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Review

The analysis of onion and garlic

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Abstract

Onion (*Allium cepa* L.) and garlic (*Allium sativum* L.), among the oldest cultivated plants, are used both as a food and for medicinal applications. In fact, these common food plants are a rich source of several phytonutrients recognized as important elements of the Mediterranean diet, but are also used in the treatment and prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia). These activities are related to the thiosulfinates, volatile sulfur compounds, which are also responsible for the pungent of these vegetables. Besides these low-molecular weight compounds, onion and garlic are characterized by more polar compounds of phenolic and steroidal origin, often glycosilated, showing interesting pharmacological properties. These latter compounds, compared to the more studied thiosulfinates, present the advantages to be not pungent and more stable to cooking. Recently, there has been an increasing scientific attention on such compounds. In this paper, the literature about the major volatile and non-volatile phytoconstituents of onion and garlic has been reviewed. Particular attention was given to the different methodology developed to perform chemical analysis, including separation and structural elucidation.

Keywords: Onion; Garlic; Analysis; Volatile compounds; Thiosulfinates; Polar compounds; Flavonoids; Phenolics; Sapogenins; Saponins; Reviews

Contents

1. Introduction

Since ancient times garlic, *Allium sativum* L., and onions, *Allium cepa* L., have been used as common foods and for the treatment of many diseases. The first citation of these plants is found in the Codex Ebers (1550 BC), an Egyptian medical papyrus reporting several therapeutic formulas based on garlic and onions as useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumours [1]. Cloves of garlic have been found in the tomb of Tutankhamen and in the sacred underground temple of the bulls of Saqqara. Egyptian thought garlic and onions aided endurance and assumed large quantities of them. Raw plants were routinely given to asthmatics and to those people suffering bronchial-pulmonary complains.

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Later on, these food plants were known by Greeks and Romans, that used them as important healing agents just as they still are used from most of the people of the Mediterranean area [2]. In fact, *Allium* species are a rich source of phytonutrients, useful for the treatment or prevention of a number of diseases, including cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) [3–7].

Allium is the largest and most important representative genus of the Alliaceae family and comprises 450 species, widely distributed in the northern hemisphere. Besides the well known garlic and onion, several other species are widely grown for culinary use, such as leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* Hort.), wild garlic (*Allium ursinum* L.), elephant garlic (*Allium ampeloprasum* L. var. *ampeloprasum*), chive (*Allium schoenoprasum* L.), Chinese chive (*Allium tuberosum* L.).

Scientific research on these plants started in the second half of the 19th century with the work of Louis Pasteur that in 1858 first noted antibacterial properties of garlic [8]. Later on, in 1932 Albert Schweitzer treated amoebic dysentery in Africa with garlic [9]. It was also used for several epidemic diseases (e.g. typhus, cholera, diphtheria, and tuberculosis). More recently, attention has focused on the cancer preventive properties of garlic on the basis of the data obtained from epidemiological studies, conducted in China, showing a decrease of gastric cancer risk proportional to the increase of garlic intake [10]. This evidence has been related to the ability of garlic to reduce the nitrite concentrations in the gastric tract [11].

Many of these biological effects are related to the thiosulfinates, volatile sulfur compounds, typical of the *Allium* plants, which are also responsible of their characteristic pungent aroma and taste. However, these compounds are unstable and give rise to transformation products. For this reason, recent attention has been focused on polar compounds that are more stable to cooking and to the storage. Among these compounds, sapogenins, saponins, and flavonoids are the main classes found.

This growing interest follows a general trend that is oriented to the analysis of secondary metabolites from foods. These compounds, recently named with the terms of "nutraceutical" or "phytochemicals", are classified as non-essential micronutrients and are able to contribute to human homeostasis, playing a role in the maintenance of health [12,13]. Such interest was due to the results of epidemiological studies that have correlated a semi-vegetarian diet and a decreased incidence of chronic- and acute-inflammatory diseases such as arteriosclerosis and cancer. The identification of organic compounds responsible of these activities has increased scientific studies of food plants [14]. Thus, nutritional sciences usually interested in quality and safety of foods has moved to the issue of promotion of well-being, with an increased attention to the analysis of secondary metabolites from edible plants. In this intense scientific debate also garlic and onion received a renewed attention.

Some reviews have been published on the analysis of garlic and onion, mostly dealing with organosulfur and/or organoselenium components found in these food plants [15–18]. This paper summarizes the methods developed for the isolation and structural elucidation of the main classes of compounds isolated from garlic and onion. The data published on related food plants belonging to the *Allium* genus, such as shallot or leek, will be omitted because of the high number of published papers. Biological and pharmacological studies on the isolated metabolites, when present, will be also described.

2. Volatile compounds

2.1. Thiosulfinates and other organosulfur compounds: chemical structure and bioactivity

Thiosulfinates are the best studied compounds arising from *Allium* species and most of our knowledge of their structure and biogenesis is due to Block [18]. These molecules originate from *S*-alk(en)yl-L-cysteine-*S*-oxide (**1a–1d**, Fig. 1), located in the cytoplasm, through an enzymatic reaction catalyzed by allinase, a C-S lyase present in the vacuoles, giving initially sulfenic acids (**2a–2d**, Fig. 1). These are highly reactive intermediates that immediately produce thiosulfinates by condensation reaction (**3a–3q**, Fig. 1).

The thiosulfinates are found in all the *Allium* species examined by now, the differences among the species due to the structure and relative amounts of their precursors (**1a–d**). For example, in garlic the compound with propyl residue (**1d**) is absent, while those with allyl residue, alliin (**1a**), is the major compound. This last compound is the precursor of allicin (**3a**). On the contrary, in onion **1a** is totally absent while **1b**, the compound with the 1-propenyl residue, named isoalliin, is a major metabolite. Compounds possessing a 1-propenyl residue at the thiolic site exist as a mixture of the *E*, *Z* isomers (**3e–3f**, **3g–3h**, **3l–3m**) because of a sigmatropic [2,3] rearrangement. The sulfenic 1-propenyl acid intermediate (**2b**) gives rise also to other compounds (Fig. 2), such as (*Z*,*E*)-propanethial *S*-oxide, named lachrymatory factor (LF) (**4**, *Z*-isomer) [18,19], cepaenes (e.g. **5**) [18,19], whose name derives from *cepa*, and 2,3-dimethyl-5,6-dithiabicyclo[2.1.1] hexane 5-oxides, named *cis* and *trans*-zwiebelane (**6a** and **6b**, respectively) [20], from "zwiebel", onion in German.

Thiosulfinates are very unstable compounds and give rise to further rearrangements leading to a wide variety of derived sulfur compounds (Fig. 3), which take part in further transformations and still exhibit biological activity [18]. Among them, thiosulfonates (e.g. **7a** and **7b**), di- and tri-sulfur compounds (e.g. **8a–8f**), 2-vinyl-2,4-dihydro-1,3-dithiin (**9**), 3-vinyl-3,4-dihydro-1,2-dithiin (**10**), and ajoene (**11**, from the Spanish word "ajo" that means garlic). At higher temperatures (about 100 °C) poly-sulfur compounds are formed (e.g. **12a**, **12b** and **13**) containing up to five sulfur atoms.

Recently Kubec et al. [21] have studied the precursors involved in the formation of pink and green-blue pigments generated during onion and garlic processing. It has been confirmed that the pigment formations are similar, with (*E*)-*S*-(1-propenyl)

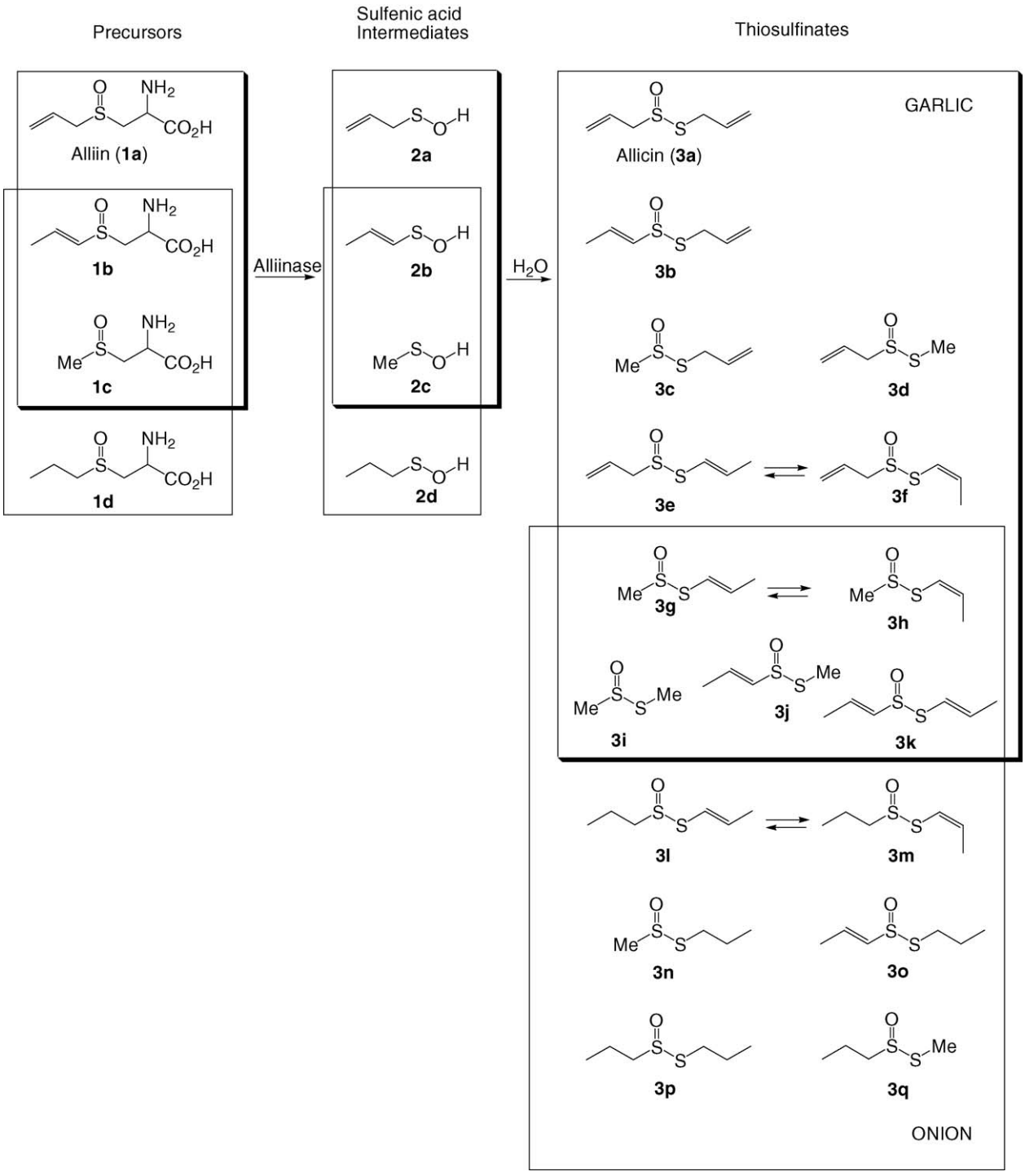


Fig. 1. Biosynthetic pathway of thiosulfinates.

cysteine sulfoxide (**1b**, Fig. 1) named isoalliin, serving as the primary precursor. Upon disruption of the tissue, isoalliin and other *S*-alk(en)ylcysteine sulfoxides are enzymically cleaved, yielding 1-propenyl-containing thiosulfinates (**3j**, **3b**, **3o**, with a Me, allyl or propyl groups at the thiolic side, respectively). A thiosulfinate possessing 1-propenil residues at both sides (**3k**, Fig. 1) of the molecule has also been found. These compounds have been shown to subsequently react with amino acids to produce the pigments. Whereas the Pr, 1-propenyl, and Me derivatives (**3o**, **3k**, and **3j**, respectively) form pink, pink-red, and magenta compounds, those containing the allyl group (**3b**, Fig. 1) give rise to blue products after reacting with glycine at pH 5.0.

Concerning the pharmacological activities attributed to onion and garlic species, a number of chemical and pharmacological reports have testified the effectiveness of their extracts as antioxidant [22], antimicrobial [23], antiasthmatic [24], antitumor and cancer preventing agents [25–27], as platelet-antiaggregating agent [28], for reducing the hypercholesterolemia [29], and

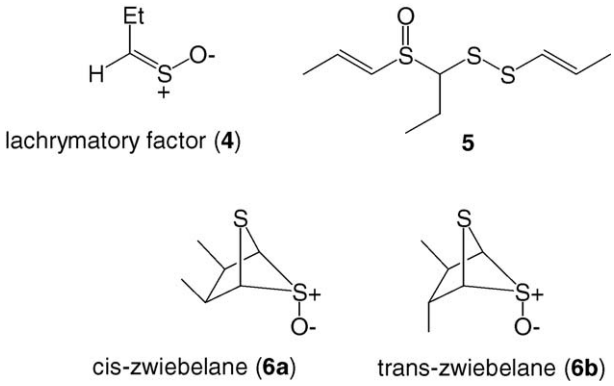


Fig. 2. Volatile organosulfur compounds in onion.

bacteriostatic actvity against *Helicobacter pylori*, that is responsible of ulcer and gastric cancer [30,31]. For example, nine epidemiological studies from different parts of the world (China, Italy, Argentina, U.S.A., etc.) have consistently shown that a significant reduction of gastrointestinal cancer risk is associated with the consumption of garlic [32]. Since nitrites have been implicated in gastric cancer, it is relevant that gastric juice nitrite concentrations were lower in individuals consuming garlic compared to those who rarely take it. Furthermore, in vitro and in vivo tests showed that these actions are not merely due to allicin (**3a**, Fig. 1) and other thiosulfinates but have to be attributed to the synergic action of thiosulfinates with non-volatile components of the plant [33].

2.2. Identification methods

Table 1 lists all the paper published on the analysis of the volatile compounds from onion and garlic, indicating the methods applied. The methods used and the data obtained will be synthetically described as follows.

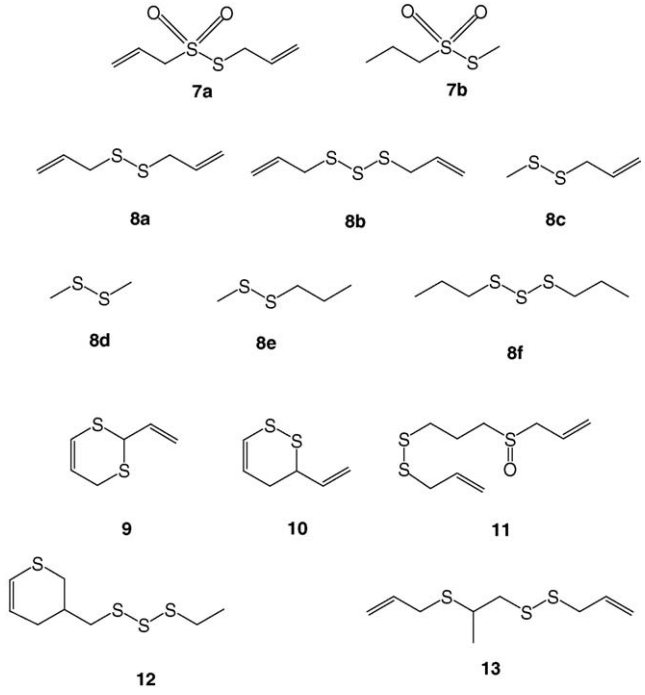


Fig. 3. Organosulfur compounds formed by thiosulfinate degradation.

The first method developed in 1973 by Freeman and McBreen [34] was based on a rapid spectrophotometric determination for the detection of thiosulfinates in fresh, freeze-dried, and oven-dried onion after extraction into hexane. Results correlated with concentration of pyruvate, which is linked to the olfactory threshold concentration of onion juice.

Only about 20 years later many other scientists started to work on this subject. Bauer et al. [35] developed a HPLC method using chiral stationary phases for the enantiomeric separation of racemic thiosulfinate esters [alk(en)ylsulfinothioic acid alk(en)yl esters] from natural source (onion) or synthetic origin. Aromatic substituted thiosulfinate esters could be baseline resolved using helical (+)-poly(triphenylmethyl methacrylate)-coated silica gel [Chiralpak OT (+)] as chiral stationary phase and MeOH as eluent. A correlation between chromatographic resolution and the structures of the thiosulfinate esters have been established. A preparative separation of diphenyl thiosulfinates was achieved with cellulose triacetate (CTA) as stationary phase. The elution sequence of diphenyl thiosulfinates enantiomers on the CTA column was reversed when compared to that on the Chiralpak OT (+).

In the same period, Lawson and et al. [36,37] first performed a quantitative analysis of all the detectable alkyl and alkenyl thiosulfinates, including configurational isomers, of garlic homogenates by using a reversed-phase HPLC method. Pure thiosulfinates were synthesized or isolated and identified by ^{1}H NMR, and their extinction coefficients detected. Some configurational isomers required separation by silica HPLC. The variation in thiosulfinates yield between different countries, bulbs, cloves, and storage times was also investigated. A method for standardizing the quantitation of allicin (**3a**, Fig. 1) yield from garlic was proposed and compared to other methods of allicin analysis [36]. The analysis was also extended to commercial garlic products and the data obtained indicated that thiosulfinates were released only from garlic cloves and garlic powder products. The vinyldithiins (**9**, **10**, Fig. 3) and ajoene (**11**, Fig. 3) were found only in products containing garlic macerated in vegetable oil. The disulfide compounds containing two allyl (**8a**, Fig. 3), or methyl allyl (**8c**, Fig. 3), or two methyl (**8d**, Fig. 3) residues were found only in products containing the oil of steam-distilled garlic. Typical steam-distilled garlic oil products contained about the same amount of total sulfur compounds as the total thiosulfinates released from freshly homogenized garlic cloves. However, oil-macerated products contained only 20% of that amount, while for garlic powder products the amount varied from 0 to 100%. Products containing garlic powder suspended in a gel or garlic aged in aqueous alcohol did not contain detectable amounts of these non-ionic sulfur compounds. A comparison of several brands of each type of garlic product revealed a large range in content (four-fold for oil-macerates and 33-fold for steam-distilled. garlic oils), indicating the importance of analysis before using garlic products for clinical investigations or commercial distribution [37].

In 1992, Sinha et al. [38] published a method based on the supercritical carbon dioxide extraction (SC-CO_2) of onions and

Table 1
Methods developed for the analysis of volatile compounds from onion and garlic

Material	Methods[a]	Reference
Onion	E/RSD	Freeman and McBreen [34]
	E/HPLC CHIRAL	Bauer et al. [35]
	SC-CO_2/GC-MS	Sinha et al. [38]
	SD/GC-MS	Sinha et al. [38]
	E/CRYOGENIC GC-MS	Block et al. [39,41]
	E/HPLC/^{1}H-NMR	Block et al. [40,41]
	SFE-MS	Calvey et al. [42]
	SFE/GC-MS	Calvey et al. [43]
	SFE/LC-MS	Calvey et al. [44]
	E/GC	Schmidt et al. [51]
	E/GC	Hong et al. [52]
	E/GC-MS	Mondy et al. [53]
	E/HPLC-UV (DAD)	Mondy et al. [53]
	SPME/GC-MS	Mondy et al. [54]
	SPME/LC-MS	Mondy et al. [54]
	E/PTV-GC-AED	Junyapoon et al. [55]
	PTV-GC-ITD/MS	Junyapoon et al. [55]
Garlic	SD/HPLC REVERSED/^{1}H NMR	Lawson et al. [36]
	SD/HPLC REVERSED/^{1}H NMR	Lawson et al. [37]
	E/HPLC/^{1}H NMR	Block et al. [40,41]
	SFE-MS	Calvey et al. [42]
	SFE/GC-MS	Calvey et al. [43]
	SFE/LC/MS	Calvey et al. [44]
	E/RSD (NTB)	Miron et al. [45]
	E/RSD (4-MP)	Miron et al. [46]
	SYNTHESIS/SPE/UV	Cruz-Villalon [47]
	E/TLC	Keusgen [48]
	GLDE/GC	Lee et al. [49]
	SD/GC	Lee et al. [49]
	E/HPLC	Nikolic et al. [50]
	E/PTV-GC-AED	Junyapoon et al. [55]
	PTV-GC-ITD/MS	Junyapoon et al. [55]
	E/HPLC REVERSED/UV	Bocchini et al. [56]
	E/HPLC REVERSED/ED	Bocchini et al. [56]
	E/HPLC/UV (DAD)/MS ELECTROSPRAY	Arnault et al. [57]
Human breath (after garlic ingestion)	GC-MS	Ruiz et al. [61]

[a] CRYOGENIC GC-MS, cryogenic gas chromatography-mass spectrometry; E, extraction; ED, electrochemical detection; GC-AED, gas chromatography-atomic emission spectrometry; GC-ITD/MS, gas chromatography with ion trap mass spectrometer; GLDE, gas-in-liquid-dispersion extraction; LC, liquid chromatography; HPLC CHIRAL, high performance liquid chromatography with chiral column; HPLC REVERSED, high performance liquid chromatography with reversed column; LC-MS, liquid chromatography-mass spectrometry; PTV, programmed temperature vaporization injection; RSD, rapid spectrophotometric determination; RSD(4-MP), rapid spectrophotometric determination 4-mercaptopyridine; RSD(NTB), rapid spectrophotometric determination 2-nitro-5-thiobenzoate; SD, steam distillation; SC-CO_2, supercritical extraction with CO_2; SFE, supercritical fluid extraction; SMD, statistical molecular design; SPE, solid phase extraction; SPME, solid phase micro extraction; SYNTHESIS, allicin synthesis; UV(DAD), UV with a diode-array detection.

their quantitative analysis by gas chromatography-mass spectrometry. This analysis evidenced the presence of 28 sulfur compounds, including allicin (**3a**, Fig. 1) (or its isomer, di-1-propenyl thiosulfinate, **3k**, Fig. 1), Pr methanethiosulfonate (**7b**, Fig. 3), dithiin derivatives (**9**–**11**, Fig. 3), diallyl sulphide (**8a**, Fig. 3), and diallyl trisulfide (**8b**, Fig. 3). A common steam-distilled onion oil, analysed under similar conditions, did not contain detectable amounts of these listed compounds while other compounds were in common with the SE-CO_2 onion extract. The compounds Me Pr trisulfide (**8e**, Fig. 3), di-Pr trisulfide (**8f**, Fig. 3), and di-Pr tetrasulfide (**12b**, Fig. 3) were detected only in the commonly steam-distilled onion oil and were present in high concentration.

In the same year, Block and his group published several papers on this matter. They first performed a quantitative GC-MS analysis [39] of thiosulfinates and related compounds from onion and other common related *Allium* species, such as leek, scallion, shallot, and chive. A wide-bore capillary column was used with cryogenic (0 °C) on-column injection and initial column temperature conditions, slow column heating rates (2–5 °C/min), and GC−MS transfer line temperatures of 80–100 °C. Authentic samples of suspected components were used to verify identities and quantify the compounds, using benzyl alcohol as internal standard. Under these conditions, the thiosulfinates Me*S*(*O*)*S*Me (**3i**, Fig. 1), Pr*S*(*O*)*S*Pr (**3p**, Fig. 1), Me*SS*(*O*)Pr (**3q**, Fig. 1), Me*S*(*O*)*S*Pr (**3n**, Fig. 1), (*Z*)- and (*E*)-Me*S*(*O*)*S*CH=CHMe (**3h** and **3g**, Fig. 1, respectively), (*E*)-Me*SS*(*O*)CH=CHMe (**3j**, Fig. 1), and (*Z*)- and (*E*)-Pr*S*(*O*)*S*CH=CHMe (**3m** and **3l**, Fig. 1, respectively), and *cis*- and *trans*-zwiebelanes (**6a** and **6b**, Fig. 2, respectively) were all identified in onion and in leek,

scallion, shallot, and chive. No satisfactory peaks were obtained for MeCH=CH*S*(*O*)*S*Pr isomers (**3o**, Fig. 1, (*E*)-isomer) or for thiosulfinates containing a 2-propenyl (allyl) group (**3a**–**3f**, Fig. 1), which were best identified by HPLC.

The data regarding quantitative HPLC analysis were reported in an accompanying paper [40] in which room-temperature vacuum distillates and extracts of onion, garlic, and other related *Allium* species such as leek, scallion, shallot, chive, wild garlic, and elephant (or great-headed) garlic were analysed by HPLC and ^{1}H NMR by using authentic samples of suspected thiosulfinate components to evaluate the methods. It was concluded that gas chromatography, as typically performed with high injector and column temperatures, it is a wrong picture of the volatile extracts of *Allium* species, and that HPLC provides instead a reliable measure, both qualitative and quantitative of the compounds present. In addtion, a simple vacuum distillation procedure, facilitating qualitative analysis of *Allium* volatiles, was also described. A number of significant trends were also noted regarding the varying proportions of different thiosulfinates in each *Allium* species. In particular, garlic grown in cooler climates has a higher allicin/Me thiosulfinate ratio than garlic grown in warmer climates.

In a further paper, Block et al. [41] summarized the results obtained by using different techniques (HPLC, cryogenic gas chromatography-mass spectrometry, and ^{1}H NMR spectroscopy) in the analysis of organosulfur compounds from garlic and onion in comparison with other *Allium* plants (wild garlic, leek, scallion, shallot, elephant garlic, chive, Chinese chive). Authentic samples of suspected thiosulfinate components were used to evaluate the methods.

Later, Calvey et al. started a project based on the application of supercritical fluid extraction (SFE) technique to the analysis of organosulfur compounds present in *Allium* species. Their goal was to develop a robust method for the routine analysis of fresh plants and derived products, as well as in vivo measurements. To this purpose, the performance of the SFE process paired with different calibration approaches for HPLC and LC-MS were presented. In a first paper, SFE-MS has been also used successfully to identify allicin (**3a**, Fig. 1), the predominant thiosulfinate in freshly cut garlic [42]. A low oven temperature (50 °C) and low restrictor tip temperature (115 °C) were needed in order to obtain a chemical ionization (Cl) mass spectrum of allicin with the protonated molecular ion, *m*/*z* 163, as the major ion. The effects of tip temperature on the Cl mass spectrum of allicin have been also studied. Another study [43] regarded the SFE of fresh garlic and fresh onion and the successive analysis of the obtained extracts by the same technique based on GC-MS analysis. Allicin was readily extracted from fresh garlic by using supercritical CO_2 (SC-CO_2). Under SFE conditions using a solvent trap, the yield of allicin from a water homogenate of fresh garlic was 98.2%; however, when a solid-phase trap (i.e., condensation on glass beads) was used, the yield of allicin was higher, reaching 124.6%. An increase of the quantity of thermal decomposition products with respect to allicin was evident when the garlic was extracted at temperatures higher than 36 °C. The identity of allicin in the garlic SF extracts was confirmed by thermospray MS. The SFE of yellow onion was about 69% as efficient as the extraction with diethyl ether, as detected by GC-MS.

In a third paper, Calvey et al. [44] characterized the thiosulfinate profile of homogenized SFE of garlic, onion and ramp (*A. tricoccum* Ait.) by liquid chromatography (LC) and chemical ionization mass spectrometry. The major thiosulfinates from garlic and ramp were readily identified and quantified by this technique. Small quantities of ajoene (**11**, Fig. 3), which has been shown to be a potent antithrombotic agent, were also found in SF extracts of garlic homogenates. The profiles of onion juice extracts revealed the usual thiosulfinates, zwiebelanes, and disulfides reported in prior studies, as well as cepaenes previously identified in extracts of onion juice through extensive isolation steps and spectroscopic methods. However, the presence of trace quantities of allyl compounds (**3a**–**3f**, Fig. 1) in onion juice and Pr compounds (**3l**–**3q**, Fig. 1) in garlic and ramp homogenates has been verified by the use of LC−MS. The presence of these compounds was not readily evident in previous analyses using GC−MS with cold-on-column injection and reversed-phase or normal phase LC with UV detection.

As described before, allicin (**3a**, Fig. 1) is produced upon the interaction of the non-protein amino acid alliin (**1a**, Fig. 1) with the enzyme alliinase (alliin lyase). A simple and rapid spectrophotometric procedure for detection of allicin and alliinase activity, based on the reaction between 2-nitro-5-thiobenzoate (NTB) and allicin, was described by Miron et al. [45]. NTB reacted with the activated disulfide bond –*S*(*O*)–*S*– of allicin, forming the mixed-disulfide allylmercapto-NTB, as characterized by NMR. The method can be used for detection of allicin and total thiosulfinates in garlic preparations and garlic-derived products. It can also applied for detection of alliinase activity in crude garlic extracts. However, this method presents the disadvantage that NTB reagent is not commercially available and must be synthesized.

This was the reason that pushed Miron et al. to publish a further paper [46] describing the quantitative analysis of alliin (**1a**, Fig. 1), allicin (**3a**, Fig. 1), and the alliinase activity by the use of 4-mercaptopyridine (4-MP), a commercially available chromogenic thiol. The assay was based on the reaction of 4-MP (λ_{max} = 324 nm) with the activated disulfide bond of thiosulfinates –*S*(*O*)–*S*–, forming the mixed disulfide, 4-allylmercaptothiopyridine, which had no absorbance at this region. The structure of 4-allylmercaptothiopyridine was confirmed by mass spectrometry. The method was used for detection of alliin and allicin concentration in their pure form, and for the determination of alliin and total thiosulfinates concentrations in crude garlic preparations and garlic-derived products at μM concentrations. The 4-MP assay was demonstrated to be an easy, sensitive, fast, no costly, and highly efficient assay detection of allicin, alliin, and alliinase activity in garlic preparations.

Although bacteriostatic, antifungal, and antithrombotic properties have been attributed to allicin, its industrial development was limited because of its unstable nature. For this reason, research has been oriented to the development of synthetic routes for obtaining the compound. A method for the synthesis of allicin (**3a**, Fig. 1) has been published by Cruz-Villalon [47], based on the oxidation of diallyldisulfide (**8a**, Fig. 3) with an

aqueous solution of magnesium monoperoxyphthalate (MMPO) and tetrabutylammonium hydrogen sulfate, as a phase transfer catalyst. The allicin obtained was isolated by solid-phase extraction and its concentration detected spectrophotometrically, allowing the allicin solution to be used also as a standard for HPLC calibration.

A improvement in TLC analysis of garlic constituents has been obtained for separating alliin from other compounds [48]. A modified ninhydrin detection reagent was used to optimize the differentiation between cysteine sulfoxides and other amino acid derivatives. In addition, a sensitive and specific color reaction was developed for detection of allicin after TLC.

Extraction yields of thiosulfinates from garlic were studied by Lee et al. [49] by gas-in-liquid-dispersion (GLD) system to maximize the thiosulfinate extraction. The extracted thiosulfinates were spectrophotometrically quantified using synthetic allicin (**3a**, Fig. 1). The conditions for a complete extraction were optimized by response surface methodology. The volatile components extracted by various methods, such as hexane extraction, simultaneous steam distillation and GLD system, were compared by using GC. The results indicated that the thiosulfinate yield was increased by increasing temperature and nitrogen gas flow rate, while the effects of bubble sizes on thiosulfinate extraction were not significant at the ranges tested. Application with GLD system resulted into extraction of more volatile components compared to other extraction methods. Therefore, it was concluded that GLD system was a very efficient extraction method for thiosulfinate extraction.

The thiosulfinate content of aqueous extracts of garlic powder, as well as from fresh garlic bulbs has been also analysed by Nikolic et al. [50]. The stability of thiosulfinates in freshly prepared extracts, in extracts prepared from garlic powder stored 4 days at room temperature (293 K), and in extracts from powder stored for 18 months at 278 K was analysed by HPLC. Allicin (**3a**, Fig. 1), allyl-methyl-/methyl-allyl-thiosulfinate (**3c** and **3d**, Fig. 1) and dimethyl-thiosulfinate (**3i**, Fig. 1) were the most representative components in freshly extracts from garlic bulbs and from garlic powder. The stability of these compounds resulted very low at room temperature. The highest stability was detected in the case of extracts prepared from powder sample stored for 18 months at 273 K. The content of allicin and other thiosulfinates in such extracts was almost equal to the content of thiosulfinates in fresh garlic bulbs.

Concerning the onion chemistry, a rapid extraction method has been developed by Schmidt et al. [51] to quantify the lachrymatory factor (LF, **4**, Fig. 2)) and other flavour compounds in onion, using gas chromatography. This method involved crushing the onion and extracting the resulting juice with a solution of methylene chloride and an internal standard. The resulting extract was concentrated and injected onto a gas chromatograph. This method was shown to be simple, fast, and reproducible. Results showed the onion juice to contain 1–22 μmol of the LF/mL.

To maintain high quality and to extend the shelf life of intact and minimally processed (removal of roots and compressed stem) green onions, the potential benefits of controlled atmospheres (CA) and heat treatment were evaluated by Hong et al. [52]. Atmospheres of 0.1–0.2% O_2 or 0.1–0.2% O_2 containing 7.5–9% CO_2 were the CA conditions that best maintained the visual appearance and prolonged shelf life to more than 2 weeks at 5 °C in both intact and cut onions. No CA treatment completely controlled extension, and growth of the inner white leaf bases of the minimally processed onions at 5 °C. Heat treatment (55 °C water for 2 min) of the white leaf bases effectively controlled quality of cut onions stored at 5 °C. Total sugars generally decreased in intact and minimally processed green onions, but were maintained in heat-treated cut onions. Heat treatment did not affect thiosulfinate concentrations during 14 days at 5 °C, except for treated cut onions not stored under CA.

Recently, volatile sulfur compounds produced enzymically from *Allium* species have been identified by Mondy et al. [53] by combined GC-MS method and the results obtained have been compared to those of HPLC-UV analysis (with a diode-array detection, DAD). Analysis of the same onion sample by successive SPME-GC-MS or by solvent extraction followed by GC-MS enabled the authors to identify and quantify the true onion volatiles including lachrymatory factor (**4**, Fig. 1) and thiosulfinates (**3g–3q**, Fig. 1). Conversely, HPLC analysis of garlic samples was shown to be more suitable because of the substantial degradation of allicin (**1a**, Fig. 1) during GC-MS analysis.

From the same research group it followed a paper [54] dealing with the analysis of the aroma of fresh and preserved onion and leek by dual solid-phase micro extraction-liquid extraction and GC-MS. In particular, the lachrymatory factor (**4**, Fig. 1) was directly analysed on fresh onion juice by solid-phase micro extraction on polyacrylate fiber, using a fast routine GC-MS method on a 10 m × 0.32 mm I.D. (4 μm thick polydimethylsiloxane film) column with splitless mode injection. The identification and quantification of thiosulfinates and zwiebelanes (**6a** and **6b**, Fig. 2) were performed on the same juice extracted by diethyl ether after 80 min maceration using the same GC-MS method. Selected ion recording enhanced both differentiation possibility and detection limit. This dual method was then applied to evaluate flavour differences between onion and shallot varieties as it provided accurate profiles of all initially formed compounds. Moreover, this method allowed to compare qualitative and quantitative transformed products, such as frozen, freeze-dried powders and sterilized products. With the exception of the lachrymatory factor, frozen onion compounds were similar to those of fresh onion sample. Conversely, the other transformed samples have lost most of the initially formed compounds and produced mainly di- and trisulfides (e.g. **8–11**, Fig. 3) corresponding to the degradation of thiosulfinates and zwiebelanes. These dramatic changes explained the very different flavours of these manufactured products compared to fresh material.

An analysis applied to garlic and onion by Junyapoon et al. [55] regarded the use of injection by programmed temperature vaporization injection (PTV) of gaseous samples for gas chromatography-atomic emission spectrometry (GC-AED). To evaluate the effectiveness of the developed technique, a series

of headspace samples of foods, including garlic and onion, and of landfill gases were analysed. The volumes of gaseous samples varied from a few mL up to L depending on analyte dilution, through focusing onto a sorbent trap, then rapid liberation into the GC-AED system by programmed thermal desorption. Despite the high carrier gas flow rates associated with direct PTV-GC, AED performance and sensitivity were unaffected. The detailed elemental information obtained from the PTV-GC-AED analyses was confirmed using a PTV coupled to a gas chromatograph with ion trap mass spectrometer (PTV-GC-ITD/MS).

An improvement in the analytical method for the detection of allicin (**3a**, Fig. 1) in garlic samples, using reversed-phase HPLC with both UV and electrochemical detection (ED) and online post-column photochemical reaction, was recently published by Bocchini et al. [56]. Standard allicin was synthesized and its behaviour at the chosen analytical conditions was tested. The post-column irradiation at 254 nm on the one hand decreased the response of allicin to UV detector, and on the other hand, allowed the detection of this compound, which was otherwise electrochemically inactive, using the electrochemical detector. Detection limits of 0.1 and of 0.01 mg/L using UV and ED detectors, and linearity of response in the range 1–8 mg/L were obtained. Samples of garlic extracts, obtained following two different extraction procedures, were analyzed and the results obtained using both UV and electrochemical detectors were compared.

The quality of garlic and its derived products is related to the amounts of allicin (**3a**, Fig. 1) and of course of its biogenetic precursors and there was no methods in the literature able to simultaneously quantify them. So, it was recently developed an analytical method by Arnault et al. [57] to quantify allicin (**3a**, Fig. 1), alliin (**1a**, Fig. 1), SAC (*S*-allyl-L-cysteine) and various dipeptides that acts in garlic as storage compounds. It is well known that all these intermediates are involved in the allicin biosynthetic pathway. The method was based on a simple and rapid HPLC analysis, using high-performance ion-pair chromatography for separation and multiple mass spectrometry and UV for detection. Among the eluents containing an ion-pairing, heptanesulfonate showed to guarantee a sufficient separation between alliin and the more retained dipeptides at very low pH value. Allicin was eluted after 18 min on a 15 μm × 3 mm column. Synthetic reference compounds were characterized by the same chromatographic method using a diode-array UV detector and an ion trap mass spectrometer (electrospray ionization) in the multiple MS mode. In routine analysis of garlic bulbs, powders and other products, the diode-array detector has showed to be sufficient for a relevant quantification.

The differences of thiosulfinates in onion has been also correlated to sulfur fertility by Randle et al. [58]. Thus, onions were greenhouse grown with various sulfur fertilities and assayed for changes in thiosulfinates and related compounds. The concentration of thiosulfinates (**3g**–**3q**, Fig. 1), zwiebelanes (**6a** and **6b**, Fig. 2), and onion lachrymatory factor (LF, **4g**, Fig. 2) increased with a linear trend as sulfur fertility increased with the exception of methanesulfinothioic acid *S*-propyl ester (**3m**, Fig. 1) and methanesulfinothioic acid *S*-methyl ester (**3i**, Fig. 1). The thiosulfinate plus zwiebelanes:LF ratio increasing sulfur fertility, suggested that the former were being formed at a greater rate at higher sulfur fertility.

In a second paper, Randle [59] deeply investigated onion flavour chemistry and factors regulating flavour formation. This is because of the importance of onions for their nutritional value in diets around the world, and for their distinctive flavour and for their ability to enhance flavours in other foods. They have demonstrated that onion flavour, which is dominated by organosulfur compounds arising from the enzymatic decomposition of *S*-alk(en)yl-L-cysteine *S*-oxide flavour precursors (**1a**–**1d**, Fig. 1) following tissue disruption, depends from environmental and genetic factors which influence precursor composition and accumulation. In particular, the formation of thiosulfinates and other onion flavourants are dependent on the concentration of three different alkyl groups present in the flavour precursors and the action of alliinase, the enzyme responsible for precursor decomposition, are influenced by the precursor alkyl group. Alliinase concentration also varies and can lead to differences in onion flavour.

In a third paper, Randle [60] reviewed the literature about the onion flavour chemistry which is dominated by organosulfur compounds arising from precursor decomposition, such as the lachrymatory factor (**4g**, Fig. 2) and various thiosulfinates (**3g**–**3q**, Fig. 1) that give onions their characteristic flavours. Sulfate is absorbed by the plant and incorporated through cysteine to glutathione. From glutathione, sulfur can proceed through several peptide pathways and terminate in the synthesis of one of three flavour precursors. Flavour intensity is governed by genetic factors within the onion and environmental conditions under which the onions grow. Onion cultivars differ in the ability to absorb sulfate and in the efficiency of synthesizing flavour precursors. Increased sulfate fertility, higher growing temperatures and dry growing conditions all contribute to increased flavour intensity in onion.

In addition, it was also developed a method by Ruiz et al. [61] for determining the garlic-borne volatile compounds by breath of human subjects in a time course study following the ingestion of various garlic samples. The method was based on the use of GC-MS with a combined adsorbent trapping and a short-path thermal desorption. In detail, after time intervals, 1 to 1.2 L of breath were purged and trapped through porous polymer resins and analyzed via short-path thermal desorption GC-MS method. Quantification of the compounds was achieved by GC with flame ionization detection using an internal standard. Allyl methyl sulfide, diallyl sulfide, diallyl disulfide, *p*-cymene and D-limonene were found consistently in all samples, while allyl thiol was detected only occasionally. Hydrogen sulfide, a potential breath odour compound, was not efficiently trapped due to its low breakthrough volume in the adsorbent resins, therefore it was analyzed by direct injection of breath samples using sulfur sensitive flame photometric GC methodology. The data obtained have showed that the acid pH values in the stomach cause transformation in the compound structures during digestion. The procedure described proved to be an useful and non-invasive technique for measuring low levels of volatile food-borne phytochemicals on the breath of human subjects.

3. Non-volatile compounds

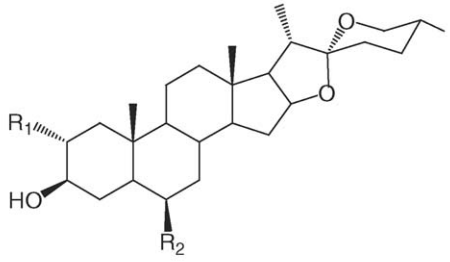
3.1. Sapogenins and saponins: chemical structure and bioactivity

Although the study on plant saponins started in the 1970s, only recently they have received an increasing attention in consideration of the involvement of saponins in important biological processes and of their ability to act as natural drugs in many diseases. In fact several papers have demonstrated antifungal, antitumor, cytotoxicity, blood coagulability, anti-spasmodic and cholesterol-lowering effects of saponins isolated from onion and garlic [62–64].

The first study of onion sapogenins and saponins was performed by Russian scientists [65–67] that have analysed the saponin contents of onion, garlic and leek. The saponins were hydrolyzed and the resulting aglycones were identified by gas chromatography-mass spectrometry, aided by NMR, IR and UV data, as sitosterol, gitogenin (**14**, Fig. 4), oleanolic acid and amyrin. Investigations of the sapogenins isolated from white onion by Ismailov and Aliev [68] have indicated the presence of diosgenin (**15**, Fig. 4) both in flowers and in bulbs. In the same period, Eristavi [69] has compared the sapogenin contents of onion and garlic with many *Allium* species showing that, in addition to diosgenin, the sapogenin β-chlorogenin (**16**, Fig. 4) is a common compound and this can be used for the chemosystematic of the genus.

Later on, Kravets et al. [70–72] have reported from onion the isolation of the sapogenin cepagenin (**17**, Fig. 5) and the saponins alliospirosides A–D (**18**–**21**, Fig. 5) and alliofuroside A (**22**, Fig. 5).

More recently, from bulbs of red onion, var. Tropea, has been reported the presence of four new antispasmodic saponins by Corea et al. [62]. These compounds, based on a furostanol skeleton, have been named tropeosides A1/A2 (**23**, Fig. 6) and tropeosides B1/B2 (**24**, Fig. 6). High concentrations of ascalonicosides A1/A2 (**25**, Fig. 6) and ascalonicoside B (**26**, Fig. 6), previously isolated from shallot, were also found. The new saponins were found to possess antispasmodic activity in the guinea pig isolated ileum; such an effect might contribute to explain the traditional use of onion in the treatment of disturbances of the gastrointestinal tract.



	R_1	R_2
gitogenin (**14**)	OH	H
diosgenin (**15**)	H	H
β-chlorogenin (**16**)	OH	OH

Fig. 4. Major sapogenins found in onion and garlic.

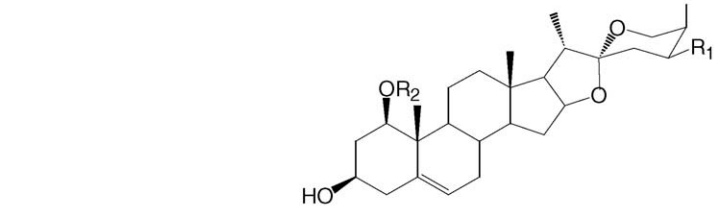
Concerning garlic, proto-eruboside B (**27**, Fig. 7) was the first furostanol saponin isolated from the bulbs [73]. The spirostanol analogue, eruboside B (**28**, Fig. 7), has been obtained by enzymatic hydrolysis of the furostanol derivative proto-eruboside B. Later on, it has been reported [74] from fresh bulbs of garlic the isolation of two closely related saponins, named proto-*iso*-eruboside B (**29**, Fig. 7) and *iso*-eruboside-B (**30**, Fig. 7), the 25*S* epimers of proto-eruboside B and eruboside B, respectively. Their effects on platelet aggregation, blood coagulation and fibrinolysis were examined by in vitro tests [74].

Further studies from garlic bulbs led to the isolation of a new furostanol saponin, named sativoside B1 (**31**, Fig. 8) and characterization of the new proto-desgalactotigonin (**32**, Fig. 8) [75]. In the same paper the structure elucidation of two new steroidal glycosides, named sativoside R1 and sativoside R2 (**33** and **34**, respectively, Fig. 8) have been reported from garlic roots. In a further paper, Matsuura has described from aged garlic extract (AGE) a number of saponins (**35**–**51**, Fig. 9), two of which, a furostanol saponin and its spirostanol derivative (**49** and **51**, Fig. 9, respectively), based on new structures [63]. Thus, this paper has demonstrated the high stability of these saponin compounds during the time and their ability to inhibit the cholesterol absorption from the intestinal lumen in experimental animals, and consequently to reduce the concentration of plasma cholesterol.

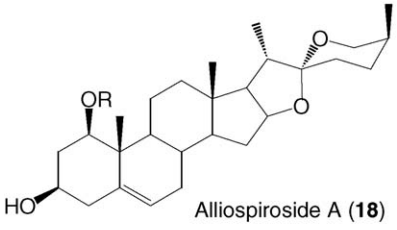
Recently, an analytical method has been developed based on UV derivatization of steroidal saponin in garlic and commercial garlic products as *p*-nitrobenzoate for liquid chromatographic determination [76]. The method was used for detection of eruboside B as the *p*-nitrobenzoyl chloride (PNBC) derivative by reversed-phase liquid chromatography with UV detection at 260 nm. The furostanol saponin, proto-eruboside B was extracted and purified from garlic by SPE with a C18 cartridge and it was enzymically converted to eruboside B, its spirostanol derivative, which was applied as an external standard. Steroidal saponins in garlic and commercial garlic products were extracted and purified by the same protocol followed by treatment with enzyme. The enzyme transformed the furostanol saponin proto-eruboside B into the spirostanol saponin, eruboside B. After reaction with PNBC, the saponin derivative was chromatographed on a C8 column with a gradient elution of (A) 80% aqueous acetonitrile and (B) 100% acetonitrile. The detection limit of the developed method was 1 μg/g for the samples. The method was successfully applied to the saponin analysis of garlic and garlic health food products.

3.2. Flavonoids and phenolics: chemical structure and bioactivity

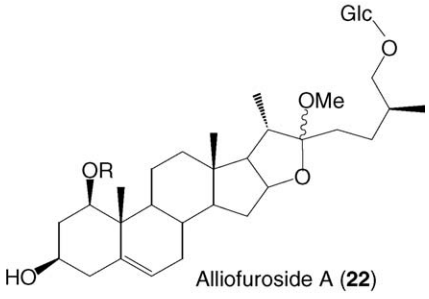
With regard to the flavonoids, a recent study [77] have shown that onion leaves are characterized by the highest total flavonoid content in comparison to other 62 common vegetables. In Table 2



	R_1	R_2
Cepagenin (**17**)	OH	H
Alliospiroside B (**19**)	H	α - L - Rha - (1→2) - β - D - Gal
Alliospiroside C (**20**)	OH	α - L - Rha - (1→2) - α - D - Ara
Alliospiroside D (**21**)	OH	α - L - Rha - (1→2) - β - D - Gal



Alliospiroside A (**18**)



Alliofuroside A (**22**)

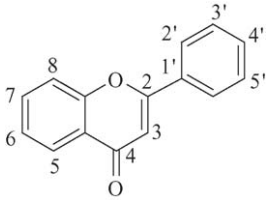
R = α - L - Ara (1→2) 6 - deoxy - α - L - Man

Fig. 5. Sapogenin and saponins from white onion.

are reported the flavonoids found in onion and garlic and their relative amounts [77,78].

From the biological point of view, flavonoids are considered as important factors of the overall antioxidant activity of dietary plants [79–82]. In particular, quercetin has shown anti-HIV property [83] and the ability to protect LDL cholesterol from oxidation, so reducing the risk of cardiovascular diseases [84–86].

Table 2
Flavonol content in onion and garlic[a]



Compound	Subst.	Content in onion (mg/Kg)	Content in garlic (mg/Kg)
Apigenin (**52**)	4′, 5, 7 $(OH)_3$	n.d.	217
Isorhamnetin (**53**)	3, 4′, 5 $(OH)_3$; 7 (OCH_3)	m.a.	n.d.
Kaempferol (**54**)	3, 4′, 5, 7 $(OH)_4$	832	n.d.
Luteolin (**55**)	3′, 4′, 5, 7 $(OH)_4$	391	n.d.
Quercetin (**56**)	3, 3′, 4′, 5, 7 $(OH)_5$	1497	47
Myricetin (**57**)	3, 3′, 4′, 5′, 5, 7 $(OH)_6$	n.d.	693

m.a., minor amounts; n.d., not detected.

[a] Data taken from Miean and Mohamed [77]; Leighton et al. [78].

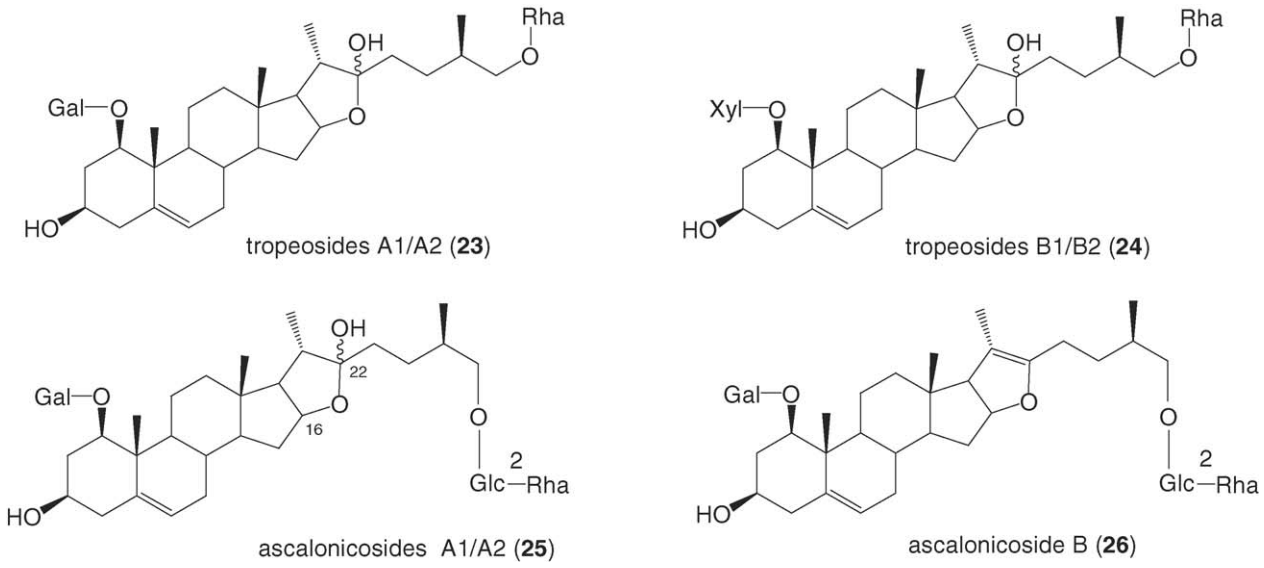


Fig. 6. Saponins from Tropea red onion.

Recently, Nuutila et al. [87] compared the antioxidant activities of onion and garlic extracts by using two different methods, inhibition of lipid peroxidation and radical scavenging activity. Thus, the antioxidant activities of the methanol extracts of selected varieties and parts of garlic and onion were detected by inhibition of lipid peroxidation induced by *tert*-butyl hydroperoxide in isolated rat hepatocytes and scavenging activity on diphenylpicrylhydrazyl radical. The total phenolics and the main flavonoids of the hydrolyzed onion and garlic samples were analyzed and the antioxidant activities obtained by the two methods compared. Both methods gave similar antioxidant activities for pure compounds and crude extracts. However, the radical scavenging method had many benefits compared to the lipid peroxidation method, being easier, cheaper, more specific and reproducible. The radical scavenging activities also correlated with the total phenolics of the extracts. Onions, as expected, had higher radical scavenging activities than garlic, while red onion was more active than yellow onion, with the skin extracts of onion possessing the highest activities observed.

Recently attention has been focused on cancer preventive effects of some foods and in particular onion and garlic [88]. Although, in recent years, experimental studies have provided growing evidence for the beneficial action of flavonoids on multiple cancer-related biological pathways (carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, inflammation), the epidemiological data on flavonoids and cancer are still limited and further studies are needed. However, a protective association against lung cancer has been observed for people consuming onion [89].

In addition, flavonoids including quercetin and taxifolin also affect the gastrointestinal tract by possessing antiulcer, antispasmodic, and antidiarrhoeal activity [62].

With regard to the analysis of flavonoids and phenolics, Table 3 summarizes the paper published on onion and garlic, indicating the methods applied that will be detailed as follows.

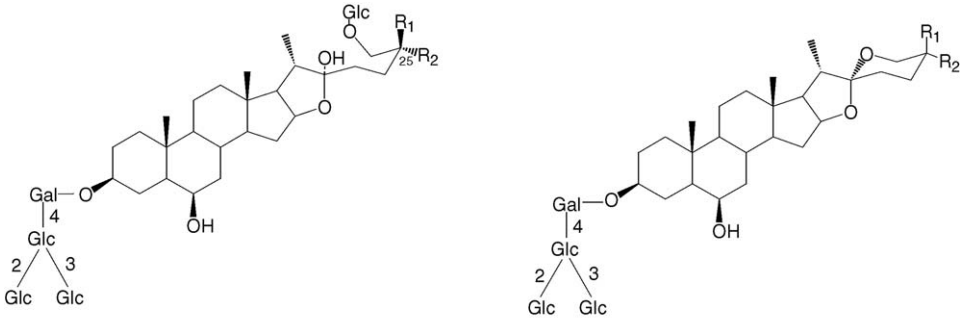
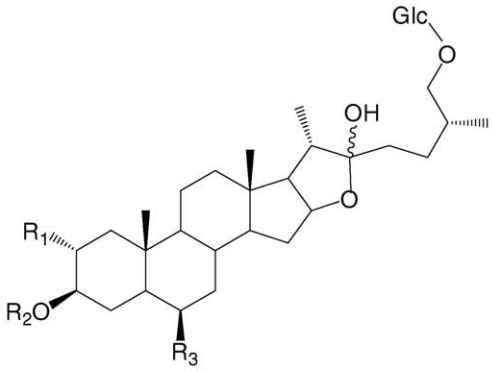
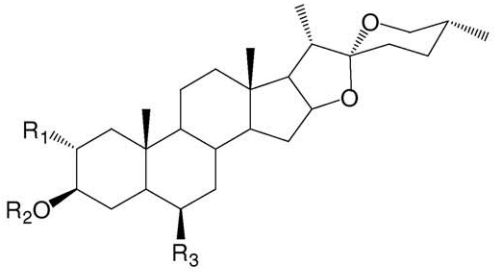


Fig. 7. Saponins from garlic bulbs.



	R_1	R_2	R_3
sativoside B1 (**31**)	H	—Gal(4)—Glc(2)—Glc(3)—Glc; Glc(3)	OH
proto-desgalactotigonin (**32**)	H	—Gal(4)—Glc(2)—Glc; Glc(3)—Xyl	H
sativoside R1 (**33**)	H	—Gal(4)—Glc(2)—Glc(3)—Glc; Glc(3)—Xyl	H



	R_1	R_2	R_3
sativoside R2 (**34**)	H	—Gal(4)—Glc(2)—Glc(3)—Glc; Glc(3)—Xyl	H

Fig. 8. Saponins from garlic bulbs and roots.

The first analysis was dated on 1996 [90] with the detection from onion of seven major flavonoid compounds, separated by Sephadex LH-20, thin-layer and high-performance liquid chromatography. Compounds have been identified by NMR and mass spectrometry. The flavonoids were quercetin, quercetin monoglucoside, quercetin diglucoside, isorhamnetin, isorhamnetin glycoside, rutin, and kaempferol.

In the same year, another paper has appeared on the analytical problems in the study of flavonoid compounds in onion [91]. In particular, it was developed a method

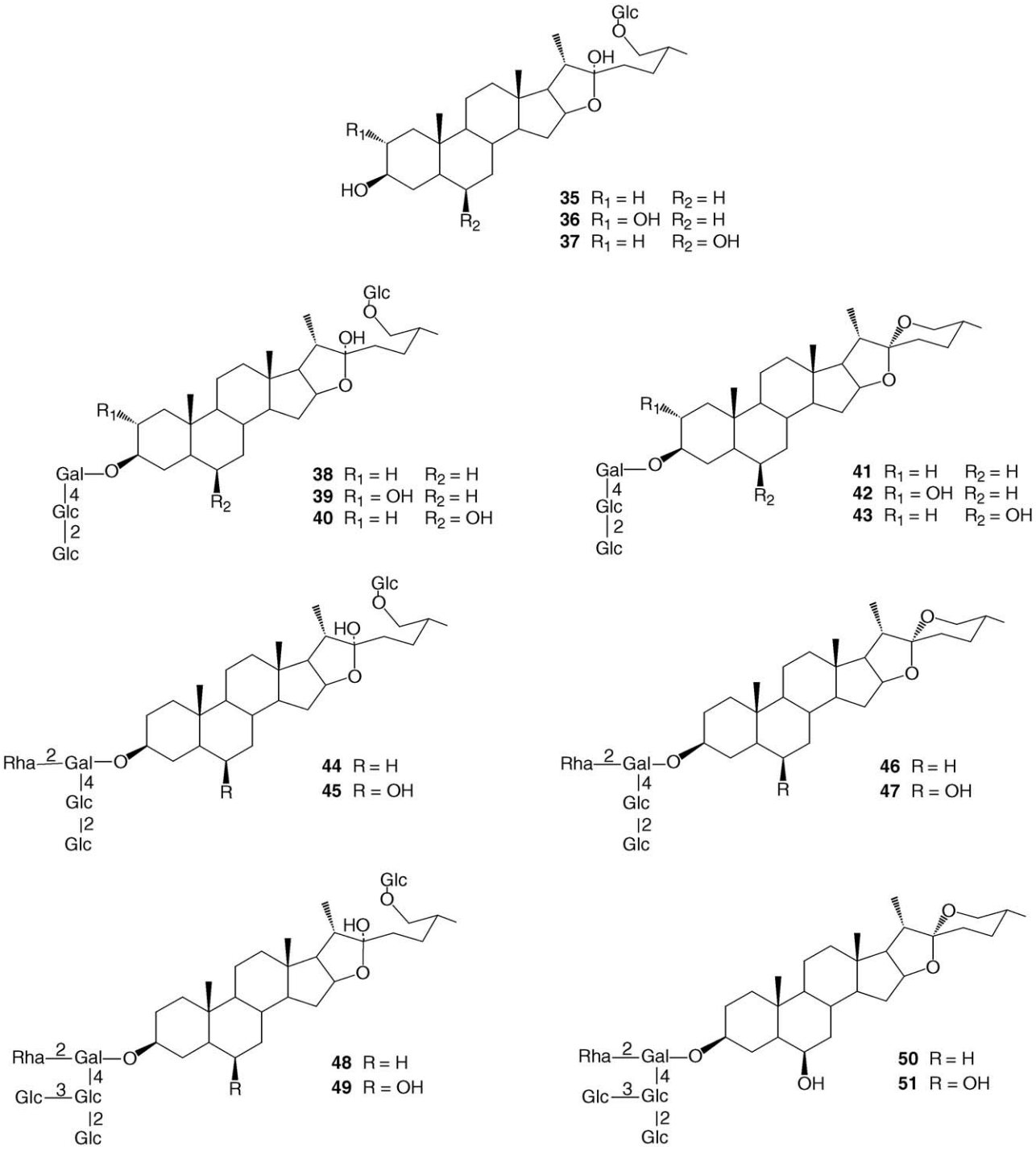


Fig. 9. Sapogenin and saponins from aged garlic extract (AGE).

to study flavonoids in their natural form in plant foods as conjugates. Two major components, quercetin monoglucoside and quercetin diglucoside, accounting for 80% of the total flavonoids, have been detected in onion. Anthocyanins are only minor components of the flavonoid spectrum in the edible portion of red varieties. A preliminary study of flavonols in onion, which had been finely chopped, have suggested that in most varieties there is only a small loss in total flavonol content but that there was a progressive loss of the diglucoside component with an accompanying quantitative accumulation of the monoglucoside and the aglycon.

Quantitative analysis of flavonoids, both in a free and conjugated forms, by reversed-phase high-performance liquid chromatography [92] has been performed, thus finding large variations in the effectiveness of using five different columns. The best results were obtained with C18 Nova-Pak, C18 Symmetry and C18 Genesis columns, but substantial

Table 3
Methods developed for the analysis of flavonoids of onion and garlic

Material	Methods[a]	Reference
Onion	SEPHADEX/HPLC/TLC/MS/NMR	Park and Lee [90]
	C/MS	Rhodes and Price [91]
	HPLC REVERSED	Crozier et al. [92,93]
	C/MS/NMR	Price et al. [94]
	TLC/HPLC/NMR	Fossen et al. [95]
	HPLC/MS/NMR	Furusawa et al. [96]
	HPLC (DAD)	Marotti and Piccaglia [96]
	HPLC/HPLC-MS	Gennaro et al. [97]
	HPLC-DAD/ESI-MS-MS	Bonaccorsi et al. [99]
	HPLC	Lombard et al. [100]
	SD	Lombard et al. [100]
	FLAVONOID HYDROLISYS/HPLC	Nuutila et al. [101]
	E/HPLC REVERSED	Miean et al. [77]
Garlic	HPLC/FAB-MS/NMR	Kim et al. [81]
	E/HPLC REVERSED	Miean et al. [77]
Human plasma and urine (after red onion ingestion)	HPLC REVERSED (DAD)/MS-MS	Mullen et al. [106]

[a] C, chromatographyc comparition; HPLC (DAD), high-performance liquid chromatography with a diode-array detection; HPLC REVERSED, high-performance liquid chromatography with a reverse phase column; HYDROLISYS, flavonoid glycosides hydrolysis; SD, spectrophotometric determination; SEPHADEX, sephadex LH-20 column.

band broadening and peak asymmetry were observed when free flavonoids were chromatographed on ODS-Hypersil and LiChrospher RP-18 supports. The Symmetry and Genesis columns provided gradient elution separations of rutin, quercetin-3-glucoside, quercitrin, myricetin, luteolin, quercetin, apigenin, kaempferol and isorhamnetin. This procedure was used for the quantitative analysis of endogenous flavones and flavonols in acid hydrolyzed onion extracts. The quercetin levels in onions ranged from 185 to 634 μg of quercetin g^{-1} fresh weight [93]. Cooking lowered the quercetin content of onions with greater effects by microwaving and boiling than frying.

In the same year, the investigation on four varieties of onion has been performed [94] by using a combination of chromatographic comparison, mass spectrometry and NMR spectroscopy, confirming the major flavonoids of mature onion bulbs as the 3,4′-*O*-diglucoside (Qdg) and 4′-*O*-monoglucoside (Qmg) of quercetin. These two components accounted for over 85% of the total flavonoids in three varieties of onion with Qdg as the main component. Quercetin was detected in these long stored onions but only at low levels of less than 2% of the total. The remaining flavonoid fraction (approximately 15%) comprised up to 17 different components of which quercetin-3-*O*-glucoside and isorhamnetin glucoside were prominent members although each contribute less than 1% of the total flavonoid fraction. There were significant differences in the levels of Qdg and Qmg between the four different onion varieties analyzed; Qdg ranging from 50 to 1300 mg kg^{-1} fresh onion tissue and Qmg ranging from 36 to 394 mg kg^{-1}.

In addition to these compounds, from the Red Baron onion variety, quercetin 3,7,4′-*O*-triglucoside has been found [95]. Minor amounts of taxifolin-4′-*O*-glucoside, a rare dihydroflavonol, was also detected. The structures were established on the basis of acid hydrolysis, chromatographic (TLC and HPLC) and homo- and heteronuclear NMR spectroscopic techniques.

A new flavonol glucoside based on a rare dimeric structure (**52**, Fig. 10) was further reported by Furosawa et al. [96] from onion and its structure elucidated by the use of spectral data.

In the same year, caracterization of the flavonoids in 12 different coloured cultivars of onion (white, golden, and red) was performed [97]. The study reported data on the flavonoid yields and contents for fresh bulbs. The fresh bulb yields ranged from 3.1 to 6.7 kg m^{-2} as found in golden cultivars

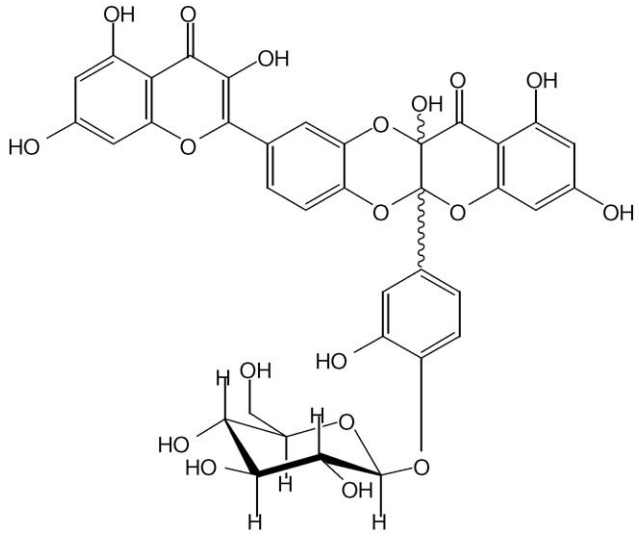


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Fig. 10. Flavonol glucoside dimer isolated from onion.

Santana and Castillo, respectively. The flavonoids, extracted with methanol/water/acetic acid (50:42:8, v/v/v) from fresh bulbs and characterized by HPLC with a DAD detector, were mainly made up of quercetin and isorhamnetin in the form of aglycons and glycosides. The highest amount of free quercetin was detected in the fresh bulbs of Tropea (557.8 mg kg^{-1}) whereas that of total flavonoids was found in Dorata D (979.1 mg kg^{-1}). The golden cultivar Castillo resulted in the highest bulb and flavonoid yields (6.7 kg m^{-2} and 5.2 g m^{-2}, respectively).

Regarding the Tropea variety, the content of anthocyanins, flavonols, and carbohydrates was analysed by HPLC and HPLC-MS [98]. Cyanidin derivatives was showed to constitute >50% of total anthocyanins, but delphinidin and petunidin derivatives, which were not reported in red onions thus far, were also detected. The flavonoid distribution in the different layers of the bulbs indicates that, after homelike peeling, the edible portion contains 79% of the total content of quercetin-4′-glucoside but only 27% of the anthocyanins. Storage of onions for 6 weeks at different conditions, all of them mimicking home storage habits, resulted in a decrease to 64–73% of total anthocyanins. The same trend was verified for the total antioxidant activity, which was reduced to 29–36%. A decrease in glucose and fructose contents correlated with anthocyanin degradation was also observed. Storage at low temperature seems to better preserve the onion anthocyanins.

A very recent study also regarded Tropea variety with the analysis of the flavonol glucoside profile by the aid of high-performance liquid chromatography-diode array detector (HPLC-DAD) coupled with electron spray mass spectrometry (ESI-MS-MS) [99]. This technique allowed the identification of seven flavonols in southern Italian red onion, quercetin-4′-glucoside and quercetin-3,4′-diglucoside being the most abundant components. Five minor flavonols have been recognized, offering a characteristic profile of such compounds in red onions under study. Quercetin-3-glucoside, quercetin-7,4′-diglucoside, quercetin-3,7,4′-triglucoside, and isorhamnetin-4′-glucoside have been previously reported as minor flavonoid components in onion, while isorhamnetin-3,4′-diglucoside was previously found in shallot. Traces of isorhamnetin-3-glucoside and free quercetin were also detected.

Flavonoid quantification in onion was performed by using a spectrophotometric determination and a high-performance liquid chromatography analysis [100]. Quercetin content in five onion varieties was monitored at 362 nm and quantified using simple spectrophotometric and HPLC methods. HPLC revealed that 3,4′-Qdg and 4′-Qmg comprised up to 93% of total flavonol content detected in the studied varieties. These major quercetin conjugates combined (3,4′-Qdg + 4′-Qmg) and total flavonol conjugates quantified by HPLC correlated closely with spectrophotometer values. Correlation coefficients were 0.96 ($P < 0.0001$) for 3,4′-Qdg + 4′-Qmg and 0.97 ($P < 0.0001$) for total flavonol conjugates in onion. Simple spectrophotometric procedure proved to be a valid, efficient, and cost-effective method for the quantification of total quercetin in onion.

For the quantitative detection of individual flavonoid glycosides in plant materials, the glycosides are normally hydrolyzed and the resulting aglycons are identified and quantified. However, the hydrolysis conditions which result in optimal breakdown of glycosides are too harsh for some of the other phenolic compounds present in the same plant material. Therefore, the effects of different hydrolysis conditions and different antioxidants on pure flavonoid glycones and aglycons were studied by Nuutila et al. [101]. The results obtained allowed the development of a suitable hydrolysis method for red spring onion. The best results were obtained by refluxing at 80 °C for 2 h with 1.2 M HCl in 50% aqueous methanol with addition of 2 mg of ascorbic acid as an antioxidant.

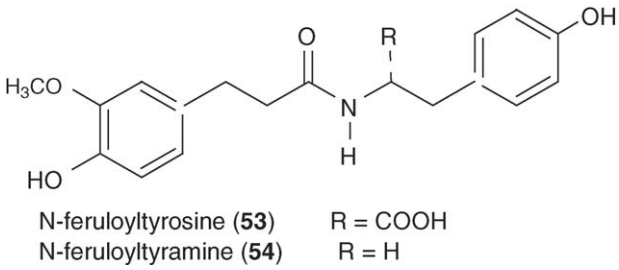

Fig. 11. Phenolics isolated from garlic roots.

Analysis of roots of garlic [102] revealed the presence of two phenolics, the new *N*-feruloyltyrosine (**53**, Fig. 11) together with *N*-feruloyltyramine (**54**, Fig. 11), the latter already described in the literature as the main component of the suberization polyaromatic domain. Because garlic roots are known in traditional medicine to be used in infusion for eliminating worms, *N*-feruloyltyrosine and *N*-feruloyltyramine have been subjected to antifungal assays that evidenced a good activity against *Fusarium culmorum* for both compounds [102]. Antifungal activity was also described for the flavonoid quercetin (**56**, Table 2) and for isoflavones such as genistein (4′,5,7-trihydroxyisoflavone). A resistance mechanism was found in *Armillaria mellea* that is able to metabolize genistein giving rise to metabolization products that are inactive [103].

A study has been conducted to evaluate varietal differences in phenolic and flavonoid content and antioxidant and antiproliferative activities of onions [104]. To characterize these differences, shallots and ten onion varieties, commonly available in the United States (Western Yellow, Northern Red, New York Bold, Western White, Peruvian Sweet, Empire Sweet, Mexico, Texas 1015, Imperial Valley Sweet, and Vidalia), were evaluated for total phenolic and flavonoid contents and antioxidant and antiproliferative activities. The data obtained indicated for all varieties a strong correlation between total phenolic and flavonoid contents and total antioxidant activity. In particular, shallots contained the highest total phenolic content (114.7 ± 10.0 mg/100 g of sample) among the onion varieties tested.

The flavonoid content in onion cooked by various methods has been analysed [105]. In particular, it has been investigated the effect of common cooking, such as boiling, frying with oil and butter, and microwave cooking, on the antioxidant content. Quercetin derivatives, total phenol compounds, and ascorbic acid were selected to estimate the amount of flavonoid ingestion

from onion. Among the cooking methods evaluated, microwave cooking without water better retains flavonoids and ascorbic acid of onion. Also frying does not affect flavonoid intake, while boiling leads to about 30% loss of quercetin glycosides, which are transferred to boiling water. In addition, the effect of additives on quercetin derivatives has been studied. Thus, addition of seasonings, such as glutamic acid, accelerate hydrolysis of quercetin glycosides and addition of ferrous ions accelerate the total loss of flavonoids.

More recently, a further study appeared in the literature on this aspect. In particular, the loss of onion antioxidants by cooking has been investigated by comparison of onion soups prepared in different ways [106]. The data obtained indicated that major onion antioxidants (quercetin-4′-glucoside and quercetin-3,4′-diglucoside) were quite stable in a simple cooking model of boiling and oven heating at 100 °C but considerably degraded in that of oven heating at 200 °C. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of browned onions was similar to that of raw onions, and fried onions exhibited much higher activity based on equivalent amounts of raw onion. Fried onions had much higher browning degree than browned onions, and the browning substance was found to contribute to the radical scavenging activity. The onion soup prepared with sautéed onions had DPPH radical scavenging activity similar to plain browned onions, but the onion soup prepared with fried onions had much lower activity than fried onions alone.

Finally, flavonoid metabolites in human plasma and urine after the consumption of red onions have been analysed by liquid chromatography with photodiode array and full scan tandem mass spectrometric detection [107]. Blood was collected from six human volunteers 1 h after the ingestion of lightly fried red onions. Urine excreted 0–4 h after ingestion was also collected. Plasma and urine were analyzed by reversed-phase HPLC with photodiode array and MS-MS detection. Twenty-three flavonols as a range of mixed sulfate, Me, glucuronide and glucoside derivatives of quercetin were detected. Among them, quercetin-3-glucuronide, quercetin-3′-glucuronide, quercetin-4′-glucuronide, quercetin-3′-sulfate and isorhamnetin-3-glucuronide in samples from all volunteers have been identified by comparison with reference compounds. Samples from one volunteer also contained trace amounts of quercetin-3,4′-diglucoside, quercetin-3-glucoside, isorhamnetin-3-glucoside and the aglycon quercetin. Despite a high dosage, neither anthocyanins nor anthocyanin metabolites accumulated in either plasma or urine in detectable quantities.

3.3. Isolation and identification methods

Polar compounds are extracted from the plant material with an appropriate organic solvent. It could been used a series of solvent of increasing polarities, such as hexane, $CHCl_3$, $CHCl_3$/MeOH 9:1, and MeOH to obtain a fractionation based on the intrinsic polarities of the organic compounds. Sapogenins are present in the $CHCl_3$/MeOH 9:1 extract, while saponins and flavonoids are found in the MeOH extract. This last also contains sugars, nucleotides, aminoacids and salts. To eliminate these compounds, the MeOH extracts is suspended in water and then extracted with butanol. The butanol extract, containing saponins and flavonoid, is concentrated under vacuum and then subjected to consecutive purification steps.

An alternative protocol to this extraction procedure is the use of a solvent, such as acetone, with unique solubility properties, which makes it able to extract compounds with very different polarities, both less and high polar metabolites. The organic extract is taken to dryness under vacuum to eliminate the acetone and then the organic material is suspended in water. The water phase is first partitioned with ethyl acetate, solubilizing less polar compounds, including fatty acids, then is partitioned with butanol. This last phase, containing sapogenins, saponins and flavonoids, is concentrated under vacuum and then subjected to consecutive purification steps.

The choice between these two extraction methods strongly depends from the nature of the starting material. In fact, in the case of fresh plant material, thus containing water, is more convenient the use of the acetone extraction, being acetone completely mixable with water. On the other hand, when the starting material is dried it is more efficient to use the first protocol that starts with a very apolar solvent, such as hexane.

The extracts containing polar compounds ($CHCl_3$/MeOH 9:1 and butanol, in the first protocol; butanol, in the second protocol) are concentrated and then subjected to silica-gel C-18 reversed-phase chromatography, using a medium pressure liquid chromatograph (MPLC) and eluting with a gradient solvent system from water 100% to methanol 100%. The obtained fractions after TLC analyses, (SiO_2, butanol/acetic acid/water 60:15:25, v/v/v) are combined, subjected to a preliminary ^{1}H NMR analysis, and purified by a semi-preparative C-18 reversed-phase HPLC. Final purification is obtained by analytical C-18 reversed-phase HPLC, which affords pure compounds.

The approach for structural elucidation is mainly based on the use of spectroscopic techniques, including high-resolution FAB-MS and advanced 2D NMR experiments. Chemical methods are only applied for stereostructure elucidation of sugar moieties. The use of 2D NMR methods has simplified the structure elucidation in the past based on the use of chemical methods combined to spectral analysis, and reveals the interactions between nuclei. The interpretation of 2D NMR spectra is usually straightforward but such spectra can be obtained also for few mg of pure metabolite. A suitable selection of the NMR experiments are used to give chemical shift correlation and their choice depends on the complexity of the structural problem to be solved.

The more used techniques include direct correlation through homonuclear coupling (COSY, HOHAHA or TOCSY, ROESY) [108–111] and correlation through heteronuclear coupling using ^{1}H detected experiments (HMQC or HSQC, HMBC) [112–115]. In these last experiments, the signal is acquired on ^{1}H (reverse detection) instead of ^{13}C, thus resulting in a higher sensitivity of the experiments. The fact that the sensitivity of ^{1}H is higher compared to ^{13}C makes these experiments more sensible and feasible on a rather small quantity of compound. The sensitivity

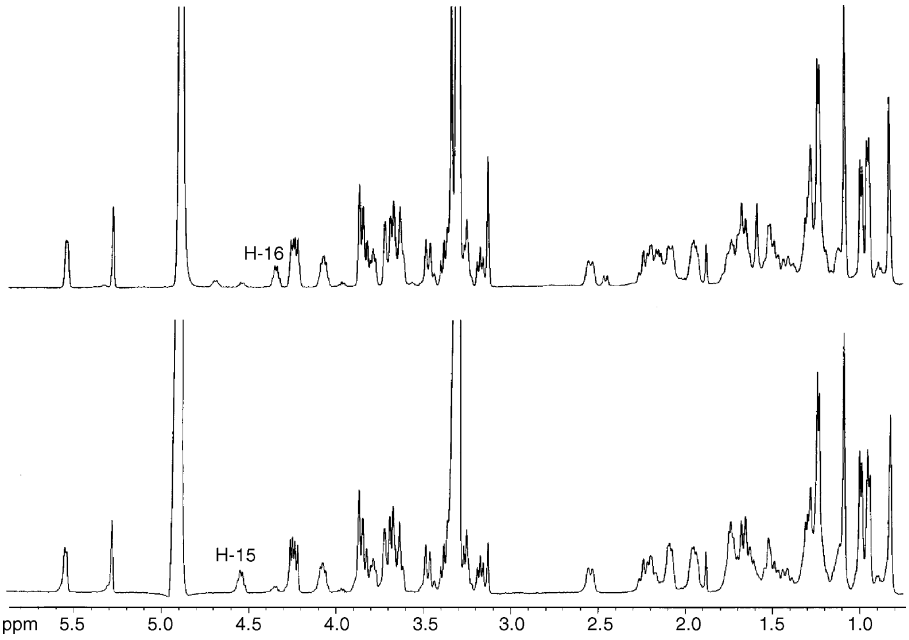

Fig. 12. ^{1}H NMR spectra (500 MHz, in CD_3OD) of ascalonicosides A1/A2 (25, Fig. 6). The ^{1}H NMR spectra of ascalonicoside A1 (the 22α-OH epimer) and of ascalonicoside A2 (the 22β-OH epimer) are placed at bottom and top of the picture, respectively.

is further increased in HSQC experiment, where the introduction of field gradients allows to reduce the solvent interference [115].

Due to the extreme structural complexity of saponin compounds, one of the most time-consuming parts of the work is the structural elucidation. The complexity of this approach is illustrated by the ^{1}H NMR spectrum of the trisaccharide saponin ascalonicoside A1/A2 (**25**, Fig. 6), reported in Fig. 12 [62,116]. The ^{1}H NMR spectra of ascalonicoside A1 (the 22α-OH epimer) and of ascalonicoside A2 (the 22β-OH epimer) are placed at bottom and top of the picture, respectively. The two spectra appeared almost superimposable except for the H-16 signal, which was strongly influenced by the change of configuration at C-22. In fact, ascalonicosides A1/A2 are hemiacetals at C-22 that equilibrate if left in solution overnight (40% of **25a** and 60% of **25b**).

To investigate these spectra, which show a severe overlapping of a great number of signals, 2D NMR techniques are applied on both native saponins (to elucidate the aglycone structure) and their peracetyl derivatives (used to infer the structure of the saccharide portion). Otherwise, it is also possible to determine all the chemical structure on the native saponin. In this case, it is necessary to determine (i) the aglycon structure, (ii) the number of sugar residues, (iii) the nature of each monosaccharide, including the configuration at the anomeric center, (iiii) the glycosilation sites and the interglycosidic linkages. To obtain these informations by 2D NMR approach, a concerted and systematic application of the techniques is necessary. To identify the aglycon and sugar nature, it is necessary to obtain the unambiguous assignment of the ^{1}H and ^{13}C resonances and the first task to accomplish this is to perform through-bond connectivity analysis in order to determine the number of spin systems. This will be obtained by TOCSY and HOHAHA experiments in which magnetization is transferred via ^{1}H–^{1}H J couplings. However, as the number of monosaccharides increases, the ^{1}H spectrum become more crowded and the signal overlap increase. Although many signals are overlapped, the anomeric protons, which resonate at lower fields, are easily recognizable in the spectra and can act as starting point to delineate each sugar spin system. In fact, in these experiments the signals of each spin system are clearly detected and can be identified. Once the proton resonances are assigned, attribution of the carbon resonances can be obtained by tracing the ^{1}H–^{13}C connectivities observed in HMQC or HSQC spectra. Usually, the anomeric configuration can be obtained by the vicinal coupling constant of the anomeric proton. Glycosilation sites are indicated by an accurate analysis of α, β, and γ effects determined by glycosilation, in ^{13}C NMR spectra of the aglycon and of each sugar unit. In this case the chemical shift have to be compared to reported values of steroidal saponins [117] and methyl glycosides [118]. An alternative method applied is the use of an HMBC experiment that indicate the ^{1}H–^{13}C ^{2,3}J couplings and thus can be useful to connect the spin system among them. With this experiment, glycosilation sites can be also easily determined by observing correlation peaks between the sugar anomeric proton and the carbon nuclei of the adjacent unit and/or the sugar anomeric carbon and the proton directly linked to the carbon involved in the glycosidic bond. Further confirmation of the glycosilation sites can be obtained by measuring the dipolar couplings of each anomeric proton with the linkage site proton by a ROESY spectrum, since nuclear Overhauser effects (nOe) trasversing the glycosilation linkage are invariably detected [119].

Considering that these molecules are isolated in relatively low amounts, 10 kg of fresh plant typically providing 5–10 mg of pure saponin, the use of an entirely spectroscopic approach,

mainly based on NMR analysis, proved successful in the definition of the structures because it, being non-destructive method, preserves the required amounts of natural products for pharmacological screening.

4. Conclusion

4.1. New perspectives in onion and garlic analysis

Onion and garlic, commonly used as foods, are among the oldest herbal remedies, prescribed as antimycotic, antibacterial, hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic drugs, both for prevention and for therapy. Responsible of their pungent taste and also of some of these bioactivities are volatile organosulfur compounds, named thiosulfinates. These compounds, characteristic for onion and garlic taxa, have been studied in detail and have shown to be chemically reactive. They easily give rise to several organosulfur compounds, mainly disulfides and trisulfides, which also exhibit pharmacological activities [1,15,18]. In addition, these plants also have been shown to contain polar compounds that have recently received an increasing scientific attention. In particular, many saponins [62,63] and flavonoids [90,94] have been characterized and tested for their interesting biological activities.

More recently, the research interest focused on antioxidant and anticancer activities of onion and garlic. In fact, oxidative modification of DNA, proteins and lipids by reactive oxygen species (ROS) have been recognized to play a role in aging and disease, including cardiovascular, neurodegenerative and inflammatory diseases and cancer [22,88]. Furthermore, antioxidant health effects also for aged garlic extracts have been reported by Borek [120]. Aged garlic extract (AGE) has been shown to exert its action by scavengng ROS, enhancing the cellular antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase, and inhibiting lipid peroxidation, thus protecting endothelial cells from the injury by the oxidized molecules, which contributes to arteriosclerosis. AGE inhibited the activation of the oxidant-induced transcription factor, nuclear factor (NF)-kB, which has clinical significance in human immunodeficiency virus gene expression and atherogenesis. AGE was also able to reduce the risk of cardiovascular disease, stroke, cancer and aging, including the oxidant-mediated brain cell damage that is implicated in Alzheimer's disease. Future studies, besides the traditional investigation approach, should focus on the relationship between chemical structure and activity (SAR) on the onion and garlic compounds [121,122], but also on clinical tests to evaluate the potential effects both of the crude extracts and of the isolated metabolites in human health.

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