# Distribution of *S*-alk(en)yl-L-cysteine Sulfoxides in Garlic (*Allium sativum* L.)

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**Abstract**: This study was devoted to distribution of *S*-alk(en)yl-L-cysteine sulfoxides (particularly alliin, methiin, and isoalliin) in plants of *Allium sativum* L. and to diversity of selected groups of garlic clones representing the three basic garlic morfotypes (i.e. flowering plants, scape absent and semi-bolters – this classification is based on IPGRI descriptors). The content of *S*-alk(en)yl-L-cysteine sulfoxides, important non-volatile precursors flavour-active compounds, was determined in four parts (roots, bulbs, false stems, and leaves) of six different clones of garlic (*Allium sativum* L.) plants collected during the whole of vegetation – in March, April, May, June, and July. The contents of target *S*-alk(en)yl-L-cysteine sulfoxides were dependent not only on part of garlic plants but also on morfotype of garlic plants and vegetation period.

**Keywords**: garlic; *Allium sativum*; *S*-alk(en)yl-L-cysteine sulfoxides; alliin; methiin; isoalliin

## INTRODUCTION

Garlic is a plant of the genus *Allium* (*Alliaceae*). It belongs to the oldest cultivated plants and is used as a vegetable and characteristic pungent flavouring agent for foods and medicinal applications. The intact garlic bulb contains unique sulphur amino acids, *S*-alk(en)yl-L-cysteine sulfoxides (ACSO). When the garlic is crushed or cut, the C-S lyase (alliin lyase, EC 4.4.1.4) is released and converts *S*-alk(en)yl-L-cysteine sulfoxides into thiosulphinates and other volatile sulfur compounds, which are responsible for the characteristic flavour of garlic and its antimicrobial and other beneficial properties. Garlic (*Allium sativum* L.) contain three main *S*-alk(en)yl-L-cysteine sulfoxides, namely *S*-2-propenyl-L-cysteine sulfoxide (alliin), *S*-methyl-L-cysteine sulfoxide (methiin) and *S*-1-propenyl-L-cysteine sulfoxides (isoalliin) (Kubec *et al.* 2000; Bloem *et al.* 2004; Ichikawa *et al.* 2006; Velíšek *et al.* 2006).

The genus *Allium* includes many different species and clones of garlic. The species most commonly cultivated belong to *Allium sativum* L. After thousands of years of cultivation and selection, *A. sativum* has lost the ability to produce fertile seeds. Some clones have even lost the ability to form flower stalks and flowers. This is presumably due to random mutations which have been selected over the years. In accordance with the ability to form inflorescences (classification based on IPGRI descriptors) garlic genotypes are possible separate into three basic morfotypes (i.e. flowering plants – F, scape absent – N, and semi-bolters – S) (IPGRI 2001).

Clones of flowering plant morfotype produce a flower stalk and are, therefore, also known as hard neck type. They are presumably closely related to wild garlic species. The hard flower stalk makes braiding difficult. Another drawback of flowering plant type is that they cannot be stored for longer periods because they form roots and start to dry out within a few months after harvest. Clones of semi-bolter and scape absent morfotype are subgroups of *Allium sativum* var. *sativum*. Clones of scape absent morfotype do not produce a flower stalk at all. Semi-bolter clones produce flower stalks, but not flowers. However, flower stalk formation can depend on climatic conditions. It is not under laid

by genetic changes and probably it is connected with gene expression or epigenetic changes. Clones of scape absent type never produce flower stalks at all. Clones of semi-bolter and scape absent morfotype exhibit a high degree of geographical diversity (IPGRI 2001).

The aim of this study was devoted to distribution of *S*-alk(en)yl-L-cysteine sulfoxides (particularly alliin, methiin, and isoalliin) in individual parts of garlic plants picked during vegetation and to diversity of selected groups of garlic clones representing the basic garlic morfotypes (flowering plants, scape absent and semi-bolters).

## MATERIAL AND METHODS

***Plant material.*** The distribution of *S*-containing amino acids was determined in six samples (two samples of each morfotypes: semi-bolters (S), scape absent (N), and flowering plants (F)) representing the garlic collection of Gene bank in the Czech Republic. The samples were collected six times during vegetation: first on March 4$^{th}$ (sampling I), second on April 5$^{th}$ (II), third on May 20$^{th}$ (III), fourth on June 2$^{nd}$ (IV), fifth on June 23$^{rd}$ (V) and sixth on July 7$^{th}$ (VI) by the Research Institute of Crop Production in Olomouc (Czech Republic).

Plants of each clones were divided into four portions: roots, bulbs, false scape and leaves (the clones of flowering plants collected in June were divided into roots, bulbs, false scape, leaves and stems), in July into roots, bulbs, false stems, leaves, stems and spathe with bulbils and flowers (flower)), and each portion was immediately analysed for alliin, methiin and isoalliin content.

***Chemicals and reagents.*** *S*-alk(en)yl-L-cysteines were synthesised by alkylating L-cysteine with the appropriate alk(en)yl halides. ACSOs were then synthesised by oxidising the *S*-alk(en)yl-L-cysteines with hydrogen peroxide (Kubec *et al.* 2000).

Buffers: sodium dihydrogen phosphate (7.8 g) was dissolved in 1000 ml of water and the resulting solution was adjusted to either pH 9.5 or 6.5 with sodium hydroxide. Buffers of pH 6.5 were diluted with water (1:9, v/v) and used as the mobile phase for HPLC.

Derivatisation reagent (OPA): *o*-phthaldehyde (140 mg) was dissolved in 5 ml of methanol and 0.1 ml of 2-methylpropane-2-thiol and 50 ml of phosphate buffer (pH 9.5) were added (Velíšek *et al.* 1993).

***Sample preparation.*** Plants of each clones were divided into separate portions and *S*-alk(en)yl-L-cysteine sulfoxides were extracted using boiling methanol to inactivate alliinase. Then norleucin was added as an internal standard and the mixture was homogenised using a laboratory blender. The homogenised sample was filtered through a 0.45 μm cellulose acetate HPLC syringe-tip filter (Alltech). An aliquot of the filtrate was derivatised using the OPA reagent and analysed by HPLC.

***High-performance liquid chromatography.*** The quantitative analysis was performed using an HPLC system composed of ConstaMetric pump system, Watrex, autosampler AS 100 Thermo Instrument system, an UV detector SpectroMonitor 320, Thermo Instrument System Inc., USA, Hypersil column, 5 μm (4.5 × 250 mm), ThermoQuest, Hypersil Division, UK.

The separation of the target analytes was done using a gradient HPLC employing a mobile phase composed of the phosphate buffer and methanol. The gradient elution started at 44% of methanol and was followed by raising the concentration of methanol linearly to 75% within 37 min. After maintaining these conditions for 2 min, the concentration of methanol was changed back to the initial one. The mobile phase flow rate was 0.8 ml/min and 20 μl of the derivatised sample were injected onto the column.

## RESULTS AND DISCUSSION

The average contents of target analytes, namely alliin, methiin and isoalliin, in the individual parts of garlic plants sampled during their vegetation are shown in Figures 1–3.

In plants collected in March, during first sampling at the start of vegetation, the highest content of alliin, the major *S*-alk(en)yl-L-cysteine sulfoxide in garlic, was in leaves of semi-bolters morfotype, the lowest in bulbs of semi-bolters morfotype. During the next two months (April and May) the content of alliin in garlic plants of morfotype F and S was decreased. In the next stage of vegetation, the increase of alliin content is constantly evident in the bulbs until the end of vegetation. By contrast in leaves, false stems and roost the content of alliin decreased (Figure 1). So, the highest contens of alliin were in bulbs sampling in July, just before harvest. From Figures 1 and 2 it is evident, that high contents of alliin and next target analyte

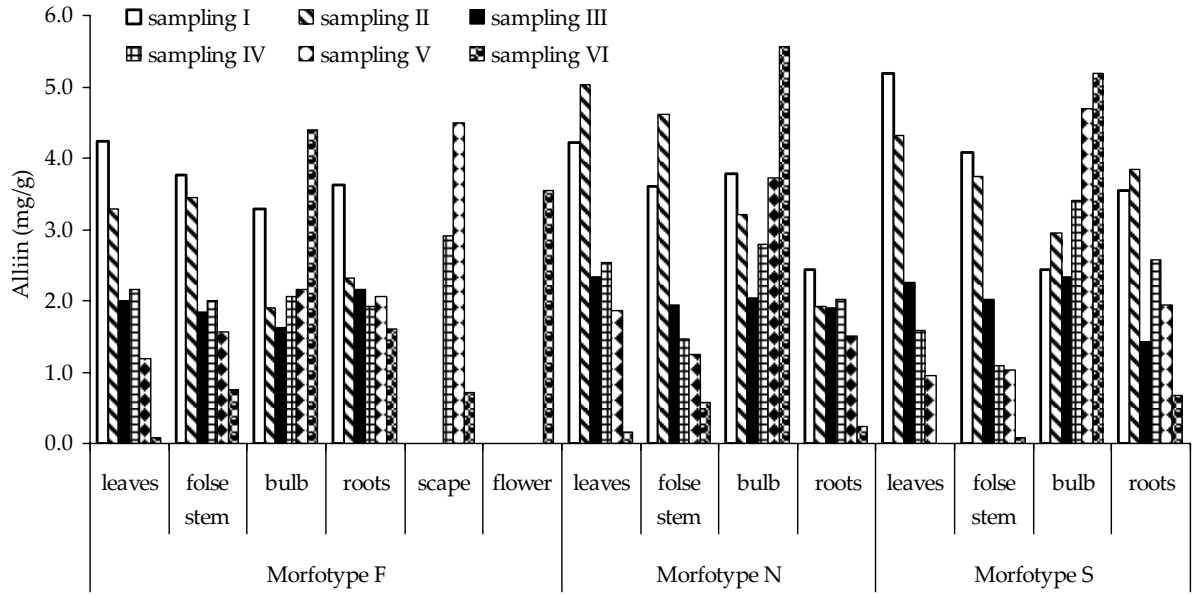


Figure 1. Influence of the vegetation period on the alliin content in parts of garlic plants

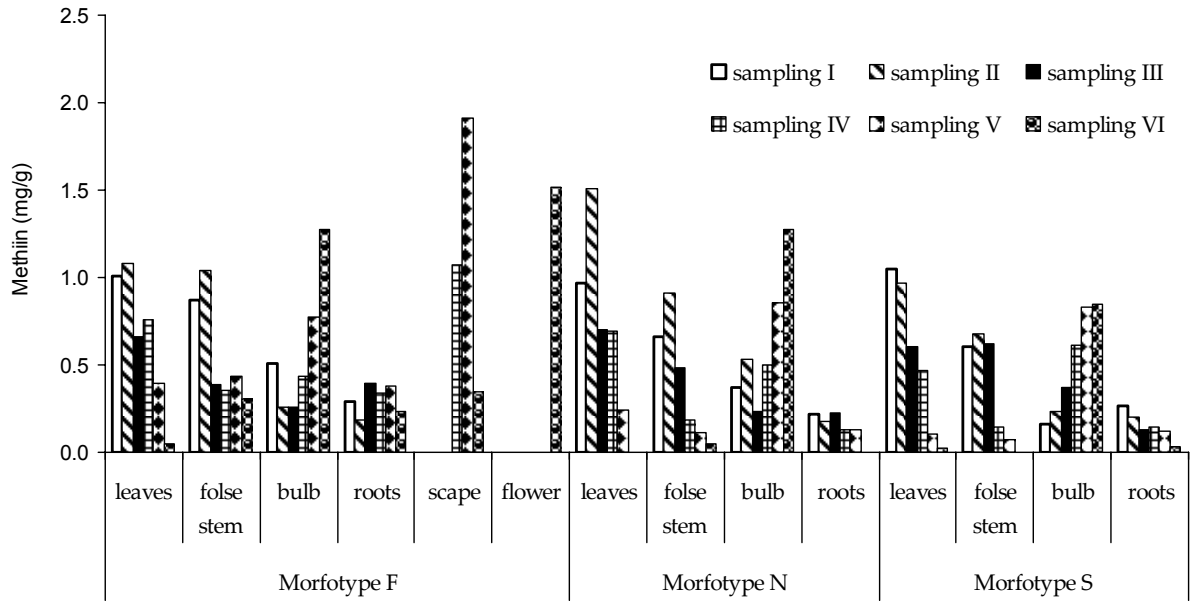


Figure 2. Influence of the vegetation period on the methiin content in parts of garlic plants

methiin were, at the end of vegetation, also in scape and spathe with bulbils and flowers. Also, changes of contents of methiin in the next analysed parts of garlic plants are similar to changes of alliin contents. In leaves and false stems contents of methiin decreased contrary to the content in bulbs, the higher methiin concentration in bulbs was found at the end of vegetation, in July.

The higher contents of isoalliin were in roots, namely in roots collected in March. However after that, the content of isoalliin was decreased. Most significant decrease of isoalliin concentration was at the end of vegetation season of garlic growth.

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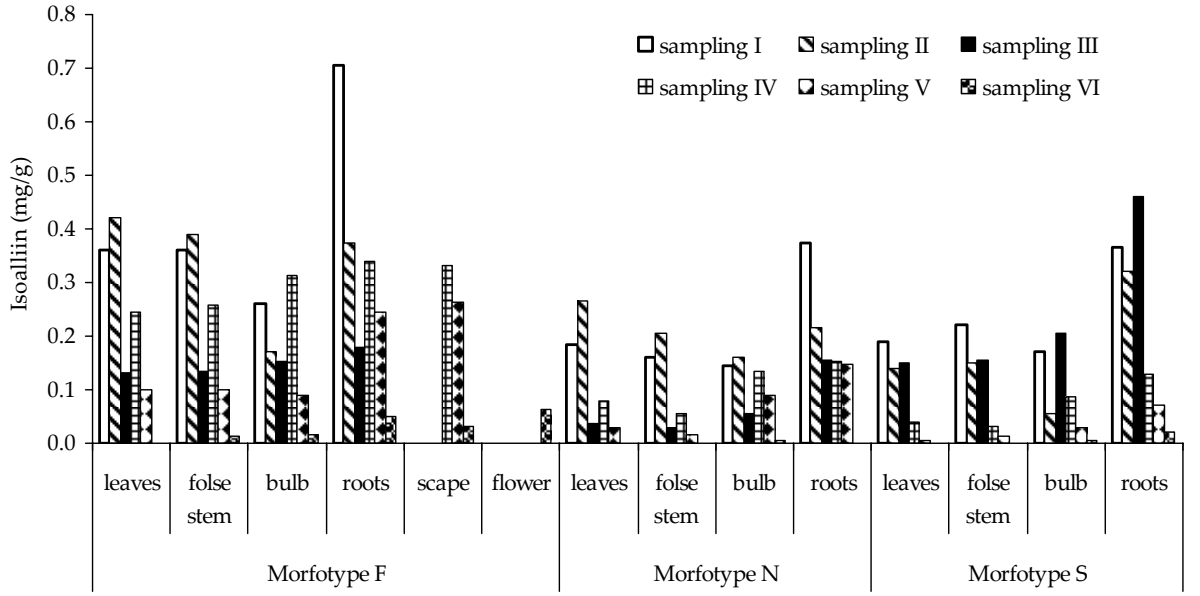


Figure 3. Influence of the vegetation period on the isoalliin content in parts of garlic plants

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