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Home-cooked garlic remains a healthy food

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ABSTRACT

Numerous studies have demonstrated that garlic has many biological properties due to its phytochemicals. These components include organosulphur compounds (OSCs) such as allicin, which is a chemically unstable metabolite. The aim of this study was to evaluate whether garlic could still be considered a healthy food after home cooking procedures. For that purpose, an experimental design with two factors and three levels was used. Pre-cooking and cooking procedures were the selected factors. Allicin, ajoenes, 2-vinyl-4H-1,3-dithiin (2-VD), diallyl sulphide (DAS), diallyl disulphide (DADS) and diallyl trisulphide (DATS) were the target analytes. Samples were analyzed by high performance liquid chromatography coupled to ultraviolet detector (HPLC-UV). The results showed that it was possible to find OSCs with important biological activities after all pre-cooking and cooking treatments. This is the first study to our knowledge to investigate cooked garlic using an analytical methodology, which avoid artifacts formation.

1. Introduction

Garlic (*Allium sativum* L.) has been used as an ingredient, spices and flavouring since ancient times. Several studies have shown that garlic has numerous biological properties, (Fujisawa, Suma, Origuchi, Seki, & Ariga, 2008; Iciek, Kwiecien, & Wlodek, 2009; Lee, Lee, Kim, Rhee, & Pyo, 2015; Santhosha, Jamuna, & Prabhavathi, 2013) due to the presence of phytochemicals, which make garlic a healthy food. These compounds include organosulphur compounds (OSCs) that are synthesized using sulphate absorbed by roots as a source of sulphur. From that, the first OSC is formed in the plant, until the garlic bulb is ready for consumption; the OSCs undergo biological transformations resulting in different products (Block, 2010). The first group of OSCs that is generated when garlic tissue is broken are the thiosulphinates, allicin being the most abundantly found in fresh garlic. These compounds are responsible of the characteristic garlic pungency. The most stable among the OSCs are the polysulphides, which are the last set of transformation compounds. Within the group of OSCs with important biological activities diallyl sulphide (DAS), diallyl disulphide (DADS), diallyl trisulphide (DATS), ajoenes and vinyldithiins may be mentioned (Table 1). These compounds can occur upon cooking processes, distillation, storage of garlic or in aged garlic extracts (Kamel & Saleh, 2000; Kim, Wu, Kobayashi, Kubota, &

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Chemical compounds: Allyl sulphide (PubChem CID: 11617); Diallyl disulphide (PubChem CID: 16590); Diallyl trisulphide (PubChem CID: 16315); Allicin (PubChem CID: 65036); E-Ajoene (PubChem CID: 5386591); Z-Ajoene (PubChem CID: 9881148); Vinildithiin (Pub Chem CID: 54113692).

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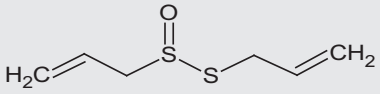
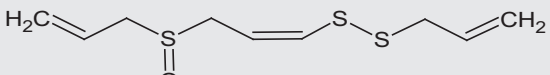
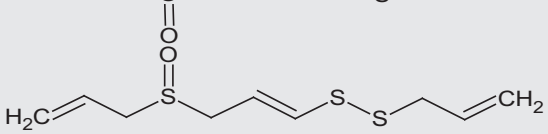
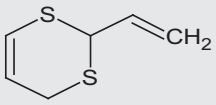
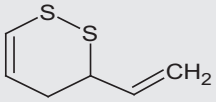
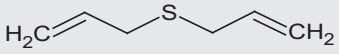
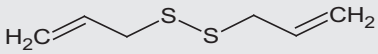
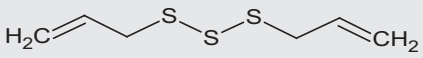
1756-4646/

Table 1 – Target organosulphur compounds.

Compound	Chemical structure
Diallyl thiosulphinate (Allicin)	
Trans-ajoene (Z-ajoene)	
Cis-ajoene (E-ajoene)	
2-vinyl-4H-1,3-dithiin (2-VD)	
3-vinyl-4H-1,2-dithiin (3-VD)	
Diallyl sulphide (DAS)	
Diallyl disulphide (DADS)	
Diallyl trisulphide (DATS)	

Okumura, 1995; Weinberg, Manier, Richardson, & Haibach, 1993; Yu, Lin, & Ho, 1994; Yu, Wu, & Ho, 1993) leading to different OSCs profiles. Sensory as well as biological or functional characteristics have been determined for OSCs in each garlic preparations (Amagase, Petesch, Matsuura, Kasuga, & Itakura, 2001; Fujisawa et al., 2008; Liang et al., 2015). Moreover, it has been reported that cooking processes induce changes in the chemical composition, which influence the concentration and bioavailability of bioactive compounds in vegetables (Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009). However, OSCs levels in garlic samples under different pre-cooking or cooking treatments have received little attention. There are few reports regarding the analysis of these compounds in cooked garlic samples (Cavagnaro, Camargo, Galmarini, & Simon, 2007; Kim, Wu, Kobayashi, et al., 1995; Kim, Wu, Kubota, & Kobayashil, 1995; Yu & Wu, 1994; Yu et al., 1993). Works reported have been based on the analysis of OSCs by GC-MS (Artacho Martin-Lagos, Olea Serrano, & Ruiz Lopez, 1995; Tocmo, Lin, & Huang, 2014; Yan, Wang, & Barlow, 1992, 1993), using Lickens–Nickerson distillation and solvent extraction techniques (Kim, Wu, Kobayashi, et al., 1995; Kim, Wu, Kubota, et al., 1995; Yu et al., 1993, 1994; Yu & Wu, 1994; Yu, Wu, & Chen, 1989; Yu, Wu, & Liou, 1989).

It is worth emphasizing that those sample preparation techniques, as well as the subsequent analysis by GC, are inadvisable (Block, Putman, & Zhao, 1992). Consequently, at present the data available about the OSCs profiles in cooked garlic samples are controversial. In a previous work we demonstrated that OSCs should be analyzed by using techniques to avoid artifacts formation along the analysis due to their thermolability (Locatelli, Altamirano, Luco, Norlin, & Camargo, 2014).

The aim of this work was to determinate the phytochemical profile of garlic after home-cooking and to evaluate whether cooked garlic could still be considered as a healthy food. An experimental design with two factors (pre-cooking and cooking treatments) and three levels each was used. The OSCs profile of all samples was determined by using an HPLC technique. This is the first time that the OSCs profiles in home-cooked garlic samples were studied taking into account an analytical methodology in order to avoid artifacts formation.

2. Materials and methods

2.1. *Plant materials*

Red garlic clone "Rubi" from the germplasm collection of Instituto Nacional de Tecnología Agropecuaria (INTA) La Consulta, Mendoza, was used in all the experiments. Garlic was grown at INTA's experimental field located in La Consulta, Mendoza, Argentina (33°44′ S, 69° 07′ W) in 2013. Bulbs were harvested when leaves were senesced and then were fully cured. During postharvest the bulbs were stored at ambient condition in sheds for 2 months.

2.2. *Experimental design*

Multifactorial experimental design combining two factors and three levels was used. The selected factors were: pre-cooking and cooking treatments. For the first factor, the levels were: whole (uncrushed) (WG), sliced (SG) and chopped garlic cloves (ChG). For the second the levels were the cooking treatments: simmering (HS), rolling boil (RB) and stir-frying (SF); using raw garlic (R) as control (Barham et al., 2010).

2.3. Sample preparation

Four kilograms of cloves from several bulbs were pooled and peeled. Sub-samples of 150 g were prepared by treatments mentioned above including pre-cooking and cooking treatments. Pre-cooking treatment involve different disruption degree of tissues: whole garlic (WG) (uncrushed cloves), sliced (SG) (garlic cloves were cut into pieces of around 2 mm thickness), and chopped (ChG) (using a garlic press). These samples were kept standing for 15 min in order to promote allicin formation prior to cooking treatments.

Samples were cooked in a gas-stove (Longvie 2600) until they reach the end point for each preparation. ChG, SG and WG were cooked by simmering, rolling boil and stir-frying methods. The final time for each treatment was considered when samples reached the colour and texture of home-cooked products (Table 2). An informal testing panel, which includes three trained panellist, determined it.

2.3.1. Simmering (HS)

Simmering is a way to boil water with a minimum heat input, just enough to ensure the boiling point of water (Barham et al., 2010). A water aliquot of 600 mL was added to an aluminium pot and was covered with a lid. It was low fire-heated until the water reached its boiling point. The samples were immersed in the boiling water and cooked within the time required to get its end point (previously tested for each pre-cooking treatment). Afterward, both simmer cooked garlic as well as the cooking water were separated and preserved for subsequent analysis. It is described in the "Sample Conditioning" section of this work.

2.3.2. Rolling boil (RB)

Rolling boil is a way to boil water with a high heat input, indicating the large-scale movements in the water (Barham et al., 2010). A water aliquot of 600 mL was added to an aluminium pot and was covered with a lid. It was high fire-heated until the water reached its boiling point. The samples were immersed in the boiling water and cooked within the time required to reach the cooking end point. Afterward, both garlic cooked in rolling boil as well as the cooking water were separated and preserved for subsequent analysis. It is described in the "Sample Conditioning" section of this work.

2.3.3. Stir-frying (SF)

A standardized volume of sunflower oil (50 g) was added to a pan and fire-heated until the oil temperature reached 180 °C. The samples WG, SG and ChG were put into the pan and cooked within the time required to reach the cooking end point for each treatment. Subsequently, stir-fried garlic was removed and the vegetable oil was collected and conditioned for preservation.

Table 2 – Cooking treatments and times to cook.

Pre-cooking treatments	Cooking methods		
	Simmering (HS)	Rolling boil (RB)	Stir-frying (SF)
	Time [min]		
Whole cloves (uncrushed) (WG)	30	10	2
Sliced garlic (SG)	15	6	2
Chopped garlic (ChG)	15	6	1

2.3.4. Sample conditioning

Garlic samples were frozen in liquid nitrogen, and freeze-dried at –58 °C for 72 h in a vacuum (Freeze Dry Systems LabConco Model Freezone 2.5, Kansas, MO, USA). The resulting lyophilized material was ground by using a mortar and stored at –80 °C. Additionally, cooking mediums (water (W) and vegetable oil (O)) were also preserved in a closed flask in the dark at –18 °C for preservation until analysis.

All the treatments were carried out by triplicate.

2.4. Analytical standards

DAS (97%) and DADS (80%) were purchased from Sigma Aldrich (Buenos Aires, Argentina). DATS (98%) was purchased from LKT Laboratories, Inc (St. Paul, MN, USA). Acetonitrile (ACN), methanol (MeOH), acetone, hexane, isopropanol and dichloromethane (DCM) were chromatography grade purchased from Merck (Kenilworth, NJ, USA). Ultrapure water (18 MΩcm) was obtained from a Milli-Q water purification system (Millipore, Molsheim, France). Allicin was synthesized by oxidation of diallyl disulphide DADS with hydrogen peroxide following a previously reported paper by the group (González, Camargo, & Burba, 2007). To obtain E-Z Ajoene isomers, synthesized allicin was heated while stirring in acetone/water (40:60, v/v) (Block, Ahmad, Catalfamo, Jain, & Apitz-Castro, 1986; Locatelli et al., 2014; Soto, Camargo, González, & Galmarini, 2007). Vinyldithiin compounds were synthesized by heating allicin in acetone/methanol (60:40, v/v) following the procedure described by Iberl, Winkler, and Knobloch (1990); with slight modifications in temperature (84 °C) and time (3.5 h).

2.5. Extraction of OSCs

One (1) gram lyophilized garlic powder was reconstituted with 30 mL distilled water. Then, it was extracted with 50 mL dichloromethane three times. The organic phases of each extraction were combined and concentrated almost to dryness by using a rotary evaporator (DecalabFbr®, Buenos Aires, Argentina). The remaining extract (ca. 500 μL) was reconstituted in acetonitrile reaching a final volume of 2 mL. Then, it was filtered and analyzed by HPLC.

2.6. Analysis of OSCs

The analysis was carried out by HPLC Konik KNK 500-series, UV/Vis detector (Konik, Barcelona, Spain). The HPLC column used was Waters C_{18} column (254 × 4.6 mm id; 5 μm particle size) (Milford, MA, USA). HPLC data were processed by EZChrom Chromatography Data System Version 6.8 software. Operating conditions were adapted from those previously reported by Iberl et al. (1990) as follows: isocratic elution at 1 mL min^{-1} using ACN/water/MeOH (50:41:9, v/v/v) mobile phase; and a wave length of 254 nm for detection. Peak identification in samples was carried out by comparing retention times with reference standards.

2.7. Statistical analysis

All data were expressed as mean ± standard deviation (SD). The data were analyzed by ANOVA using the software INFOSTAT. Mean of each treatment group were compared by Tukey test. P-values < 0.05 were considered to be significant.

3. Results and discussion

3.1. OSCs profile changes

The data were analyzed by Factorial ANOVA (Supplementary Table S1). The ANOVA revealed significant differences among OSCs profiles. Interaction effects were observed between the factors: pre-cooking and cooking treatments for all analytes considered ($P < 0.05$). Nevertheless, for ajoene levels, pre-cooking treatments resulted the determining factor of variations (41.3 %), while cooking treatments influenced significantly only on DADS and DATS levels (58.4 and 58.2%, respectively).

Significant variations on allicin levels were observed for all pre-cooking and cooking garlic treatments ($P < 0.05$) (Table 3). However, there were no significant differences in allicin levels for the stir-frying treatment and for uncrushed garlic simmering. This can be explained considering mainly two aspects. One of them is the thermolability of allicin to high temperatures, and the other is the presence of non-polar solvents like vegetable oil, which favours allicin decomposition to other OSCs (Block, 1985; Fujisawa et al., 2008; Iberl et al., 1990; Ilić et al., 2012). The allicin levels ranged between 48.7 ± 5.3 and 1574.6 ± 23.9 μg g^{-1}. High levels of allicin were observed in sliced and chopped garlic samples cooked by rolling boil. Table 3 shows that for these treatments, high allicin levels were also detectable in the cooking water samples. Based on these results it might be reasonable to assume that the pre-cooking treatments determined the levels of allicin found.

Regarding difference in allicin levels between simmering and rolling boil, the results are in agreement with those previously described by Cavagnaro et al. (2007). They reported that allicin levels varied with the time and temperature of cooking.

Concerning ajoene, it is possible to observe that its level increased proportionally with the degree of disruption of garlic tissues, temperature in the different cooking treatments and the polarity of the cooking medium. The highest amounts of ajoene were detected in chopped garlic under stir-frying conditions, while the lowest levels of ajoene were observed in sliced garlic cooked by simmering.

Another important aspect to highlight was the relationship between the levels of ajoene in garlic tissues and cooking mediums (water or vegetable oil). The ajoene levels in cooking water were lower than in the corresponding tissues. Instead, when the cooking medium was vegetable oil this relationship resulted favourably for the less polar medium.

The 2-VD showed a behaviour similar to the one previously described for ajoene. The highest amounts of 2-VD were detected in stir-fried chopped garlic. It is noteworthy that for all the OSC studied, the analyte found in the highest concentrations was the 2-VD in the vegetable oil of fried chopped garlic.

DAS was only detected in chopped garlic cooked by simmering and stir-frying and it could not be detected in any cooking mediums. This is the polysulphide with the highest vapour pressure among those studied in this work. Therefore, the observed result could be associated with evaporation losses.

DADS variability ($P < 0.05$) could be related to cooking treatment but not to the degree of disruptions of garlic tissues. The levels found ranged between 4.8 ± 0.3 and 92.7 ± 2.7 μg g^{-1}. Chopped garlic cooked by stir-frying presented the highest amount of this compound. With respect to cooking mediums, DADS was the only detected compound in cooking water of rolling boil treatment for chopped garlic, and in vegetable oils of chopped and sliced garlic cooked by stir-frying.

Significant differences in DATS levels were observed for all pre-cooking and cooking treatments. The highest levels were detected in chopped garlic cooked by stir-frying. This OSC was absent in the whole garlic cooked by simmering and rolling boil. DATS was quantified in water and vegetable oil during the strongest heat treatments (rolling boil and stir-frying). It has been previously reported that thiosulphinates found in garlic are very unstable and could be transformed to polysulphides in water (Yu et al., 1993). It was found that the transformation rate of these thiosulphinates to polysulphides is proportional to the cooking temperature increase. Our results are in agreement with this observation since the highest levels of polysulphides were found in samples under the strongest heat cooking treatments. In the case of whole garlic, DATS was only present in garlic tissues cooked by stir-frying but not in vegetable oil. Previous studies have reported that polysulphides are also formed from flavour precursors, this fact could explain our findings (Yu et al., 1994).

3.2. Pre-cooking and cooking treatments

Table 4 shows the OSCs concentrations expressed in μmol g^{-1}. It can be observed that allicin levels detected in raw garlic evidenced significant differences for all the pre-cooking treatments. Chopped garlic presented the highest allicin concentration (29.26 μmol g^{-1}) followed by sliced garlic (14.97 μmol g^{-1}). These levels were determinant on the levels of the other OSCs formed during cooking treatments.

The results suggest that chopped garlic was the treatment that elicited the highest qualitative and quantitative OSCs levels for all cooking treatments. This fact could be a consequence of the highest allicin levels present in raw garlic. In the same way, as can be seen from the results, stir-frying is the best cooking treatment because it gave rise to the formation of significant quantities of bioactive compounds.

Based on this approach the best combination treatments to obtain the highest bioactive compound levels were SFChG followed by RBChG.

3.3. Principal component analysis (PCA)

Fig. 1 (scores and loading plot) shows the outcomes of PCA for garlic samples cooked by different methods. The first two principal components (PC1and PC2) account for 69.0% of the total variance of the data. They were chosen because these eigenvalues were higher than 1, and therefore, they explain better the data variance than the original variables, and are distributed

Table 3 – OSCs levels in different pre-cooking treatments: Chopped garlic (ChG), Sliced garlic (SG), Whole cloves (WG). Cooking treatments: simmering (HS), rolling boil (RB) and stir-frying (SF).

Garlic samples	OSCs levels [μg g^{-1}][1]											
	Allicin		E-Z-ajoene		2VD		DAS		DADS		DATS	
		Total		Total		Total		Total		Total		Total
HS-ChG	15.6 ± 0.3^{b}	274.3 ± 5.9^{b}	26.0 ± 3.1^{c}	41.6 ± 3.3^{b}	8.5 ± 2.2^{b}	31.2 ± 2.6^{b}	9.2 ± 3.4	9.2 ± 3.4	4.8 ± 0.3^{a}	4.8 ± 0.3^{a}	222.7 ± 8.2^{b}	222.7 ± 8.2^{b}
W-HS-ChG*	258.6 ± 6.0^{d}		15.6 ± 0.5^{b}		22.7 ± 0.7^{d}		nd[2]		nd[2]		nd[2]	
HS-SG	5.8 ± 0.2^{a}	48.7 ± 5.3^{a}	1.0 ± 0.1^{a}	2.6 ± 0.8^{a}	5.0 ± 1.1^{a}	5.0 ± 1.1^{a}	nd[2]	nd[2]	5.5 ± 0.2^{b}	5.5 ± 0.2b	95.9 ± 4.0^{c}	95.9 ± 4.0^{a}
W-HS-SG*	42.9 ± 5.2^{c}		1.6 ± 0.8^{a}		nd[2]		nd[2]		nd[2]		nd[2]	
HS-WG	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]
W-HS-WG*	nd[2]		nd[2]		nd[2]		nd[2]		nd[2]		nd[2]	
RB-ChG	166.2 ± 3.1^{d}	1574.6 ± 23.9^{c}	96.6 ± 4.6^{d}	142.8 ± 7.3^{b}	35.6 ± 9.3^{a}	166.7 ± 39.9^{b}	nd[2]	nd[2]	nd[2]	8.9 ± 1.1^{a}	172.0 ± 24.3^{c}	415.2 ± 22.9^{b}
W-RB-ChG*	1408.4 ± 22.9^{g}		46.2 ± 2.8^{c}		131.1 ± 30.8^{b}		nd[2]		8.9 ± 1.1^{a}		243.2 ± 2.5^{d}	
RB-SG	97.0 ± 4.9^{c}	157.9 ± 5.4^{b}	9.1 ± 0.9^{a}	12.7 ± 3.3^{a}	3.8 ± 1.1^{a}	3.8 ± 1.1^{a}	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	24.3 ± 1.0^{a}
W-RB-SG*	60.9 ± 4.6^{b}		3.6 ± 3.3^{a}		nd[2]		nd[2]		nd[2]		24.3 ± 1.0^{a}	
RB-WG	14.4 ± 2.1^{a}	14.4 ± 2.1^{a}	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]
W-RB-WG*	nd[2]		nd[2]		nd[2]		nd[2]		nd[2]		nd[2]	
SF-ChG	nd[2]	nd[2]	61.5 ± 1.6^{c}	644.2 ± 4.1^{b}	68.9 ± 19.1^{b}	2964.5 ± 60.0^{b}	nd[2]	517.3 ± 88.5^{a}	22.4 ± 0.4^{b}	92.7 ± 2.7^{c}	598.0 ± 5.3^{c}	1324.3 ± 45.6^{c}
O-SF-ChG*	nd[2]		582.7 ± 3.8^{d}		2895.5 ± 68.4^{c}		517.3 ± 88.5^{a}		70.3 ± 2.5^{e}		726.3 ± 41.2^{e}	
SF-SG	nd[2]	nd[2]	9.5 ± 3.8^{a}	17.9 ± 9.7^{a}	8.4 ± 0.9^{a}	66.1 ± 1.0^{a}	nd[2]	nd[2]	20.8 ± 0.7^{b}	48.7 ± 0.7^{b}	651.9 ± 31.1^{d}	892.9 ± 27.4^{b}
O-SF-SG*	nd[2]		8.5 ± 6.3^{a}		57.7 ± 1.5^{a,b}		nd[2]		27.9 ± 1.2^{c}		241.0 ± 4.9^{b}	
SF-WG	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	nd[2]	12.5 ± 0.8^{a}	12.5 ± 0.8^{a}	262.9 ± 23.4^{b}	262.8 ± 23.4^{a}
O-SF-WG*	nd[2]		nd[2]		nd[2]		nd[2]		nd[2]		nd[2]	

[1] Results expressed as a mean ± SD (n = 5); 95% confidence interval; μg g^{-1} DW.

[2] Not detectable.

* Cooking medium (water (W-) and vegetable oil (O-)).

Values followed by the same superscript letters are not significantly different according to the Tukey test ($p < 0.05$).

Pre-cooking treatments: ChG, Chopped garlic (garlic cloves crushed with a garlic press); SG, Sliced garlic (garlic cloves cut into pieces (around 2 mm thickness)) and WG, whole garlic cloves (uncrushed).

Cooking treatments: R, Raw; HS, Simmering (boil water with a minimum heat input); RB, rolling boil (boil water with a high heat input); SF, Stir-frying.

Table 4 – OSCs moles formed in the several pre-cooking and cooking treatments, using raw garlic as a control.

Treatments	OSCs level [μmol g^{-1}][a]					
	Allicin	E-Z-ajoene	2VD	DAS	DADS	DATS
R-ChG	29.26 ± 0.58	0.00	0.00	0.00	0.00	0.00
HS-ChG	1.69 ± 0.04	0.18 ± 0.01	0.22 ± 0.02	0.08 ± 0.03	0.03 ± 0.002	1.25 ± 0.05
RB-ChG	9.70 ± 0.15	0.61 ± 0.03	1.16 ± 0.28	0.00	0.06 ± 0.01	2.33 ± 0.13
SF-ChG	0.00	2.75 ± 0.02	20.55 ± 0.42	4.53 ± 0.77	0.63 ± 0.02	7.43 ± 0.26
R-SG	14.97 ± 0.50	0.00	0.00	0.00	0.00	0.00
HS-SG	0.30 ± 0.03	0.01 ± 0.003	0.03 ± 0.01	0.00	0.04 ± 0.001	0.54 ± 0.02
RB-SG	0.97 ± 0.03	0.05 ± 0.01	0.03 ± 0.01	0.00	0.00	0.14 ± 0.01
SF-SG	0.00	0.08 ± 0.04	0.46 ± 0.01	0.00	0.33 ± 0.005	5.01 ± 0.15
R-WG	0.00	0.00	0.00	0.00	0.00	0.00
HS-WG	0.00	0.00	0.00	0.00	0.00	0.00
RB-WG	0.09 ± 0.01	0.00	0.00	0.00	0.00	0.00
SF-WG	0.00	0.00	0.00	0.00	0.09 ± 0.01	1.47 ± 0.13

[a] Results expressed as mean ± SD (n = 5); 95% confidence interval; μmol g^{-1} DW.
Pre-cooking treatments: ChG, Chopped garlic (garlic cloves crushed with a garlic press); SG, Sliced garlic (garlic cloves cut into pieces (around 2 mm thickness)) and WG, whole garlic cloves (uncrushed).
Cooking treatments: R, Raw; HS, Simmering (boil water with a minimum heat input); RB, rolling boil (boil water with a high heat input); SF, Stir-frying.

as follows: PC1 accounts for 50.0%, and PC2 for 19.0% of the total (Supplementary Table S2).

Fig. 1 shows that the "fresh garlic samples" are placed on the negative area of PC1 and PC2 and are highly influenced by their allicin level. "Fried cooked samples" are positioned in the positive score area of the PC1, and negative score area of the PC2 mainly due to their DADS and DATS content. The samples "Simmering cooked" are positioned in the central areas of the score plot. HSSG is placed in the lower left space due to its allicin levels, while HSChG is placed in the upper right space because of its DAS levels. It is noteworthy that DAS was only detected in this treatment. The RBChG samples are placed in the positive area of PC1 and PC2; these are linked to a high content of E-Z-ajoene and 2-VD. Samples with identical cooking

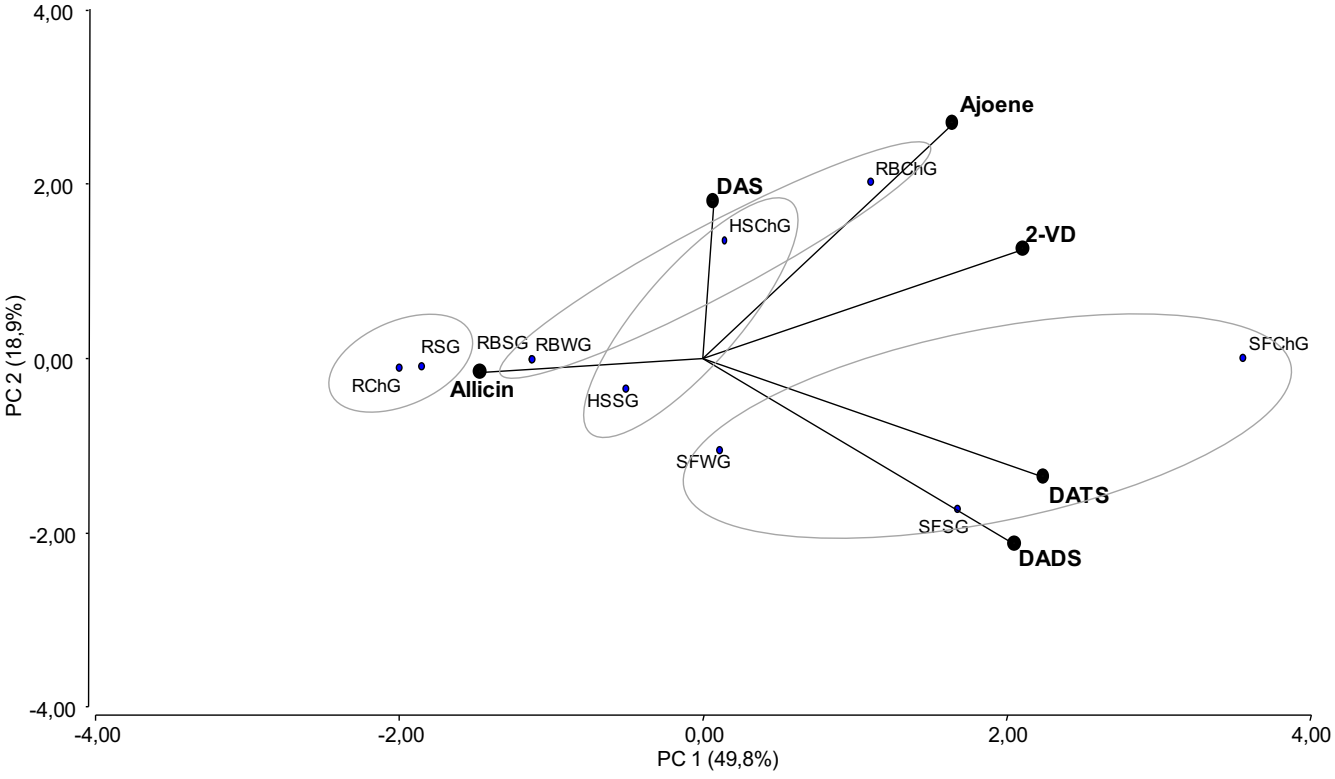


Fig. 1 – Principal components analysis. Biplot scores and loading plot for garlic samples after different cooking methods. R, Raw; HS, Simmering; RB, Rolling boil; SF, Stir-frying; ChG, Chopped garlic; SG, Sliced garlic and WG, Whole garlic cloves (uncrushed).

treatment and different pre-cooking treatment (RBSG and RBWG) are distributed near fresh samples (left lower quadrant), due to their allicin content.

It is interesting to point out that the samples distribution according to the first two PC suggests that they tend to group together mainly due to the cooking treatments, rather than the pre-cooking treatments. Although it is evident that pre-cooking treatment also influenced significantly in OSCs profile.

4. Conclusion

The data obtained highlight the relevant potential functional value of all combined garlic treatments under study. This is the first time, to our knowledge, that an analytical methodology to prevent artifacts formation is used to evaluate the remaining OSCs in home-cooked garlic. Due to the high content of OSCs detected in HSChG, SFChG, and RBChG, we could suggest their use as ingredient in both therapeutic preparations as functional foods (bread, condiments, flavourings, instant soup and so on). An important aim for future studies is to evaluate the bioavailability of these target analytes.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2015.04.012.

REFERENCES

Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., & Itakura, Y. (2001). Intake of garlic and its bioactive components. *The Journal of Nutrition*, *131*, 955–962.

Artacho Martin-Lagos, R., Olea Serrano, M. F., & Ruiz Lopez, M. D. (1995). Determination of organic sulphur compounds in garlic extracts by gas chromatography and mass spectrometry. *Food Chemistry*, *53*, 91–93.

Barham, P., Skibsted, L. H., Bredie, W. L. P., Frøst, M. B., Møller, P., Risbo, J., Snitkjaer, P., & Mortensen, L. M. (2010). Molecular gastronomy: A new emerging scientific discipline. *Chemical Reviews*, *110*(4), 2313–2365. http://doi.org/10.1021/cr900105w.

Block, E. (1985). The chemistry of garlic and onions. *Scientific American*, *252*, 114–119.

Block, E. (2010). Chemistry in a salad bowl: Allium chemistry and biochemistry. In E. J. Corey (Ed.), *Garlic and other alliums. The lore and the science* (pp. 100–222). New York: The Royal Society of Chemistry.

Block, E., Ahmad, S., Catalfamo, J. L., Jain, M. K., & Apitz-Castro, R. (1986). Antithrombotic organosulfur compounds from garlic: Structural, mechanistic, and synthetic studies. *Journal of the American Chemical Society*, *108*(22), 7045–7055. http://doi.org/10.1021/ja00282a033.

Block, E., Putman, D., & Zhao, S. H. (1992). Allium chemistry: GC-MS analysis of thiosulfinates and related compounds from onion, leek, scallion, shallot, chive, and Chinese chive. *Journal of Agricultural and Food Chemistry*, *40*(12), 2431–2438. http://doi.org/10.1021/jf00024a018.

Cavagnaro, P. F., Camargo, A., Galmarini, C. R., & Simon, P. W. (2007). Effect of cooking on garlic (*Allium sativum* L.) antiplatelet activity and thiosulfinates content. *Journal of Agricultural and Food Chemistry*, *55*(4), 1280–1288. http://doi.org/10.1021/jf062587s.

Fujisawa, H., Suma, K., Origuchi, K., Seki, T., & Ariga, T. (2008). Thermostability of allicin determined by chemical and biological assays. *Bioscience, Biotechnology, and Biochemistry*, *72*(11), 2877–2883. http://doi.org/10.1271/bbb.80381.

González, R., Camargo, A., & Burba, J. L. (2007). Obtention of a quantitative secondary standard for allicin synthesis and purification. *Revista de La Facultad de Ciencias Agrarias*, *39*, 61–70.

Iberl, B., Winkler, G., & Knobloch, K. (1990). Products of allicin transformation: Ajoenes and dithiins, characterization and their determination by HPLC. *Planta Médica*, *56*, 202–211.

Iciek, M., Kwiecien, I., & Wlodek, L. (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environmental and Molecular Mutagenesis*, *50*, 247–265. http://doi.org/10.1002/em.20474.

Ilić, D., Nikolić, V., Stanković, M., Nikolić, L., Stanojević, L., Mladenović-Ranisavljević, I., & Šmelcerović, A. (2012). Transformation of synthetic allicin: The influence of ultrasound, microwaves, different solvents and temperatures, and the products isolation. *The Scientific World Journal*, *2012*, 1–7. http://doi.org/10.1100/2012/561823.

Jiménez-Monreal, A. M., García-Diz, L., Martínez-Tomé, M., Mariscal, M., & Murcia, M. A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, *74*(3), H97–H103. http://doi.org/10.1111/j.1750-3841.2009.01091.x.

Kamel, A., & Saleh, M. (2000). Recent studies on the chemistry and biological activities of the organosulfur compounds of garlic (*Allium Sativum*). *Studies in Natural Products Chemistry*, *23*, 455–485.

Kim, S. M., Wu, C. M., Kobayashi, A., Kubota, K., & Okumura, J. (1995). Volatile compounds in stir-fried garlic. *Journal of Agricultural and Food Chemistry*, *43*(11), 2951–2955. http://doi.org/10.1021/jf00059a033.

Kim, S. M., Wu, C. M., Kubota, K., & Kobayashil, A. (1995). Effect of soybean oil on garlic volatile compounds isolated by distillation. *Journal of Agricultural and Food Chemistry*, *43*, 449–452.

Lee, C. G., Lee, H.-W., Kim, B.-O., Rhee, D.-K., & Pyo, S. (2015). Allicin inhibits invasion and migration of breast cancer cells through the suppression of VCAM-1: Regulation of association between p65 and ER-α. *Journal of Functional Foods*, *15*, 172–185. http://doi.org/10.1016/j.jff.2015.03.017.

Liang, D., Wang, C., Tocmo, R., Wu, H., Deng, L.-W., & Huang, D. (2015). Hydrogen sulphide (H2S) releasing capacity of essential oils isolated from organosulphur rich fruits and vegetables. *Journal of Functional Foods*, *14*, 634–640. http://doi.org/10.1016/j.jff.2015.02.007.

Locatelli, D. A., Altamirano, J. C., Luco, J. M., Norlin, R., & Camargo, A. B. (2014). Solid phase microextraction coupled to liquid chromatography. Analysis of organosulphur compounds avoiding artifacts formation. *Food Chemistry*, *157*, 199–204. http://doi.org/10.1016/j.foodchem.2014.02.010.

Santhosha, S. G., Jamuna, P., & Prabhavathi, S. N. (2013). Bioactive components of garlic and their physiological role in health maintenance: A review. *Food Bioscience*, *3*, 59–74. <http://doi.org/10.1016/j.fbio.2013.07.001>.

Soto, V. C., Camargo, A. B., González, R. E., & Galmarini, C. R. (2007). Synthesis and purification of ajoene and its quantificationin commercial garlic oils. *Revista de La Facultad de Ciencias Agrarias*, *39*, 93–100.

Tocmo, R., Lin, Y., & Huang, D. (2014). Effect of processing conditions on the organosulfides of shallot (*Allium cepa* L. Aggregatum group). *Journal of Agricultural and Food Chemistry*, *62*(23), 5296–5304.

Weinberg, D. S., Manier, M. L., Richardson, M. D., & Haibach, F. G. (1993). Identification and quantification of organosulfur compliance markers in a garlic extract. *Journal of Agricultural and Food Chemistry*, *41*, 37–41.

Yan, X., Wang, Z., & Barlow, P. (1992). Quantitative estimation of garlic oil content in garlic oil based health products. *Food Chemistry*, *45*, 135–139.

Yan, X., Wang, Z., & Barlow, P. (1993). Quantitative determination and profiling of total sulphur compounds in garlic health products using a simple GC procedure. *Food Chemistry*, *47*(3), 289–294. http://doi.org/10.1016/0308-8146(93)90163-A.

Yu, T. H., Lin, L. Y., & Ho, C. T. (1994). Volatile compounds of blanched, fried blanched, and baked blanched garlic slices. *Journal of Agricultural and Food Chemistry*, *42*, 1342–1347.

Yu, T. H., & Wu, C. M. (1994). Volatile compounds generated from thermal degradation of alliin and deoxyalliin in an aqueous solution. *Journal of Agricultural and Food Chemistry*, *42*, 146–153.

Yu, T. H., Wu, C. M., & Ho, C. T. (1993). Volatile compounds of deep-oil fried, microwave-heated and oven-baked garlic slices. *Journal of Agricultural and Food Chemistry*, *41*(5), 800–805. http://doi.org/10.1021/jf00029a023.

Yu, T. H., Wu, C. M., & Chen, S. Y. (1989). Effects of pH adjustment and heat treatment on the stability and the formation of volatile compounds of garlic. *Journal of Agricultural and Food Chemistry*, *37*, 730–734.

Yu, T. H., Wu, C. M., & Liou, Y. C. (1989). Volatile compounds from garlic. *Journal of Agricultural and Food Chemistry*, *37*, 725–730.