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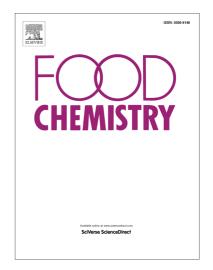
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ACCEPTED MANUSCRIPT

Alliinase and cysteine synthase transcription in developing garlic (Allium sativum L.) over time

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Abstract

Garlic is a valuable source of healthy compounds, including secondary metabolites rich in sulphur such as cysteine sulphoxides (CSOs). Here, we present new qRT-PCR assays analysing the transcription of two genes encoding key enzymes in CSO biosynthetic pathways (cysteine synthase and alliinase) in developing garlic. we also identified a set of genes (ACT I, GAPDH, and TUB) to use as transcription normalisation controls. We showed that the (normalised) transcription of both enzymes was highest during sprouting and decreased significantly in fully developed leaves, which are the major CSO-producing organs. Transcriptional activity further declined at the end of the growing season. different cultivars show similar sulphur metabolism gene expression when European garlics were compared to Chinese and American genotypes. The qRT-PCR assays presented are also suitable for investigating the effects of agricultural practices on CSO formation in garlic to satisfy consumer demands.

Key words: garlic, *Allium sativum* L., transcription profiling, normalisation, alliinase, cysteine synthase, housekeeping genes

Abbreviations:

18S - 18S ribosomal RNA

ACT - Actin

CSO - Cysteine sulphoxide

CSase - Cysteine synthase: O3-acetyl-L-serine: hydrogen-sulphide 2-amino-2-carboxyethyl transferase

GAPD - Glyceraldehyde-3-phosphate dehydrogenase

TUB - β-Tubulin

UBQ - Polyubiquitin

qRT-PCR - Quantitative reverse transcription PCR

1 Introduction

Garlic (*Allium sativum* L.) is a vegetatively propagated species that has been used for centuries as a vegetable and natural remedy. Garlic is a valuable source of healthy substances (Corzo-Martínez, Corzo & Villamiel, 2007), including secondary metabolites rich in sulphur, such as *S*-allyl CSO (ACSO, alliin; characteristic of garlic), *S*-methyl CSO (MCSO, methiin; present in most *Alliums*), *S*-trans-prop-1-enyl CSO (PECSO, isoalliin; characteristic of onion but also present in garlic), and *S*-propyl CSO (PCSO, propiin; present in onion and related

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