Food Chemistry 215 (2017) 493–500

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

# Development of garlic bioactive compounds analytical methodology based on liquid phase microextraction using response surface design. Implications for dual analysis: Cooked and biological fluids samples

Daniela Andrea Ramirez [a,c], Daniela Ana Locatelli [a,b,e], Carolina Andrea Torres-Palazzolo [a,b,e], Jorgelina Cecilia Altamirano [c,d], Alejandra Beatriz Camargo [a,b,c,e,*]

[a] Laboratorio de Cromatografía para Agroalimentos (LabCrom) – Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina
[b] Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Mendoza, Argentina
[c] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Parque General San Martín, Mendoza, Argentina
[d] Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA)-CONICET, Mendoza, Argentina
[e] Instituto de Biología Agrícola de Mendoza (IBAM)-CONICET, Mendoza, Argentina

ARTICLE INFO

*Article history:*
Received 24 March 2016
Received in revised form 27 July 2016
Accepted 28 July 2016
Available online 29 July 2016

*Keywords:*
DLLME
Organosulphur compounds
Garlic
Biological fluids
HPLC

ABSTRACT

Organosulphur compounds (OSCs) present in garlic (*Allium sativum* L.) are responsible of several biological properties. Functional foods researches indicate the importance of quantifying these compounds in food matrices and biological fluids. For this purpose, this paper introduces a novel methodology based on dispersive liquid-liquid microextraction (DLLME) coupled to high performance liquid chromatography with ultraviolet detector (HPLC-UV) for the extraction and determination of organosulphur compounds in different matrices. The target analytes were allicin, (E)- and (Z)-ajoene, 2-vinyl-4H-1,2-dithiin (2-VD), diallyl sulphide (DAS) and diallyl disulphide (DADS). The microextraction technique was optimized using an experimental design, and the analytical performance was evaluated under optimum conditions. The desirability function presented an optimal value for 600 μL of chloroform as extraction solvent using acetonitrile as dispersant. The method proved to be reliable, precise and accurate. It was successfully applied to determine OSCs in cooked garlic samples as well as blood plasma and digestive fluids.


## 1. Introduction

Therapeutic properties of garlic (*Allium sativum* L.) have been widely known and employed since ancient times. The consumption of these vegetables has been associated with the prevention of chronic diseases, mainly due to their immunological and cardiovascular functions. It has also been associated with the prevention of carcinogenic diseases. Moreover, garlic has been reported to possesses antioxidant, antithrombotic, antibacterial, antifungal and antiviral activities (Kamel & Saleh, 2000; Kwiecien, Iciek, & Wlodek, 2009). There is evidence from several investigations that suggests that the biological and medical functions of garlic are primarily attributed to its high organosulphur compounds (OSCs) content (Corzo-Martinez, Corzo, & Villamiel, 2007).

During disruption of garlic tissue, such as cutting, crushing or chewing the vacuolar enzyme alliinase, rapidly lyses the cytosolic cysteine sulphoxides (mainly alliin) to form thiosulfinates (TS) (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001). Allicin is the predominant, and represents 70–80% of the total TS present in garlic. All of these compounds are reactive molecules and decompose to form OSCs like diallyl sulphide (DAS), diallyl disulphide (DADS) and diallyl trisulphide (DAT), vinyldithiins (E)- and (Z)- ajoene, among others (Lawson, 1992; Yu, Wu, & Liou, 1989).

Since OSCs are responsible of different biological activities, the precise determination of their quali-quantitative levels is of paramount interest. Nevertheless, nowadays, the mere presence of these compounds in food matrices is not enough to guarantee that biological properties would be verified. For these reasons, researches related to functional foods suggest the importance to develop robust analytical methodologies, not only for the analysis of food preparations but also for biological fluids.

In a previous work of our research group (Locatelli, Altamirano, Luco, Norlin, & Camargo, 2014), it was demonstrated the inconvenience to employ gas chromatography (GC) separation technique for OSCs analysis, due to its thermal instability, and it was recommended the use of High Performance Liquid Chromatography

* Corresponding author at: Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Alte. Brown 500, 5505 Chacras de Coria, Mendoza, Argentina.
*E-mail address:* acamargo@fca.uncu.edu.ar (A.B. Camargo).

http://dx.doi.org/10.1016/j.foodchem.2016.07.170
0308-8146/

(HPLC). In addition, this work proposed a Solid Phase Microextraction (SPME) technique, to avoid artifacts formation (analytes not present in the original sample product of undesired reactions) during analytes extraction. Although this methodology showed satisfactory results, we decided to develop an alternative methodology to be in line with new trends.

It is also important to note that although there are literature referred to the determination of these compounds, they were mainly focused on OSCs quantification in fresh garlic and galenic preparations (Bocchini, Andalò, Pozzi, Galletti, & Antonelli, 2001; Ichikawa, Nagatoshi, & Yoshida, 2006; Kamel & Saleh, 2000; Nikolic & Stankovic, 2001; Yoo, Lee, Lee, Seog, & Shin, 2010). Moreover, to the authors' best knowledge, regarding biological fluids very few publications can be found, and they have only determined some of the analytes studied in this work (Lawson, 1993; Rosen et al., 2000). However, validated methodologies that quantify OSCs in biological fluids are still lacking.

Currently, there is a tendency to apply greener and miniaturized extraction techniques. The literature on liquid phase microextraction, shows a variety of approaches, of which we can mention: single-drop microextraction (SDME), continuous-flow microextraction (CFME), dispersive liquid-liquid micro extraction (DLLME), directly-suspended droplet microextraction (DSDME) and hollow-fiber liquid phase microextraction (HF-LPME) (Sarafraz-Yazdi & Amiri, 2010). Accordingly, in terms of organosulphur compounds it is possible to further improve the efficiency of the analysis by employing a liquid microextraction. With this goal we chose a dispersive liquid-liquid microextraction in order to upgrade the sample preparation stage. This technique uses microliters volumes of an organic solvent as the extractant along with few milliliters of a dispersive solvent (Rezaee et al., 2006). Thus, hydrophobic solutes are enriched in the extraction solvent, which is dispersed into the bulk aqueous solution. In turn, abundant studies can be mentioned in which this technique was applied to extract organic compounds from food samples, showing their suitability of application (Bidari, Ganjali, Norouzi, Hosseini, & Assadi, 2011; Viñas, Campillo, López-García, & Hernández-Córdoba, 2014; Wu et al., 2013). The main advantages of DLLME are simplicity of operation, low cost, high recovery and specially, that involve environmental friendly analytical procedures (Sarafraz-Yazdi & Amiri, 2010).

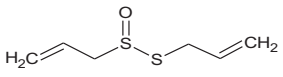
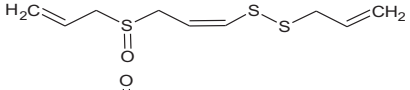
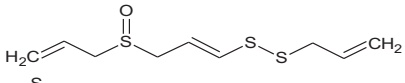
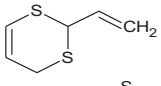
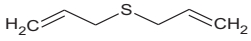
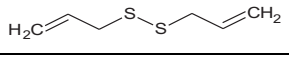
Consequently with the above mentioned, since the methodologies reported for OSCs determination have not shown a modernization, especially at sample preparation stage, and also no precedents can be found related to OSCs validated methodologies in complex matrices, the present work proposes a novel methodology using DLLME for the extraction and preconcentration of OSCs in garlic samples prior to their determination by HPLC-UV, and then evaluate its application in different matrices such as cooked samples and biological fluids (digestive fluids and blood plasma). The names and structures of the target analytes are summarized in Table 1. The optimum extraction conditions were obtained employing a response surface experimental design, and then analytical figures of merit were calculated under optimum conditions. The proposed method was successfully applied to determine the OSCs content from different matrices, therefore achieving the verification of the method suitability. Finally, it was concluded that the present methodology provides an efficient way to extract and purify these compounds from different complex matrices, and also the formation of "artifacts" is avoided. Therefore, the development of this methodology represents a reliable tool for use in bioaccessibility and bioavailability studies, thus enabling respond queries related to the release of the OSCs from the food matrix and their subsequent availability in the body to exert its beneficial effect.

## 2. Experimental

### 2.1. Reagents and analytical standards

DAS (97%) and DADS (80%) were purchased from Sigma Aldrich (Buenos Aires, Argentina). Acetonitrile (ACN), methanol (MeOH), acetone, hexane, isopropanol, chloroform and dichloromethane (DCM) were chromatography grade purchased from Merck (USA). Ultrapure water (18 MΩcm) was obtained from a Milli-Q water purification system (Millipore, France). Allicin was synthesized by oxidation of diallyl disulphide DADS with hydrogen peroxide following the previously reported by the group (González, Camargo, & Burba, 2007). To obtain E-Z Ajoene isomers, synthetized allicin was heated while stirring in acetone/water (40:60 v/v) (Block, Ahmad, Catalfamo, Jain, & Apitz-castrozd, 1986). Vinyldithiin compounds were synthesized by heating allicin in acetone/methanol (60:40 v/v) following the procedure described by Iberl et al. (Iberl, Winkler, & Knobloch, 1990); with slight modifications. The synthetized OSCs were further isolated by fractions collection after HPLC separation. Allicin and E- and Z-ajoene were purified using a normal phase Waters Spherisorb S5W HPLC column and hexane/isopropanol (92:8 v/v) as mobile phase. 2-vinyldithiin (2-VD) was purified by reverse phase-HPLC using the chromatographic conditions described in "Operating conditions" section of this work. The 3-VD purification was not successful, therefore was not included within the validation study. Then, the

**Table 1**
Chemical structures of the target OSCs.

| Name | Abbreviation | Chemical structure |
|---|---|---|
| Diallyl thiosulfinate | Allicin |  |
| *trans*-Ajoene | Z-ajoene |  |
| *cis*-Ajoene | E-ajoene |  |
| 2-Vinyl-4H-1,3-dithiin | 2-VD |  |
| Diallyl sulphide | DAS |  |
| Diallyl disulphide | DADS |  |