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 rep-
resenting 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 4th (sampling I),
second on April 5th (II), third on May 20th (III),
fourth on June 2nd (IV), fifth on June 23rd (V) and
sixth on July 7th (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 (Velišek
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 In-
strument system, an UV detector SpectroMonitor
320, Thermo Instrument System Inc., USA, Hyper-
sil column, 5 µ (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. Af-
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
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 halides. ACSOs were then
synthesised by oxidising the S-alk(en)yl-l-cysteines
with hydrogen peroxide (Kubec et al. 2000).

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et al. 1993).
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

References


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