrich, K. P. Henschel, M. Kujawa, *Ernaehr.-Umsch.* **1992**, *39*, 252 – 257.

[74] a) L. N. Kolonel, J. H. Hankin, A. S. Whittemore, A. H. Wu, R. P. Gallagher, L. R. Wilkens, E. M. John, G. R. Howe, D. M. Dreon, D. W. West, R. S. Paffenbarger, *Cancer Epidemiol. Biomarkers Prev.* **2000**, *9*, 795 – 804; b) M. G. Jain, G. T. Hislop, G. R. Howe, P. Ghadirian, *Nutr. Cancer* **1999**, *34*, 173 – 184; c) P. Terry, A. Wolk, I. Persson, C. Magnusson, *JAMA J. Am. Med. Assoc.* **2001**, *285*, 2975 – 2977; d) L. E. Voorrips, R. A. Goldbohm, G. van Poppel, F. Sturmans, R. J. J. Hermus, P. A. van den Brandt, *Am. J. Epidemiol.* **2000**, *152*, 1081 – 1092.

[75] S. S. Hecht, *Cancer Chemoprevention*, *Vol. 1*, Humana, Totowa, **2004**, pp. 21 – 35.

[76] a) J. J. P. Bogaards, H. Verhagen, M. I. Willems, G. van Poppel, P. J. van Bladeren, *Carcinogenesis* **1994**, *15*, 1073 – 1075; b) W. A. Nijhoff, M. J. A. L. Grubben, F. M. Nagengast, J. B. M. J. Jansen, H. Verhagen, G. van Poppel, W. H. M. Peters, *Carcinogenesis* **1995**, *16*, 2125 – 2128; c) W. A. Nijhoff, T. P. J. Mulder, H. Verhagen, G. van Poppel, W. H. M. Peters, *Carcinogenesis* **1995**, *16*, 955 – 957; d) J. W. Lampe, C. Chen, S. Li, J. Prunty, M. T. Grate, D. E. Meehan, K. V. Barale, D. A. Dightman, Z. Feng, J. D. Potter, *Cancer Epidemiol. Biomarkers Prev.* **2000**, *9*, 787 – 793; e) H. Steinkellner, S. Rabot, C. Freywald, E. Nobis, G. Scharf, M. Chabicovsky, S. Knasmüller, F. Kassie, *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2001**, *480 – 481*, 285 – 297; f) E. Lamy, M. Garcia-Käufer, J. Prinzhorn, V. Mersch-Sundermann, *Eur. J. Cancer Prev.* **2012**, *21*, 400 – 406; g) E. Lamy, S. Schmitz, A. Krumbein, V. Mersch-Sundermann, *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2011**, *726*, 146 – 150; h) C. I. Gill, S. Haldar, L. A. Boyd, R. Bennett, J. Whiteford, M. Butler, J. R. Pearson, I. Bradbury, I. R. Rowland, *Am. J. Clin. Nutr.* **2007**, *85*, 504 – 510; i) C. Hoelzl, H. Glatt, W. Meinl, G. Sontag, G. Haidinger, M. Kundi, T. Simic, A. Chakraborty, J. Bichler, F. Ferk, K. Angelis, A. Nersesyan, S. Knasmüller, *Mol. Nutr. Food Res.* **2008**, *52*, 330 – 341.

[77] a) M. A. Riedl, A. Saxon, D. Diaz-Sanchez, *Clin. Immunol.* **2009**, *130*, 244 – 251; b) T. A. Shapiro, J. W. Fahey, A. T. Dinkova-Kostova, W. D. Holtzclaw, K. K. Stephenson, K. L. Wade, L. Ye, P. Talalay, *Nutr. Cancer* **2006**, *55*, 53 – 62; c) "Clinical development plan: Phenethyl isothiocyanate": *J. Cell. Biochem.* **1996**, *63*, 149 – 157.

[78] http://www.cancer.gov/clinicaltrials.

[79] N. V. K. K. Murthy, M. S. N. Rao, *J. Agric. Food Chem.* **1986**, *34*, 448 – 452.

[80] L. Drobnica, P. Kristián, J. Augustín in *The Chemistry of Cyanates and Their Thio Derivatives*, *Part 2* (Ed.: S. Patai), Wiley, Chichester, **1977**, pp. 1003 – 1221.

[81] a) L. Mi, F.-L. Chung, *Nutr. Cancer* **2008**, *60*, 12 – 20; b) L. Mi, X. Wang, S. Govind, B. L. Hood, T. D. Veenstra, T. P. Conrads, D. T. Saha, R. Goldman, F.-L. Chung, *Cancer Res.* **2007**, *67*, 6409 – 6416; c) L. Mi, Z. Xiao, T. D. Veenstra, F.-L. Chung, *J. Proteomics* **2011**, *74*, 1036 – 1044.

[82] a) C. C. Conaway, D. Jiao, F.-L. Chung, *Carcinogenesis* **1996**, *17*, 2423 – 2427; b) S. S. Hecht, *Drug Metab. Rev.* **2000**, *32*, 395 – 411; c) K. Mahéo, F. Morel, S. Langouët, H. Kramer, E. Le Ferrec, B. Ketterer, A. Guillouzo, *Cancer Res.* **1997**, *57*, 3649 – 3652.

[83] a) D. James, S. Devaraj, P. Bellur, S. Lakkanna, J. Vicini, S. Boddupalli, *Nutr. Rev.* **2012**, *70*, 654 – 665; b) S. Boddupalli, J. R. Mein, D. R. James, S. Lakkanna, *Front. Genet.* **2012**, *3*, DOI: 10.3389/fgene.2012.00007.

[84] Y.-S. Keum, W.-S. Jeong, A. N. Tony Kong, *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2004**, *555*, 191 – 202.

[85] C. Gerhäuser, *Curr. Opin. Clin. Nutr. Metab. Care* **2013**, *16*, 405 – 410.

[86] a) P. Rose, M. Whiteman, S. H. Huang, B. Halliwell, C. N. Ong, *CMLS Cell. Mol. Life Sci.* **2003**, *60*, 1489 – 1503; b) L. Tang, Y. Zhang, *J. Nutr.* **2004**, *134*, 2004 – 2010; c) Y. Zhang, L. Tang, V. Gonzalez, *Mol. Cancer Ther.* **2003**, *2*, 1045 – 1052.

[87] a) S. Kalkunte, N. Swamy, D. S. Dizon, L. Brard, *J. Exp. Ther. Oncol.* **2006**, *5*, 287 – 300; b) D. Xiao, V. Vogel, S. V. Singh, *Mol. Cancer Ther.* **2006**, *5*, 2931 – 2945; c) C.-T. Yeh, G.-C. Yen, *Carcinogenesis* **2005**, *26*, 2138 – 2148.

[88] a) S. M. Meeran, S. N. Patel, T. O. Tollefsbol, *PLoS ONE* **2010**, *5*, e11457; b) E. Lamy, C. Herz, S. Lutz-Bonengel, A. Hertrampf, M.-R. Márton, V. Mersch-Sundermann, *PLoS ONE* **2013**, *8*, e53240; c) E. Lamy, D. Oey, F. Eißmann, C. Herz, K. Münstedt, H.-R. Tinneberg, V. Mersch-Sundermann, *Phytother. Res.* **2013**, *27*, 1036 – 1041.

[89] B. E. Cavell, S. S. Syed Alwi, A. Donlevy, G. Packham, *Biochem. Pharmacol.* **2011**, *81*, 327 – 336.

[90] C. Fimognari, E. Turrini, L. Ferruzzi, M. Lenzi, P. Hrelia, *Mutat. Res. Rev. Mutat. Res.* **2012**, *750*, 107 – 131.

[91] a) B. B. Aggarwal, H. Ichikawa, *Cell Cycle* **2005**, *4*, 1201 – 1215; b) K. R. Grose, L. F. Bjeldanes, *Chem. Res. Toxicol.* **1992**, *5*, 188 – 193.

[92] G. A. Reed, D. W. Arneson, W. C. Putnam, H. J. Smith, J. C. Gray, D. K. Sullivan, M. S. Mayo, J. A. Crowell, A. Hurwitz, *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 2477 – 2481.

[93] a) J.-R. Weng, C.-H. Tsai, S. K. Kulp, D. Wang, C.-H. Lin, H.-C. Yang, Y. Ma, A. Sargeant, C.-F. Chiu, M.-H. Tsai, C.-S. Chen, *Cancer Res.* **2007**, *67*, 7815 – 7824; b) K. Kunimasa, T. Kobayashi, K. Kaji, T. Ohta, *J. Nutr.* **2010**, *140*, 1 – 6.

[94] a) J. J. Michnovicz, H. L. Bradlow, *Nutr. Cancer* **1991**, *16*, 59 – 66; b) J. J. Michnovicz, H. Adlercreutz, H. L. Bradlow, *J. Natl. Cancer Inst.* **1997**, *89*, 718 – 723.

[95] a) K. J. Auborn, S. Fan, E. M. Rosen, L. Goodwin, A. Chandraskaren, D. E. Williams, D. Chen, T. H. Carter, *J. Nutr.* **2003**, *133*, 2470S – 2475S; b) Q. Meng, F. Yuan, I. D. Goldberg, E. M. Rosen, K. Auborn, S. Fan, *J. Nutr.* **2000**, *130*, 2927 – 2931.

[96] O. I. Vivar, E. F. Saunier, D. C. Leitman, G. L. Firestone, L. F. Bjeldanes, *Endocrinology* **2010**, *151*, 1662 – 1667.

[97] C. A. Rosen, P. C. Bryson, *J. Voice* **2004**, *18*, 248 – 253.

[98] a) D. J. Kim, K. K. Lee, B. S. Han, B. Ahn, J. H. Bae, J. J. Jang, *Cancer Sci.* **1994**, *85*, 578 – 583; b) G. Stoner, B. Casto, S. Ralston, B. Roebuck, C. Pereira, G. Bailey, *Carcinogenesis* **2002**, *23*, 265 – 272.

[99] a) C. Baasanjav-Gerber, B. H. Monien, I. Mewis, M. Schreiner, J. Barillari, R. Iori, H. Glatt, *Mol. Nutr. Food Res.* **2011**, *55*, 783 – 792; b) H. Glatt, C. Baasanjav-Gerber, F. Schumacher, B. H. Monien, M. Schreiner, H. Frank, A. Seidel, W. Engst, *Chem.-Biol. Interact.* **2011**, *192*, 81 – 86.

[100] F. Schumacher, K. Herrmann, S. Florian, W. Engst, H. Glatt, *Anal. Biochem.* **2013**, *434*, 4 – 11.

[101] M. Wiesner, M. Schreiner, H. Glatt, *Food Chem. Toxicol.* **2014**, *67*, 10 – 16.

[102] a) N. V. Matusheski, E. H. Jeffery, *J. Agric. Food Chem.* **2001**, *49*, 5743 – 5749; b) G. P. Basten, Y. Bao, G. Williamson, *Carcinogenesis* **2002**, *23*, 1399 – 1404; c) C. W. Nho, E. Jeffery,

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Toxicol. Appl. Pharmacol. **2004**, *198*, 40 – 48; d) H. Tanii, T. Higashi, F. Nishimura, Y. Higuchi, K. Saijoh, *J. Agric. Food Chem.* **2005**, *53*, 8993 – 8996.

[103] C. W. Nho, E. Jeffery, *Toxicol. Appl. Pharmacol.* **2001**, *174*, 146 – 152.

[104] FAOSTAT, Food and Agriculture Organization of the United Nations, **2013**.

[105] A. Steinbrecher, J. Linseisen, *Ann. Nutr. Metab.* **2009**, *54*, 87 – 96.

[106] I. Schonhof, A. Krumbein, B. Brückner, *Food/Nahrung* **2004**, *48*, 25 – 33.

[107] a) R. Verkerk, M. Schreiner, A. Krumbein, E. Ciska, B. Holst, I. Rowland, R. De Schrijver, M. Hansen, C. Gerhäuser, R. Mithen, M. Dekker, *Mol. Nutr. Food Res.* **2009**, *53*, S219 – 265; b) P. Y. Nugrahedi, R. Verkerk, B. Widianarko, M. Dekker, *Crit. Rev. Food Sci. Nutr.* **2013**, DOI: 10.1080/10408398.2012.688076; c) K. Hennig, R. Verkerk, M. van Boekel, M. Dekker, G. Bonnema, *Trends Food Sci. Technol.* **2014**, *35*, 61 – 68.

[108] a) M. Schreiner, P. Peters, A. Krumbein, *J. Food Sci.* **2007**, *72*, S585 – S589; b) F. Vallejo, F. Tomás-Barberán, C. García-Viguera, *J. Agric. Food Chem.* **2003**, *51*, 3029 – 3034.

[109] L. Song, P. J. Thornalley, *Food Chem. Toxicol.* **2007**, *45*, 216 – 224.

[110] J. Volden, G. B. Bengtsson, T. Wicklund, *Food Chem.* **2009**, *112*, 967 – 976.

[111] R. Verkerk, M. Dekker, W. M. Jongen, *J. Sci. Food Agric.* **2001**, *81*, 953 – 958.

[112] a) M. Tolonen, S. Rajaniemi, J. M. Pihlava, T. Johansson, P. E. J. Saris, E. L. Ryhänen, *Food Microbiol.* **2004**, *21*, 167 – 179; b) E. Ciska, D. R. Pathak, *J. Agric. Food Chem.* **2004**, *52*, 7938 – 7943.

[113] a) R. B. Jones, C. L. Frisina, S. Winkler, M. Imsic, R. B. Tomkins, *Food Chem.* **2010**, *123*, 237 – 242; b) N. V. Matusheski, J. A. Juvik, E. H. Jeffery, *Phytochemistry* **2004**, *65*, 1273 – 1281; c) V. Rungapamestry, A. J. Duncan, Z. Fuller, B. Ratcliffe, *J. Agric. Food Chem.* **2006**, *54*, 7628 – 7634.

[114] a) R. Verkerk, M. Dekker, *J. Agric. Food Chem.* **2004**, *52*, 7318 – 7323; b) D. Van Eylen, I. Oey, M. Hendrickx, A. Van Loey, *J. Agric. Food Chem.* **2007**, *55*, 2163 – 2170.

[115] a) A. Gliszczyńska-Świgło, E. Ciska, K. Pawlak-Lemańska, J. Chmielewski, T. Borkowski, B. Tyrakowska, *Food Addit. Contam.* **2006**, *23*, 1088 – 1098; b) J. Volden, G. I. A. Borge, M. Hansen, T. Wicklund, G. B. Bengtsson, *LWT-Food Sci. Technol.* **2009**, *42*, 63 – 73; c) J. Volden, G. I. A. Borge, G. B. Bengtsson, M. Hansen, I. E. Thygesen, T. Wicklund, *Food Chem.* **2008**, *109*, 595 – 605.

[116] a) E. Cieślik, T. Leszczyńska, A. Filipiak-Florkiewicz, E. Sikora, P. M. Pisulewski, *Food Chem.* **2007**, *105*, 976 – 981; b) M. Francisco, P. Velasco, D. A. Moreno, C. García-Viguera, M. E. Cartea, *Food Res. Int.* **2010**, *43*, 1455 – 1463; c) E. A. S. Rosa, *Phytochemistry* **1997**, *44*, 1415 – 1419; d) E. Ciska, H. Kozłowska, *Eur. Food Res. Technol.* **2001**, *212*, 582 – 587.

[117] a) J. M. Bell, M. O. Keith, J. A. Blake, D. I. McGregor, *Can. J. Anim. Sci.* **1984**, *64*, 1023 – 1033; b) P. Friis, P. O. Larsen, C. E. Olsen, *J. Chem. Soc. Perkin Trans. 2* **1977**, 661 – 665; c) F. Shahidi, J.-E. Gabon, *J. Food Sci.* **1990**, *55*, 793 – 795.

[118] O. Olsen, H. Sørensen, *J. Am. Oil Chem. Soc.* **1981**, *58*, 857 – 865.

[119] a) K. Oerlemans, D. M. Barrett, C. B. Suades, R. Verkerk, M. Dekker, *Food Chem.* **2006**, *95*, 19 – 29; b) M. Dekker, K. Hennig, R. Verkerk, *Czech J. Food Sci.* **2009**, *27*, S85 – S88; c) B. A. Slominski, L. D. Campbell, *J. Sci. Food Agric.* **1987**, *40*, 131 – 143; d) F. S. Hanschen, S. Rohn, I. Mewis, M. Schreiner, L. W. Kroh, *Food Chem.* **2012**, *130*, 1 – 8; e) S. K. Jensen, Y.-G. Liu, B. O. Eggum, *Anim. Feed Sci. Technol.* **1995**, *53*, 17 – 28.

[120] R. McDanell, A. E. M. McLean, A. B. Hanley, R. K. Heaney, G. R. Fenwick, *Food Chem. Toxicol.* **1988**, *26*, 59 – 70.

[121] a) B. A. Slominski, L. D. Campbell, *J. Agric. Food Chem.* **1989**, *37*, 1297 – 1302; b) B. A. Slominski, L. D. Campbell, *J. Sci. Food Agric.* **1989**, *47*, 75 – 84; c) L. Campbell, B. Slominski, *J. Am. Oil Chem. Soc.* **1990**, *67*, 73 – 75.

[122] a) S. Chevolleau, L. Debrauwer, G. Boyer, J. Tulliez, *J. Agric. Food Chem.* **2002**, *50*, 5185 – 5190; b) S. Chevolleau, N. Gasc, P. Rollin, J. Tulliez, *J. Agric. Food Chem.* **1997**, *45*, 4290 – 4296.

[123] F. S. Hanschen, S. Platz, I. Mewis, M. Schreiner, S. Rohn, L. W. Kroh, *J. Agric. Food Chem.* **2012**, *60*, 2231 – 2241.

[124] G. Ishii in *Biologically-active phytochemicals in food: the proceedings of the EUROFOODCHEM XI meeting, Norwich, UK, 26 – 28 September 2001* (Ed.: W. Pfannhauser, G. R. Fenwick, S. Khokhar), Royal Society of Chemistry, Cambridge, **2001**, pp. 485 – 487.

[125] a) A. J. MacLeod, G. MacLeod, *J. Food Sci.* **1970**, *35*, 734 – 738; b) A. J. MacLeod, G. MacLeod, *J. Food Sci.* **1970**, *35*, 739 – 743.

[126] A. J. MacLeod, S. S. Panesar, V. Gil, *Phytochemistry* **1981**, *20*, 977 – 980.

[127] D. J. Williams, C. Critchley, S. Pun, M. Chaliha, T. J. O'Hare, *Phytochemistry* **2009**, *70*, 1401 – 1409.

[128] F. S. Hanschen, A. Bauer, I. Mewis, C. Keil, M. Schreiner, S. Rohn, L. W. Kroh, *J. Agric. Food Chem.* **2012**, *60*, 9890 – 9899.

[129] S. Gronowitz, L. Svensson, R. Ohlson, *J. Agric. Food Chem.* **1978**, *26*, 887 – 890.

[130] I. Sarvan, R. Verkerk, M. van Boekel, M. Dekker, Innovative Food Sci. Emerging Technol. **2014**, DOI: 10.1016/j.ifset.2014.01.007.

[131] K. Hennig, R. Verkerk, G. Bonnema, M. Dekker, *J. Agric. Food Chem.* **2012**, *60*, 7859 – 7865.

[132] a) K. Hennig, R. Verkerk, M. Dekker, G. Bonnema, *Theor. Appl. Genet.* **2013**, 1 – 12; b) K. Hennig, R. C. H. de Vos, C. Maliepaard, M. Dekker, R. Verkerk, G. Bonnema, *Food Chem.* **2014**, *155*, 287 – 297.

[133] T. Oliviero, R. Verkerk, M. Dekker, *Food Chem.* **2012**, *132*, 2037 – 2045.

[134] C. G. Youngs, A. S. Perlin, *Can. J. Chem.* **1967**, *45*, 1801 – 1804.

[135] T. Sato, K. Nagata, M. Shiro, H. Koyama, *Chem. Commun.* **1966**, 192 – 192.

[136] a) F. L. Austin, C. A. Gent, I. A. Wolff, *J. Agric. Food Chem.* **1968**, *16*, 752 – 755; b) F. L. Austin, C. A. Gent, *Chem. Commun.* **1967**, 71b – 72; c) F. L. Austin, C. A. Gent, I. A. Wolff, *Can. J. Chem.* **1968**, *46*, 1507 – 1512; d) A. J. MacLeod, J. T. Rossiter, *Phytochemistry* **1986**, *25*, 855 – 858.

[137] L. M. Searle, K. Chamberlain, T. Rausch, D. N. Butcher, *J. Exp. Bot.* **1982**, *33*, 935 – 942.

[138] N. Bellostas, A. D. Sørensen, J. C. Sørensen, H. Sørensen, *J. Nat. Prod.* **2008**, *71*, 76 – 80.

[139] V. Y. Kukushkin, A. J. L. Pombeiro, *Inorg. Chim. Acta* **2005**, *358*, 1 – 21.

[140] Y. Zhang, P. Talalay, *Cancer Res.* **1994**, *54*, 1976 – 1981.

[141] K. Cejpek, J. Urban, J. Velísek, H. Hrabcová, *Food Chem.* **1998**, *62*, 53 – 57.

[142] C. Baasanjav-Gerber, H. M. Hollnagel, J. Brauchmann, R. Iori, H. Glatt, *Mutagenesis* **2011**, *26*, 407 – 413.

[143] G. R. De Nicola, M. Bagatta, E. Pagnotta, D. Angelino, L. Gennari, P. Ninfali, P. Rollin, R. Iori, *Food Chem.* **2013**, *141*, 297 – 303.

[144] C.-W. Chen, T. Rosen Robert, C.-T. Ho in *Flavor Analysis, Vol. 705*, American Chemical Society, New York, **1998**, pp. 152 – 166.

[145] a) S. Kosemura, S. Yamamura, K. Hasegawa, *Tetrahedron Lett.* **1993**, *34*, 481 – 484; b) H. Matsuoka, Y. Toda, K. Yanagi, A. Takahashi, K. Yoneyama, *Biosci. Biotechnol. Biochem.* **1997**, *61*, 2109 – 2112; c) F. S. Hanschen, N. Brüggemann, A. Brodehl, I. Mewis, M. Schreiner, S. Rohn, L. W. Kroh, *J. Agric. Food Chem.* **2012**, *60*, 7735 – 7745.

[146] S. Kawakishi, M. Namiki, *Agric. Biol. Chem.* **1969**, *33*, 452 – 459.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[147] a) Y. Ohta, K. Takatani, S. Kawakishi, *Biosci. Biotechnol. Biochem.* **1995**, *59*, 102 – 103; b) R. Pecháček, J. Velíšek, H. Hrabcová, *J. Agric. Food Chem.* **1997**, *45*, 4584 – 4588.
[148] C.-W. Chen, C.-T. Ho, *J. Agric. Food Chem.* **1998**, *46*, 220 – 223.
[149] a) G. Slater, *Chromatographia* **1992**, *34*, 461 – 467; b) P. A. S. Smith, D. W. Emerson, *J. Am. Chem. Soc.* **1960**, *82*, 3076 – 3082.
[150] a) T. H. Yu, C. M. Wu, R. T. Rosen, T. G. Hartman, C. T. Ho, *J. Agric. Food Chem.* **1994**, *42*, 146 – 153; b) E. Block, *Angew. Chem.* **1992**, *104*, 1158 – 1203; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1135 – 1178; c) G. Kiss, H. Neukom, *Experientia* **1968**, *24*, 326 – 326.
[151] J. E. R. Frijters, N. M. Griffiths, A. M. Mather, J. Reynolds, G. R. Fenwick, *Chem. Senses* **1981**, *6*, 33 – 43.
[152] Y. Jin, M. Wang, R. T. Rosen, C.-T. Ho, *J. Agric. Food Chem.* **1999**, *47*, 3121 – 3123.
[153] W. C. K. Chiang, D. J. Pusateri, R. E. A. Leitz, *J. Agric. Food Chem.* **1998**, *46*, 1018 – 1021.
[154] D. Song, H. Liang, P. Kuang, P. Tang, G. Hu, Q. Yuan, *J. Agric. Food Chem.* **2013**, *61*, 5097 – 5102.
[155] a) Y. Ozawa, Y. Uda, S. Kawakishi, *Agric. Biol. Chem.* **1990**, *54*, 1849 – 1851; b) Y. Ozawa, Y. Uda, T. Ohshima, K. Saito, Y. Maeda, *Agric. Biol. Chem.* **1990**, *54*, 605 – 611.
[156] Y. Uda, Y. Ozawa, K. Yoneyama, *Stud. Nat. Prod. Chem.* **2002**, *26*, *Part G*, 1073 – 1111.
[157] a) D. Podhradský, L. Drobnica, P. Kristian, *Experientia* **1979**, *35*, 154 – 155; b) L. Drobnica, J. Augustín, *Collect. Czech. Chem. Commun.* **1965**, *30*, 1618 – 1625; c) J. Kroll, H. J. Jancke, *Food/Nahrung* **1994**, *38*, 96 – 98.
[158] L. Drobnica, D. Podhradský, P. Gemeiner, *Collect. Czech. Chem. Commun.* **1975**, *40*, 3688 – 3697.
[159] a) S. Kawakishi, T. Goto, M. Namiki, *Agric. Biol. Chem.* **1983**, *47*, 2071 – 2076; b) S. Kawakishi, T. Kaneko, *Phytochemistry* **1985**, *24*, 715 – 718; c) S. Kawakishi, M. Namiki, *J. Agric. Food Chem.* **1982**, *30*, 618 – 620.
[160] T. Nakamura, Y. Kawai, N. Kitamoto, T. Osawa, Y. Kato, *Chem. Res. Toxicol.* **2009**, *22*, 536 – 542.
[161] a) K. Kassahun, M. Davis, P. Hu, B. Martin, T. Baillie, *Chem. Res. Toxicol.* **1997**, *10*, 1228 – 1233; b) I. M. Bruggeman, J. H. M. Temmink, P. J. van Bladeren, *Toxicol. Appl. Pharmacol.* **1986**, *83*, 349 – 359; c) G. Brüsewitz, B. D. Cameron, L. F. Chasseaud, K. Görler, D. R. Hawkins, H. Koch, W. H. Mennicke, *Biochem. J.* **1977**, *162*, 0.
[162] A. Kumar, G. Sabbioni, *Chem. Res. Toxicol.* **2010**, *23*, 756 – 765.
[163] K. Cejpek, J. Valušek, J. Velíšek, *J. Agric. Food Chem.* **2000**, *48*, 3560 – 3565.
[164] P. Edman, *Acta Chem. Scand.* **1950**, *4*, 283 – 293.
[165] H. Rawel, J. Kroll, S. Haebel, M. G. Peter, *Phytochemistry* **1998**, *48*, 1305 – 1311.
[166] a) J. Kroll, H. Rawel, R. Kröck, W. Schnaak, *Food/Nahrung* **1993**, *37*, 179 – 181; b) J. Kroll, H. Rawel, R. Kröck, J. Proll, W. Schnaak, *Food/Nahrung* **1994**, *38*, 53 – 60.
[167] a) L. Mi, Z. Xiao, B. L. Hood, S. Dakshanamurthy, X. Wang, S. Govind, T. P. Conrads, T. D. Veenstra, F.-L. Chung, *J. Biol. Chem.* **2008**, *283*, 22136 – 22146; b) L. Mi, N. Gan, A. Cheema, S. Dakshanamurthy, X. Wang, D. C. H. Yang, F.-L. Chung, *J. Biol. Chem.* **2009**, *284*, 17039 – 17051.
[168] A. Kumar, P. Vineis, C. Sacerdote, L. Fiorini, G. Sabbioni, *Biomarkers* **2010**, *15*, 739 – 745.
[169] R. Björkman, *Phytochemistry* **1973**, *12*, 1585 – 1590.
[170] a) H. M. Rawel, J. Kroll, I. Schröder, *Nahrung/Food* **1998**, *42*, 197 – 199; b) S. Kawakishi, T. Kaneko, *J. Agric. Food Chem.* **1987**, *35*, 85 – 88.
[171] J. Kroll, H. Rawel, *J. Sci. Food Agric.* **1996**, *72*, 376 – 384.
[172] K. Rade-Kukic, C. Schmitt, H. M. Rawel, *Food Hydrocolloids* **2011**, *25*, 694 – 706.
[173] M. Hernández-Triana, J. Kroll, J. Proll, J. Noack, K. J. Petzke, *J. Nutr. Biochem.* **1996**, *7*, 322 – 326.
[174] a) K. Xu, P. J. Thornalley, *Biochem. Pharmacol.* **2001**, *61*, 165 – 177; b) K. Xu, P. J. Thornalley, *Biochem. Pharmacol.* **2000**, *60*, 221 – 231; c) Y. Morimitsu, K. Hayashi, Y. Nakagawa, H. Fujii, F. Horio, K. Uchida, T. Osawa, *Mech. Ageing Dev.* **2000**, *116*, 125 – 134; d) R.-K. Lin, N. Zhou, Y. L. Lyu, Y.-C. Tsai, C.-H. Lu, J. Kerrigan, Y.-t. Chen, Z. Guan, T.-S. Hsieh, L. F. Liu, *J. Biol. Chem.* **2011**, *286*, 33591 – 33600.
[175] a) Food and Agriculture Organization of the United Nations, World Health Organization, United Nations University, **2007**, pp. 1 – 265; b) V. R. Young, P. L. Pellett, *Am. J. Clin. Nutr.* **1994**, *59*, 1203S – 1212S.
[176] M. de Onis, C. Monteiro, J. Akré, G. Clugston, *Bull. W. H. O.* **1993**, *71*, 703 – 712.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim