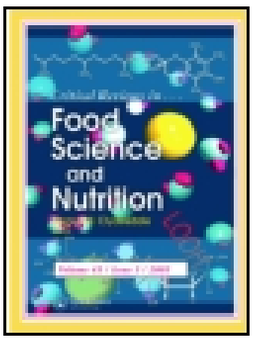

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Bitter taste of Brassica vegetables: the role of genetic factors, receptors, isothiocyanates, glucosinolates and flavor context

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Abstract:

It is well known that consumption of Brassica vegetables has beneficial effect on human's health. The greatest interest is focused on glucosinolates and their hydrolysis products isothiocyanates, due to their potential as cancer preventing compounds. Brassica vegetables are also rich in flavor compounds belonging to many chemical groups. The main sensory sensation related to these vegetable is their characteristic sharp and bitter taste, and unique aroma. Because of these features this group of vegetables is often rejected by consumers. Interestingly, for some people unpleasant sensations are not perceived, suggesting a potential role of inter-individual variability in bitter taste perception and sensibility. Receptors responsible for bitter sensation with the emphasis on Brassica are reviewed, as well as genetic predisposition for bitterness perception by consumers. Also the role of glucosinolates and isothiocyanates as compounds responsible for bitter taste is discussed based on data from the field of food science and

molecular biology. Isothiocyanates are shown in broaded context of flavor compounds also contributing to the aroma of Brassica vegetables.

Keywords

Bitter taste, Brasssica vegetables, glucosinolates, isothiocyanates, flavor, TAS2R receptor.

Introduction

The cruciferous vegetables are example of food with widely described and proven anticancerogenic potential (Higdon et al. 2007, Capuano et al. 2017). The main factor determining the health-beneficial effect of vegetables from the *Brassicaceae* family is a high glucosinolates content and their degradation products: isothiocyanates (Fenwick et al. 1983). Therefore, the consumption of Brassica vegetables should be promoted in society. On the other hand, these compounds contribute to characteristic, sharp and bitter flavor of these vegetables (Fenwick et al. 1983, Engel et al. 2006, Hansen et al. 2007, Ghawi et al. 2014). Several studies have suggested that certain flavor properties, such as bitterness or the typical pungent aroma, may be considered, by some consumers, as undesirable in food and beverage, possibly influencing their consumption habits (Drewnowski & Gomez-Carneros, 2000, Engel et al. 2006). Based on this it may be suggested that rejection of brassica vegetables by some consumers can be caused by their higher sensitivity to sulfur containing compounds responsible for sharp flavor notes and bitterness of these vegetables. In France, vegetable producers have noticed a relative decline in cauliflower purchase (Engel et al. 2002). A recent survey conducted by the regional fruit and vegetables economic committee (CERAFEL) found evidence that flavor was one of the main reasons for some consumers rarely or never purchase cauliflower (Engel et al. 2002).

The different perception of Brassica vegetables among consumers is partially a consequence of genetic differences between individuals. Almost for 80 years is has been known that bitter taste sensitivity is not the same for everyone. After identification of *TAS2R38* gene which encode for TAS2R38 receptor, many studies have confirmed its participation in bitter taste perception and capability to interact with thiocyanate moiety presented also in isothiocyanates.

Finally, specific mutations responsible for different bitter taste sensitivity in *TAS2R38* and its differential frequencies among populations have been described. Other receptors from TRP family and their interactions with isothiocyanates are also well characterized. However, formation of Brassica flavor compounds, especially in raw vegetables after tissue disruption, is a highly dynamic process, where sulfur compounds can interact both with taste and olfactory receptors, and contrary to interactions with taste receptors state of the art of brassica vegetables sulfur volatile compounds and their interactions with olfactory receptors is still insufficient.

This review is focused mainly on bitterness sensation in Brassica vegetables, with the emphasis of receptors and genetic factors involved in this process, which may cast some light on the consumers' preferences of these vegetables. Interaction mechanisms between isothiocyanates and receptors are discussed in detail. On the other hand the relations between glucosinolates and perceived bitterness is discussed based on papers where sensory analysis was involved. Finally, to show the formation of Brassica isothiocyanates in broader flavor compounds context the myrosinase mediated hydrolysis of glucosinolates is discussed along with other compounds of sensory importance.

The role of flavor in consumers acceptance of Brassica vegetables

The flavor is determined by both taste and odor-active compounds. The specialized taste receptor cells located in the mouth lead to a combined complex taste sensation. Flavor perception also consist of odor sensation, occurring because of the interaction of volatile food components with the olfactory receptors (Reineccius, 2006).

The flavor of Brassica vegetables is complex and although glucosinolates contribute little to it, there is no doubt that their hydrolytic breakdown products and other sulphur containing compounds have an important role in formation of the specific flavor of those plants (Kubec et al. 1998). The flavor of Brassica vegetables is peculiar and not always accepted by consumers. However, increasing consciousness about health and proven health benefits of cruciferous made them attractive food products in everyday diet. Technological process (like: cooking, steaming, freezing, drying) have an influence on the specific flavor of *Brassicaceae*, because of the enzymatic reactions products which play a significant role in flavor creation. According to the literature, the higher impact on the specific aroma of this group have isothiocyanates and other sulphur compounds. They are products of reaction catalyzed by myrosinase (see the part: *Myrosinase -- glucosinolates system*). In this situation, the same compounds present in *Brassicaceae* seem to have a dual role - the positive effect on consumers health and substantial impact (often negative) on consumers acceptance. It seems significant to manipulate the taste and smell of vegetables not to lose advantages of health benefits.

How genetic changes may determine food preferences in Brassica vegetables

Food preferences differ between individuals. This fact is incontestable, however taste preferences may be influenced by many different factors, especially environmental like diet habits, personal experiences, culture, religion, physiological factors and obviously state and form of food are important elements. The level of taste sensitivity also seems to play a key role, especially in bitter sensations which are a potential defense mechanism against different toxic substances threatening health of mammals (Galindo et al. 2012; Lucock 2014). Almost for about

80 years it has been known that bitter taste is more sensible for one group of people and less for others, dividing them for subgroups defined as tasters and non-tasters (Bartoshuk et al. 1994). Further, tasters can be divided into medium tasters and supertasters depending on the bitter intensity (Lucock 2014). Genetic differences between individuals have been described predominantly for TAS2R receptors, especially for TAS2R38. This receptor detects compounds with thiocyanate moiety (N = C = S), which naturally occurs in isothiocyanates. However, most of bitter taste research are based on two model compounds: phenylthiocarbamide (PTC) and 6-n-propyl-2-thiouracil (PROP), which do not occur in foods but contain mentioned thiocyanate moiety. So far many studies has been conducted to investigate how large is the input of genetic background in final bitter sensibility. This results may contribute to define food preferences in particular populations and finally allow to develop food products addressed to appropriate customers.

TAS2R38 receptor is located on the surface of taste cells of the tongue (Kim & Drayna 2005). Investigations using PROP and PTC chemicals are based on the presence of thiocyanate moiety, responsible for the receptor-ligand interactions. In case of the PTC, approximately 75% of individuals worldwide recognize this component as bitter, while to remaining 25% is relatively tasteless (Khataan et al. 2010). To predict taster status, usually standardized control and PTC/PROP papers are used. Molecular analyses mostly are based on three genetic SNP polymorphisms (Single Nucleotide Polymorphisms). These are genetic substitutions, where one nucleotide is changed for another, which finally result in amino acid change and may affect protein activity. All three changes are present in *TAS2R38* gene and are inherited as a haplotype in strong linkage disequilibrium (Mennella et al. 2005). These are substitutions at position 49

(alanine or proline, refSNP: rs714598), 262 (valine or alanine, refSNP: rs1726866) and at 296 (isoleucine or valine, refSNP: rs10246939). Haplotype PAV (Proline, Alanine, Valine) is recognized as a most sensitive and haplotype AVI (Alanine, Valine, Isoleucine) as a least sensitive in homozygous. Individuals who carry both haplotypes in heterozygous (PAV/AVI) are intermediate. Other haplotypes have been identified, however their frequency is rare or extremely rare (Risso et al. 2016; Khataan et al. 2010). Because of strong linkage disequilibrium, individuals are genotyping only for A49P substitution as a "tag" (Mennella et al. 2005), however there is a possibility that other haplotypes may also contribute to the differences in bitter taste perception.

Significance of TAS2R38 receptor to bitter perception was first time demonstrated by Kim et al. They identified non-taster AVI haplotype and taster haplotype PAV and noted, what is important, that their distribution is different among populations (Kim et al. 2003). Functional studies performed on HEK293 cells which expressed human TAS2R38 receptor in haplotype PAV and AVI combinations confirmed this results. Authors also characterized less common PVI, AAI and AAV variants as haplotypes with decreased response to PROP/PTC in comparison to PAV and suggest that thiocyanate moiety may be a necessity for influence on TAS2R38 receptor (Bufe et al. 2005). Behrens et al reiterated previous results, strengthening present knowledge about association between different bitter taste sensitivity among individuals and presence of PAV and AVI haplotypes. They performed functional *in vitro* studies confirming strong differences in PAV and AVI haplotypes association with PTC, PROP and other thioamide agonists. Results of this group also pointed out important differences in frequency of less common haplotypes among populations (Behrens et al. 2013). Different ethnic groups were

object of research performed by Roura et al. (2015), using eight compounds recognizable as bitter, previously associated with the activation of human TAS2R receptors (Roura et al. 2015). Differences in bitter taste perception between ethnical groups and their association with genetic background were also suggested by Khataan et al (Khataan et al. 2010). In 2016 Risso et al published results of analysis focused on the distribution of *TAS2R38* haplotypes in a large number of human populations, based on available datasets (Risso et al. 2016). Authors confirmed the global predominance of two main haplotypes PAV and AVI of *TAS2R38* and the distribution of rare variants. They also suggest hypothesis of *TAS2R38* haplotypes evolution, speculating that on account of ancient selection in the early stages of human evolution, PAV and AVI haplotypes were maintained at the same frequency because of both importance in detecting potential bitter toxic substations.

Analyzing the dependence between genetic background and the sensitivity to bitter taste, it is also important to note that it may be more complex process, depending on many factors, such as environmental, age, sex BMI and other. Mennella et al. (2005) has focused on how different alleles of *TAS2R38* gene may influence taste and food related behaviors in children and adults. They conducted genetic and behavioral study on 143 children and their mothers with various descent. No surprising results were found about PROP sensitivity and receptor genotypes, confirming previous studies. However, authors claimed that phenotype-genotype relationship was modified by age -- heterozygous children were more sensitive to lower concentrations of PREP than adults with the same genotype. Additionally, anyhow only children with genotypes determining increased bitterness sensitivity preferred higher concentration of sugars in liquids and food compared with non-taster genotype. This result was also correlated

with race and ethnicity (Mennella et al. 2005). Association between bitter taste sensitivity to PROP/PTC and sweet and other bitter compounds was confirmed by Chang et al, however participated subjects were adult. They additionally claimed that no statistical important differences were shown between genders (Chang et al. 2006).

These findings are especially interesting considering the interactions of taste molecules and potential masking effects for bitterness by sweet taste. Studies also raise the question about TAS2R38 genetic differences, PROP/PTC sensitivity and fat consumption. Keller et al has found no significant differences between PROP tasters group/*TAS2R38* genotype and total energy intake or fat percentage intake in 4-6 years old children with different gender end ethnicity. However, not in all participated subjects *TAS2R38* genotype predicted PROP phenotype. Additionally, PROP non-tasters males (A/A genotype) had greater BMI z-scores than non-tasters females (Keller et al. 2010). Research conducted by Inoue et al in young Japanese female students has found that individuals with non-taster haplotype were greater in weight and height than carriers with taster haplotype. Non-tasters also had a higher dietary carbohydrate intake. However, this may be just a result of increased body parameters and caloric request. In contrast, there were no differences in BMI among the *TAS2R38* haplotypes (Inoue et al. 2013).

Genetic contribution in final bitter taste sensitivity for TAS2R38 receptor, responsible for thiocyanate moiety detection which occurs in isothiocyanates, seems to be large, however it is necessary to consider a few additional points. Taste phenomenon is for sure complex process, depending on many factors. Receptors like described TRPV1, TRPA1 and many potential others may be involved in cruciferous bitter taste detection. Their potential genetic differentiation would also affect integration with ligands. Final bitter taste sensitivity also depends on odor

perception, however data about olfactory receptors and their contribution in cruciferous taste detection is incomplete.

Most of studies are based on PTC/PROP compounds, little used naturally occurring agonists (Behrens et al. 2013; Roura et al. 2015). It is important to note, that consumed vegetables contain a huge amount of potential chemical compounds in different concentrations, activating receptors responsible for bitter taste sensitivity. These agonists may compete for receptor binding. Also one receptor may be a potential target for more than one compound. As presented studies has shown, it is not clear if genetic changes in TAS2R38 may modify request for other food compounds like carbohydrates or fats consumption (Mennella et al. 2005; Chang et al. 2006; Keller et al. 2010). These results has found that food preferences are different for age groups. Important fact is also that genetic background differs between populations and PTC/PROP sensitivity haplotypes occur with different frequency, affecting number of individuals occurrence, recognized as tasters or non-tasters. Association studies also do not explain exact mechanisms of receptors activation and more functional research are needed. Finally, taste sensitivity is modifying by many environmental factors: diet habits, personal experience, culture, religion, food availability and its form of consuming.

Myrosinase activity is required for formation of isothiocyanates

Plant accumulating glucosinolates always have thioglucohydrolase thioglucoside glucohydrolase, EC 3.2.3.1), myrosinase (as more commonly known by its trivial name), which catalyzes hydrolysis of glucosinolates to numerous compounds with different biological activities. Additionally, its presence was also noted in some insects, bacteria and fungi (Martinez-Ballesta & Carvajal 2015; Rask et al. 2000). The enzyme and it's substrates --

glucosinolates are stored in different compartments in the same cell or different cells (Kissen et al. 2009).

The products of reaction catalyzed by myrosinase **(Fig. 1)** depend on the structure of the glucosinolate side chain, reaction conditions and also plant species (Bones & Rossiter 1996; Rask et al. 2000). The myrosinase activity is strongly pH depended, however the most suitable pH for the enzyme is neutral. In lower pH values the nitrile production increases. The most efficient isothiocyanates formation is observed in a range of pH 7-10 (Martinez-Ballesta & Carvajal 2015). However, studies conducted by Van Eylen (Van Eylen et al. 2007), Yen and Wei (Yen & Wei 1993) and Springett and Adams (Springett & Adams 1989) seem to be convincing evidence that the optimal pH is strongly dependent on the source of myrosinase. Another differences between myrosinase from various sources were also noted: enzyme activity value (Martinez-Ballesta & Carvajal 2015) and thermal stability (Ghawi et al. 2012; Matusheski et al. 2004).

However, the latest study (Hanschen et al. 2017) showed, that glucosinolates degradation may lead to relatively high amount of nitriles instead of bioactive isothiocyanates because of the presence of the specific protein influencing on enzymatic hydrolysis. The role of nitriles in flavor formation is not elucidated yet and not mentioned in this review. There is also a study focusing on the sulforaphan formation in digestive track during chewing of broccoli. However the study showed that the main factor for sulforaphan formation and bioaccessibility from broccoli in the oral cavity is the presence of active myrosinase. In samples where the myrosinase activity was on very low (inactivated by steaming process), almost no detectable concentration of sulforaphan was observed (Sarvan et al 2017).

Isothiocyanates and other organosulfur compounds from *Brassicaceae* interact with TRP, TAS2R and OR receptors

Isothiocyanates (ITCs) are the main bioactive compounds formed from glucosinolates occurring in cruciferous vegetables after their breakdown by myrosinase responsible for pungent, lachrymatory, bitter taste and odor of *Brassicaceae* (Ishida et al. 2014). However, the mechanism of specific isothiocyanates action and their impact on receptors have not been investigated yet for all compounds. Allyl isothiocyanate (AITC, mustard oil) is one of the most common isothiocyanates derived from sinigrin, a major glucosinolate. Singirin is present in many Brasicaccace vegetables, such as broccoli, brussel sprouts, black mustard seeds or Japanese horseradish (Mazumder et al. 2016; Ishida et al. 2014). Except the myrosynase activity, many bacterial species in the human intestine have the ability to obtain AITC from singirin (Krul et al. 2002). Isothiocyanates are responsible for the pungent taste of cruciferous vegetables because of their influence on TRP receptors, especially TRPA1 and TRPV1 (Zhang 2010). Raising the questions about isothiocyanates bitter sensations it is also worth to shed light on TAS2R receptors.

TRP channels (Transient Receptor Potential channels) play the key role in chemical senses, although they are involved in mechanical and thermal stimuli detection (Jara-Oseguera et al. 2008). These groups of receptors are expressed in taste buds, nerve fibres or keratinocytes in oronasal cavity and are located in plasma membranes (Roper 2014). The superfamily of these proteins contain 28 members divided into 6 subfamilies in mammals: canonical (TRPC), vanilloid (TRPV), ankyrin (TRPA), melastatin (TRPM), polycistin (TRPP) and mucolipin (TRPML) (Mickle et al. 2015). TRP proteins are cation channels with significant sequence

homology and structural similarities, also with different selectivity and mode of action (Montell et al. 2002). They share a similar architecture of six-transmembrane putative domains (S1 to S6) with cytoplasmic amino and carboxy termini (Patapoutian et al. 2009), mostly function as a homotetramers. Between S5 and S6 they contain pore-forming loop function as a conduit for cation permeation. Intracellular N-terminus and C-terminus are variable in length and consistence of specific domains (Nilius & Owsianik 2011). TRP channels play a role in the transcellular mechanisms responsible for non-selective transport of Ca^{2+}, Mg^{2+} and modulate ion entry driving forces which contribute to many physiological processes e.g. signal transduction, homeostasis, motile functions, also may act in carcinogenesis (Reinach et al. 2015; Shapovalov et al. 2016; Nilius & Owsianik 2011). However, from the viewpoint of isothiocyanates, the most important function is modulating pungent and bitter taste feelings. Activity of TRP channels is regulated by different mechanisms, such as voltage-dependent activation, changes in membrane phospholipids level, phosphorylation or activation related to G-protein coupled receptors after triggering by endogenous and exogenous ligands (Nilius & Owsianik 2011).

TRPV1, TRPA1 and TAS2R38 receptors seem to be the main responsible for the pungent taste of isothiocyanates and other bioactive compounds. Transient receptor potential vanilloid 1 (TRPV1) is the most investigated receptor of TRPV subgroup, which contains six TRP channels. It is well known as a target for capsaicin, painful toxin occurring in chili peppers (Cromer & McIntyre 2008). It is also activated by noxious heat, low pH, voltage, lipids compounds and other pungent substances found in vegetables (Jara-Oseguera et al. 2008). Tetrameric structure of TRPV1 is characteristic for other TRP channels, containing six transmembrane domains with pore region between fifth and sixth. On the N-terminus, six consecutive ankyrin repeats are

localized, which bind calmodulin and ATP for modulating its activation and also several putative phosphorylation sites. C-terminus interacts with cytosolic proteins and ligands, contains conserved transient receptor potential domain, phosphoinositide and calmodulin binding domains (Smutzer & Devassy 2016; Cromer & McIntyre 2008; Mickle et al. 2015). TRPV1 is widely expressed in the neurons of peripheral and central nervous system (Mickle et al. 2015). Another important receptor is transient receptor potential ankyrin 1 (TRPA1, also known as wasabi receptor). Its expression takes place in parts of the nervous system, enterochromaffin cells in the gastrointestinal tract and also in keratinocytes, the cell types of the epidermis (Mickle et al. 2015). TRPA1 is activated by many reactive compounds, e.g. mustard oil or sulfur containing particles in onion and garlic, volatile environmental toxins and endogenous agents (Brewster & Gaudet 2016; Wang et al. 2015). TRPA1 is Ca^{2+} permeable non-selective cation channel, depolarizing the plasma membrane and influx ions. A functional channel consists of 4 identical subunits. Every subunit contains six transmembrane domains (TM1-TM6) along with a long cytoplasmic N-terminal domain. In the intracellular N-terminal domain occur moieties of ankyrin repeat motifs containing cysteine and lysine residues which are essential for activation by reactive agonists. Covalent modification of these sites by electrophilic compounds causes conformational changes modifying channel functions. Within the N-terminus are also three Ca^{2+} binding EF hand domains, associated with calcium-dependent gating (Mihara & Shibamoto 2015; Mickle et al. 2015).

Considering the bitter sensations it is essential to mention about TAS2R receptors. This group of receptors belong to Taste Receptors which contain two families: type 1, responsible for sweet and umami sensations (TAS1R) and listed TAS2R activate by bitter tasting substances

(Avau & Depoortere 2016). Interestingly, taste receptors are expressed not only in the gustatory system but in respiratory epithelia, gastrointestinal tract, reproductive organs and brain, which suggest their potential function other than taste sensations and response to various, also physiological stimuli (Behrens & Meyerhof 2013). In humans, TAS2R family consist of 25 members and can be activated by structurally very different molecules (Avau & Depoortere 2016). The same ligand may activate multiple receptors and also one receptor may be activated by many different ligands, some of them are orphan. All these receptors are classified as G-protein coupled receptors (GPCRs) (Lucock 2014). GPCRs receptors are proteins built by seven transmembrane helices TM1-TM7 (Gahbauer & Böckmann 2016). Agonists, by binding to receptor lead to activation of heterotrimeric G protein (which consists of three subunits: α, β and γ). Activation induce cascade reaction which finally lead to release of intracellular calcium stores and activation of cation channel TRPM5 which by cranial nerves conducts signals to medulla (Lucock 2014). For isothiocyanates detection, TAS2R38 receptor was identified, which is probably the most studied. This receptor detects compounds with thiocyanate moiety N = C = S and it is an object of interest because of its potential genetic changes between individuals, which may affect taste preferences (Lucock 2014).

For odor perception of volatile particles in humans responsibility lies on olfactory receptors (OR). OR proteins, as mentioned TAS2R receptors, belong to seven-transmembrane domain, GPCRs superfamily (Persuy et al. 2015). However, specification of olfactory receptors is well-known, their interactions with particular volatile organosulfur compounds form *Brassicaceae* have not been investigated yet.

Allyl isothiocyanate as an example of isothiocyanates activity and its influence on receptors

Allyl isothiocyanate (AITC, also known as mustard oil) is one of the most widespread of naturally occurring isothiocyanates and it is derived from singirin, a predominant glucosinolate in many common cruciferous vegetables, particularly in mustard, horseradish and wasabi (Zhang 2010). AITC is responsible for very pungent taste of these vegetables and its ability to activate TRPV1 and TRPA1 receptors was confirmed by studies performed on cell lines and animals. AITC is an object of interest because of its potential anti-cancer, anti-bacterial and anti-inflammatory activities and potentially may be used in chemoprevention. Because of thiocyanate moiety presence, allyl isothiocyanate is also a potential activator of TAS2R38 receptor, which mutations were confirmed to be responsible for differential ability to experience bitter taste.

Studies of different irritant chemicals activating TRPA1 receptor suggest that not structure but reactivity is critical for its agonist activity. Hinman et al. (2006) provided evidences supporting a model wherein TRPA1 is activated by mechanism involving specific covalent modifications of cysteine side chains located within N-terminal domain of the channel. To test this hypothesis, authors examined whether allyl isothiocyanate and *N*-methyl maleimide (NMM), cysteine-reactive electrophile structurally unrelated to any natural agonist, could activate TRPA1. In contrast to isothiocyanates, NMM form adducts which are irreversible under physiological conditions. TRPA1 expressing oocytes and HEK293 cells were activated by NMM and AITC with similar concentration (10-200 μM). In contrast to response inducted by AITC, activation elicited by NMM was maintained even after extensive washout, confirming its irreversible covalent modification of a cysteine side chain. Authors also generated series of cysteine to serine or alanine substitutions in positions invariant among human, rat and mouse.

Mutants were expressed in HEK293 cells and response was assessed by calcium imaging. Mutant consisted of three substitutions (C619, C639, C663), closely located within N-terminus, was insensitive for MTSEA (membrane permeable, sulfhydryl reactive agent) and showed weak response for AITC at concentration in excess. This mutant was also unresponsive to NMM (Hinman et al. 2006). AITC ability to binding and activating TRPA1 receptor was confirmed by Macpherson and colleagues by similar study. They also pointed out the fact that not structure but capability to form covalent bonds with cysteine residues is important to activation of the receptor, testing the influence on TRPA1 receptor of structurally unrelated cysteine-modifying agents (Macpherson et al. 2007). Cavanaugh et al studies performed on animal cell cultures, based on electrophysiological patch clamp technic, suggest that TRPA1 receptor may exist in two different functional states and chemicals show differential ability to bind to the receptor. For TRPA1 receptor activation by pungent chemicals, it may require additional cytosolic factor. However this potential factor was not found, their previous research identified from about 30 intracellular molecules with ability to modulate ion channels and has found that pyrophosphate and polytriphosphate may mimic its potential activity, supporting activation of TRPA1 by pungent chemicals (Kim & Cavanaugh 2007; Cavanaugh et al. 2008). TRPV1 role as a direct target for allyl isothiocyanate (MO, mustard oil) was first demonstrated by Everaerts et al. (2001). They indicated that mustard oil activated mouse and human TRPV1 receptor *in vitro* (cell cultures) and mouse *in vivo*. Both TRPA1 and TRPV1 mediated the aversive response to MO (studies based on mice with gene knockouts), however TRPV1 with lower importance in comparison to TRPA1. Authors also have shown that TRPV1 receptor may act in triggering acute pain and TRPV1 is more important in the development of local inflammation (Everaerts et

al. 2011). Gees et al. (2013) have revealed that TRPV1 receptor is activated by AITC, however this process may act similar to the capsaicin activation, not critically depend on covalent cysteine modification (Gees et al. 2013). The ability of AITC to activate TRPV1 independently of TRPA1 was confirmed by Alpizar et al and authors also have found that heating enhances this response (Alpizar et al. 2014). Terada et al. (2015) investigated the ability to activate TRPA1, TRPV1 and also TRPM8 receptors by 16 isothiocyanates from different sources with different intensity of flavor, pungency and different chemical structure on the HEK cells. All compounds were able to activate TRPA1 receptor in almost the same strong level, only 6 compounds activated TRPV1 receptor with different intensification and no one of them activated TRPM8 receptor. Authors also concluded that in case of TRPA1 receptor, the ability to activation is decreased when the carbon chain length is increased and the thiocyanate moiety has higher ability to activate TRPA1 in comparison to other chemical groups (Terada et al. 2015). One aim of studies performed by Tekus et al. (2016) was to analyze involvement of TRPV1 and TRPA1 receptors in pronociceptive effect of AITC in mice wild-type and gene knockout model. They have found that TRPV1 receptor had a major contribution in AITC-induced heat hyperalgesia with no effect in comparison to TRPA1 (Tékus et al. 2016).

Many studies have uncovered functional mechanisms of TRPV1 and TRPA1 receptors activation by AITC which is functioning as a model compound in these kind of analysis and less other compounds present in *Brassicaceae* family. However, there is a lack of knowledge about genetic changes between individuals in TRPV1 and TRPA1 channels, which may affect potential differences in bitter taste sensitivity among population. In case of TAS2R38 receptor this seems to be inversely -- many studies confirmed contribution of genetic background in final bitter taste

sensibility between individuals which we reviewed exactly, however there is still a lack of knowledge presenting exact mechanism of TAS2R38 activation by PROP/PTC, isothiocyanates containing thiocyanate moiety and other bitter compounds.

Relation of glucosinolates to bitter taste in Brassica vegetables

The majority of research shown thus far on the induction of bitter taste by Brassica vegetables was attributed to isothiocyanates and their characteristic moiety interacting with bitter receptors. This moiety remains bound in glucosinolates (GLS) and the myrosinase mediated hydrolysis is required for their release **(Fig. 1)**. However in literature on Brassica flavor there are numerous works that associate bitter sensation with glucosinolates. Glucosinolates chemistry was of interest for scientists as early as in XVII century -- in 1608 volatile oil from the distillation of mustard seed was obtained, in 1831 glucosinolates - sinalbin from white mustard seeds was isolated, then singirin was extracted from the seeds of black mustard and their structures were determined at 1868. These compounds were decomposed by reaction catalyzed by myrosin (later myrosinase), isolated from mustard seed. The most intense period of basic research in glucosinolates chemistry was summarized by Fenwick et al (1982). Among glucosinolate containing crops and plants are kohlrabi, Brussels sprouts, Cauliflower, broccoli, kale, Chinese cabbage, turnip, rutabaga, rapessed, mustard, radish, horseradish, wasabi, and garden cress.

Brussels sprouts were examined in order to identify the compounds responsible for the bitter taste of these vegetables. It was found that singirin and progoitrin were the main source of their bitter taste **(Fig. 2)**. Van Doorn et al have shown that higher concentrations of both compounds were associated with a significant decrease number of consumers who evaluated the

taste of Brussels sprouts as "good." These data confirm that increase of glucosinolates (singirin and progoitrin) concentration has intensified bitter taste in consumers (Van Doorn et al. 1998).

According to the published data most members of panels rated singirin and gluconapin as bitter. In contrary to the glucobrassicin and progoitrin which were evaluated as bitter only by the small number of consumers. However, there was a linear correlation between the growing content of analyzed glucosinolates and higher level of bitter taste (Drewnowski & Gomez-Carneros 2000, Fenwick et al. 1990, Hansen et al. 1996)**.** In Brussel sprouts four glucosinolates: progoitrin, singirin, gluconapin and glucobrassicin were identified as the major fraction of total glucosinolates content. Descriptive sensory analysis of glucosinolates solution (50 mg/100 ml of glucosinolate) showed that singirin was described as bitter by 71% of assessors, gluconapin by 78%, glucobrassicin by 21% and progoitrin was bitter only to 9% of panelist members (Fenwick et al. 1983).The fact that only some part of people presented in panelists described the analyzed glucosinolates as bitter is mostly caused by polymorphism of *TAS2R38* gene. Fenwick et al. also obtained results of multiple correlation between bitterness and the analyzed glucosinolates (progoitrin, singirin, gluconapin and glucobrassicin). According to this research, only singirin had the significant influence on the bitterness.

Engel et al. (2002) quantified glucosinolates in different cauliflower varieties and correlated these results with the bitterness intensity detect by panelist for cooked vegetables (thus with inactivated myrosinase). It proved that among seven identified and quantified glucosinolates the highest impact for bitterness was observed for neoglucobrassicin and singirin. However, the impact on taste of other present components were not evaluated. It has been also presented that sensitivity to sinigrin varied between groups of cauliflower non-consumers, medium and high

consumers: in the first group more than 82% were sensitive to sinigrin concentration of 1g/L, whereas in second and third group 52% and 56% people respectively were able to detect it. Similarly, non-consumers were significantly more sensitive to an AITC (odorant) concentration of 10^{-4} g/g WMO (Engel et al., 2006). This suggests the complexity of Brassica vegetables by consumers, which may rely on combined both taste and odor sensations. The role of glucosinolates as a causal factor for Brassica bitterness in unequivocal, as there are also studies that indicate no correlation between bitterness and glucosinolates (Baik et al., 2003, Doerr et al., 2009). Another approach involving the chemical and also sensory characterization of fractioned myrosinase-free extracts from selected raw Brassica vegetables revealed that sweetness and bitterness are the most discriminating attributes. The results has shown that high sugars concentration can significantly decrease the sensation of bitterness. Moreover in contrary to earlier described research the meaning of phenolic content was also evaluated. The general conclusion is showing that glucosinolates alone may not explain the sensory of bitterness. Phenolic and other components are probably also involved in bitterness intensity (Zabaras et al. 2013). Described work was the first noted paper which took into consideration the role of phenolic and other components together with glucosinolates in bitterness creation.

In food chemistry area most papers are focused on glucosinolates influence on bitterness **(Table 1)**, what introduced some confusions about the role of isothiocyanates and glucosinolates in bitterness creation. However, data from Behrens et al. (2013) study show that the lowest detected level of sinigrin was 297 μM and the highest threshold detected by non-tasters was 12.5 mM. Engel et al. (2002) determined the level of sinigrin and other glucosinolates in different varieties of cauliflower. The highest concentration of sinigrin was 7.99 g/ kg of dry mass and the

lowest concentration of this glucosinolate was 0.15 g/kg of dry mass. Assuming, that water content in cauliflower is about 90%, the level of sinigrin expressed in mol value is between about 38 µM and 2 mM. These concentrations might be perceptible by tasters, but probably not detect by non-tasters. However, sinigrin is not the only one glucosinolate present in cauliflower, and if sum up the amount of all present glucosinolates, the concentration is relatively high and may have influence on bitterness perception. The distinction between bitterness generated by glucosinolates and their hydrolysis product in Brassica is still a challenging task to solve.

Thiocyanates in Brassica show also odor activity and are accompanied by other aroma active compounds

Research on volatile compounds in cabbage dates back to 1929 (report of methyl mercaptan and hydrogen sulfide, Konig and Kracht), then isothiocyanates in seeds and leaves in the end of 50-ties (Bailey, Clapp, Jensen). Challenger (1954) reported that odor and taste of cabbage and other Cruciferous are caused by thioglucosides that on hydrolysis yield glucose, potassium hydrogen sulfate and pungent isothiocyanate (Fenwick et al., 1982). The volatile sulfur compounds of cabbage were investigated by Bailey and coworkers in 60-ties. At this time it was known after publication by Hewitt et al., that use of hydrolysis for release of aroma compounds offers a promising concept in food processing. Experiments on dehydrated cabbage treated with myrosinase in high vacuum extracts yielded allyl isothiocyanate and other isothiocyanates (methyl, butyl, butenyl, methylthiopropyl) as well as sulfides, disulfides and trisulfides, all detected in fresh cabbage, but in enzyme treated only DMS, DMDS and carbonylsulfide and disulfide. Hydrogen sulfide was detected as a compound formed during

hydrolysis of allyl isothiocyanate (Shankaranaranyana et al., 1974). MacLeod investigated volatiles of Brussels sprouts, cauliflower fresh, cooked and frozen and identified about 30 compounds in these plants, including sulfur compounds (sulfides, isothiocyanates), aldehydes and alcohols (MacLeod and MacLeod, 1970a). Isothiocyanates were identified as main volatile compounds in horseradish roots by Gilbert and Nursten. As for products eaten raw solvent extraction (ethyl ether/pentane 1/2.5) in Soxhlet apparatus was used and they identified 2-butyl, allyl, 4-pentenyl, 2-pentenyl isothiocyanates and allyl thiocyanate. They also proposed and synthesized standards of these compounds for confirmatory purposes (Gilbert and Nursten 1972). Benzyl isothiocyanate, is presented in cress as a pungent odorant and in high levels in unripe papaya, as pungent-sour notes (Pino, 2014). It has been discovered that, in low concentration, benzyl isothiocyanate has fruity aroma (Pino, 2014). 3-butenyl isothiocyanate is one of the most important odorant in horseradish. It is also presented in wasabi and mustard, giving off a strong, penetrating aroma. Fisher isolated from kohlrabi volatile compounds which were isothiocyanates (allyl- and 3-thiomethylpropyl), as well as cyanates (3-methylthiopropyl, 4-methylthiobutyl and also dimethylsulfide, trimethylsulfide and tetramethylsulfide. Apart from sulfur containing compounds, aldehydes (nonenal, 2-hexenal, 2,4-heptadienal) were detected (Fisher, 1992).

Very few information is on impact of particular compounds on aroma of Brassica demonstrated by the use of gas chromatography -- olfactometry.. As first Ulrich et al. applied SPME and Dynamic headspace sampling to compare those two extraction techniques for sniffing experiment with broccoli. The experiment revealed only a few components taking a part in aroma creation, most of them have not been identified (Ulrich et al. 1998). Engel used headspace-GC-O to analyze odorants of cooked cauliflower and found allyl isothiocyanate,

dimethyltrisulfide, dimethyl sulfide and methanethiol as main odorants (Engel et al., 2002). GC-O was used also in the analysis of influence on packaging volatile compounds of broccoli, identifying 28 compounds, among them methyl sulfides (methyl, dimethyl, trimethyl) as well as hexanal, 3-cis-hexen-1-ol, nonanal, ethanol, methanethiol (MT), methyl thiocyanate (MTC), butyl isothiocyanate (Jacobsson et al., 2004).

Isothiocyanates, though forming and important group of flavor compounds in Brassica vegetables are not the only ones **(Fig. 3, Table 2)**. Methyl methanethiosulfide occurs in Brussels sprouts and cabbage, and for the flavor of cooked broccoli include DMS, TMS, nonanal and 4-(methylthio)-butylisothiocyanate. Similar compounds were found to be responsible for cooked cauliflower flavor with t 3-(methylthio)propyl isothiocyanate as the characteristic thiocyanate (McGorrin, (2011). S-methylcysteine sulfoxide (SMCSO) is natural chemical compound also presented in Brassica plats, such a cauliflower, broccoli, Brussel sprouts, cabbage (Marks et al. 1992). SMCSO play a significant role in flavor formation (Dateo et al. 1957; Chin & Lindsay 1994). S-methylcysteine sulfoxide is hydrolyzed by cysteine lyase, products including methyl methanethiosulfinate (MMTSO) (Marks et al. 1992). S-alkyl-l-cysteines and their sulfoxides are presented in *Cruciferae* vegetables, much of the specific odor is caused also by the degradation of those constituents by C-S lyase presented in the tissue (Hamamoto & Mazelis 1986; Virtanen 1965). Sulfides play the crucial role in the formation of characteristic aroma of Cruciferous. Dimethyl disulfide and dimethyl trisulfide were recognized as major aroma components in raw and cooked Brassicaceae (Maruyama et al. 1970, Valette et al. 2003), those results were further confirmed by use of the gas chromatography - olfactometry by Engel et al. (2002) and Jacobsson et al. (2004).

Conclusions

In Brassica vegetables glucosinolates are often considered as compounds responsible for bitterness sensation. The evidences from the molecular biology approach to bitterness perception indicate the complexity of this issue. Glucosinolates concentration is regarded as the main factor influencing the bitterness of cruciferous in food technology oriented works, in contrary to biological research where the isothiocyanates (glucosinolates hydrolysis products) are considered as compounds creating bitter tast. Taking into consideration the fact that thiocyanate N = C = S group presented in isothiocyanates interact with receptor TRPV1, it seems, that isothicoyanates may contribute more to the overall bitterness, than glucosinolates.

It has to be remembered that isothiocyanates are also volatile compounds, so are also contributing to the specific aroma of Brassica vegetables. However, the relatively small amounts of isothiocyanates probably contribute little to the odor in comparison with sulfides (dimethyl disulfide, trimethyl disulfide and others) which are presented in high amount in volatile fraction. Therefore the complex role of isothiocyanates in flavor perception of Brassica vegetables is attributed to the interaction with bitter taste receptors, as shown, as well as with odor receptors.
Genetic studies entering into food research are clarifying the knowledge why individuals differ because of feeding preferences. It seems to be especially apparent in case of cruciferous vegetables which specific taste is acceptable for some and totally repulsive for others. However, results has shown that genetic contribution in final bitter taste sensitivity of isothiocyanates and other compounds in strong. This may result in some benefits for health e.g. by modifying consumption habits by replacing food components in personal diet for more suitable for

particular individual and obviously for nutritional industry by addressing specific products for potential consumers, understood as genetic and phenotypic tasters.

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Tab.1. Glucosinolates as taste compounds in Brassicaceae

Taste compound	Taste	Occurrence	Literature source
Sinigrin	Bitter	cabbage, Brussel sprouts, cauliflower, turnip, calabrese, collards, kale, mustard greens	Carlson et al. 1987; Drewnowski& Gomez-carneros 2000
Progoitrin	Bitter	Brussel sprouts, cabbage, cauliflower, turnip. calabrese	Carlson et al. 1987; Drewnowski& Gomez-carneros 2000
Glucobrassicin	Bitter	brussel sprouts	Fenwick et al. 1983; Drewnowski& Gomez-carneros 2000
Neoglucobrassicin	Bitter	cooked cauliflower	Engel et al. 2002

Tab.2. Sulfur aroma compounds found in *Brassicaceae* vegetables.

Compound	Odor description	Source
dimethyl disulfide	cauliflower, cabbage-like	Kubec, et al. 1998; Buttery et al. 1976
Dimethyltrisulfide	spoiled, cooked cabbage-like	Kubec, et al. 1998; Engel et al. 2002; Buttery et al. 1976
dimethyl thiosulfinate	cabbage, cauliflower-like	Kubec, et al. 1998
dimethyl thiosulfonate	cooked cabbage-like	Kubec, et al. 1998
Methyl (methylthio)methyl disulfide	garlic-like, alliaceous, sulfury, and cooked cabbage-like	Spadone et al. 2006
methyl (methylthio)pyridine	ammonia, pyridine-like	Kubec, et al. 1998
Allyl-isothiocyanate	Acrid mustard oils, pungent, lachrymatory	Engel et al. 2002; Drewnowski & Gomez-carneros 2000; Buttery et al. 1976
3-Methyl-sulfinopropyl isothiocyanate	Acrid mustard oils	Engel et al. 2002
Benzyl isothiocyanate	Acrid mustard oils, garlic like	Engel et al. 2002
4-methylsulfinyl butyl isothiocyanate	Acrid mustard like	Engel et al. 2002
Phenylethylisothiocyanate	Acrid irritant, lachrymatory	Engel et al. 2002; Buttery et al. 1976
Butyl isothiocyanate	Sulfur, green, pungent	Engel et al. 2002
3-methylthiopropyl isothiocyanate	n.d.	Buttery et al. 1976
4-methylthiobutyl isothiocyanate	n.d	Buttery et al.. 1976
Methional	Cooked potatoes	Spadone et al. 2006; Engel et al. 2002
Methanethiol	Sulfur	Engel et al. 2002

Methylethyl sulfide	Sulfur	Engel et al. 2002
Methyl thiocyanate	Sulfur	Engel et al. 2002

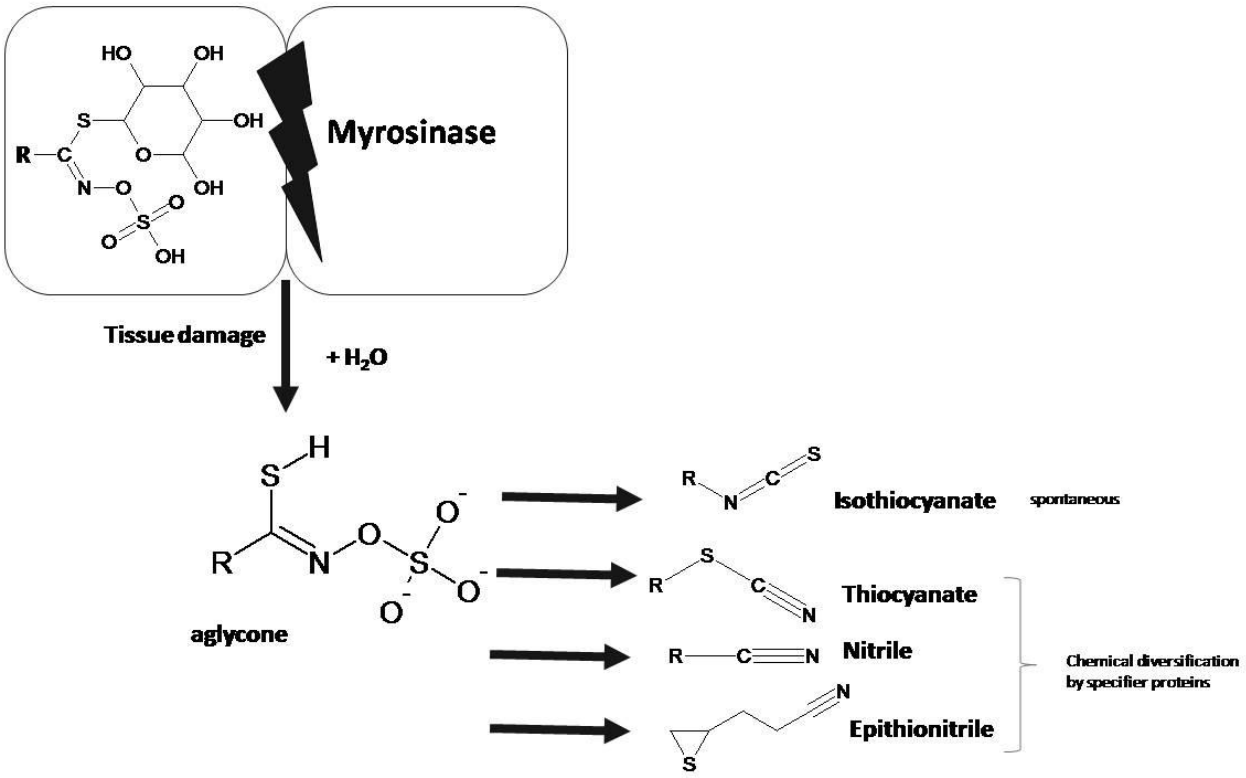

Fig.1. Hydrolysis of glucosinolates and degradation of the unstable aglycone to nitriles, thiocyanates, epithionitrile and isothiocyanates.

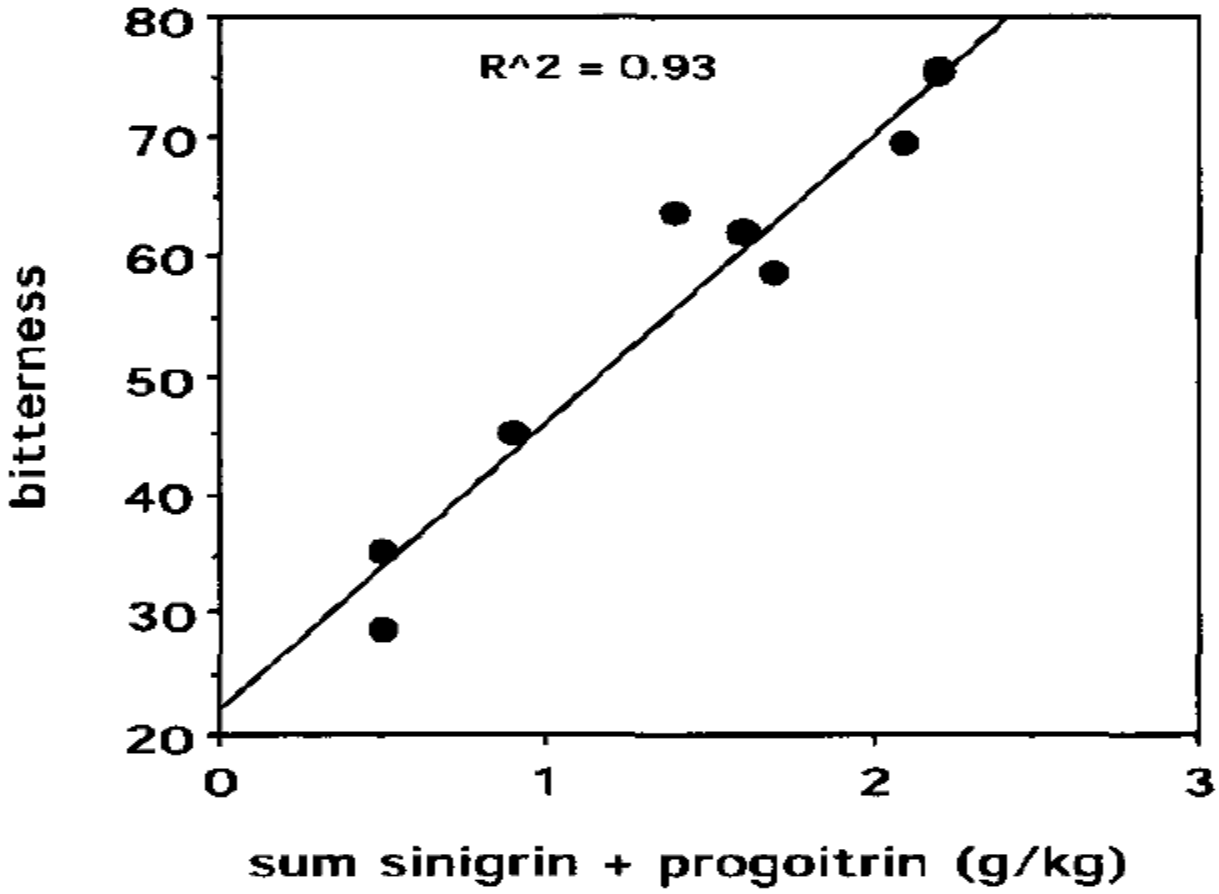

Fig. 2. Relation between bitterness (0-100 scale) of Brussels sprouts samples and the sum of sinigrin and progoitrin (g/kg). [Van Doorn, 1999]

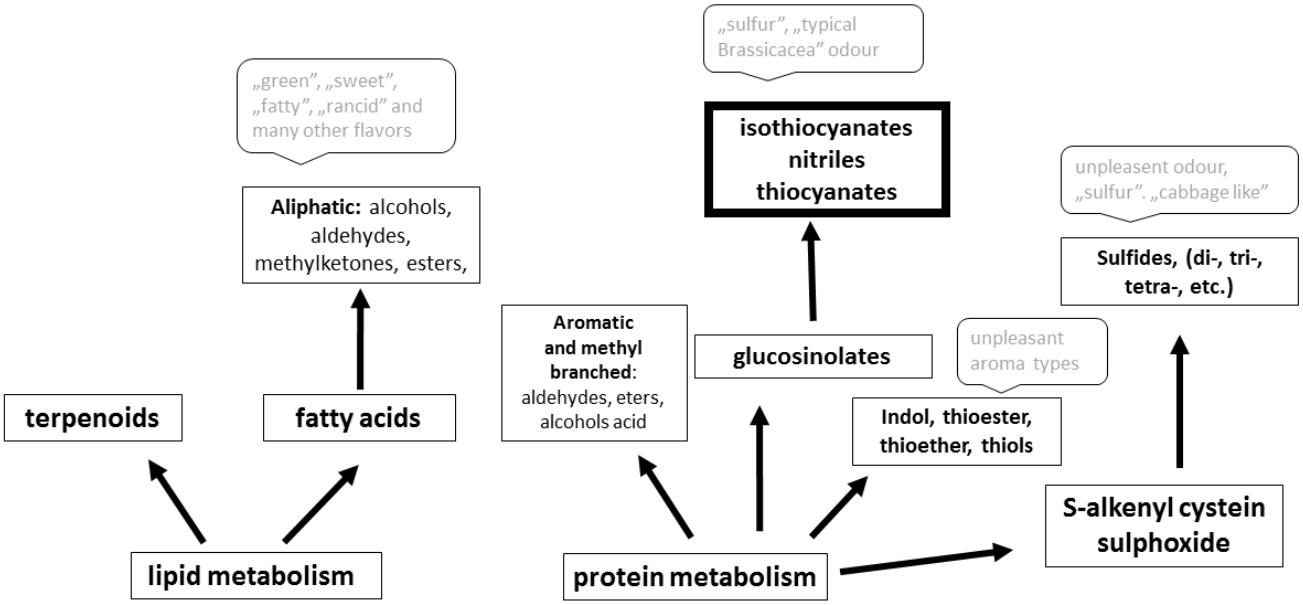

Fig.3. The general scheme of aroma compounds formation in *Brassicaeae* and specifying the source of sulfur compounds