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Matrix effect on phytochemical bioaccessibility. The case of organosulfur compounds in garlic preparations

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ABSTRACT

Phytochemicals are dietary compounds considered lifespan-essentials. Their beneficial effects strongly depend on their bioaccessible quantities, in turn determined by food matrix components. Organosulfur compounds (OSCs) are renowned phytochemicals responsible for garlic health-effects. Currently, garlic matrix role in OSCs bioaccessibility has not been studied. Furthermore, the digestive behavior of isolated OSCs added to garlic preparations is unknown. In order to decipher these questions, different garlic matrices were subjected to *in vitro* digestion. Our findings demonstrate that garlic matrix impacts significantly on OSCs digestive performance. Therefore, digestive stability and bioaccessibility varied with the garlic preparation form. Powdered garlic, in fresh and stir-fried form, presented the highest stability and bioaccessibility values because matrix components suspended in digestive fluids protected OSCs during digestion. Hence, garlic powder would be suitable for OSCs dietary incorporation and functional food design. On the other hand, naturally occurring OSCs were more stable than isolated OSCs added to garlic samples. This behavior can be explained by a more deepen interaction of endogenous compounds with the matrix components and their location inside the vegetable structure. Therefore, OSCs addition procedures in food matrices should be carefully designed to optimize the phytochemical bioefficacy.

1. Introduction

Phytochemicals are secondary plant metabolites with proved health-promoting benefits. When they are consumed, the risk of age-related chronic diseases is reduced (Kisioglu & Nergiz-Unal, 2018). However, the mere presence of these compounds in food is not enough to guarantee their bioaccessibility, which corresponds to the compound amount that is released from the food matrix into the gastrointestinal tract (GIT) and thus became available for absorption (Barba et al., 2017). In this sense, the study of phytochemical bioaccessibility is crucial to assure the bioefficacy of functional foods (Holst & Williamson, 2008; Putnik et al., 2019). Nowadays, *in vitro* digestion is the most employed methodology in bioaccessibility studies (Barba et al., 2017; Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). Two main factors influence the bioaccessibility of a bioactive compound. The first one is related to the phytochemical amount released from its original environment. This factor depends on how nutraceuticals are trapped within the vegetable matrix, such as the cellular structure. The second factor relates to the phytochemical ability to be dissolved or dispersed in the gastrointestinal fluids. Particularly, lipophilic compounds must be emulsified to disperse in digestive fluids and therefore be accessible. In this case, compound identity and food matrix composition (fat, water and fiber content) end-up determining phytochemical dispersibility (Mcclements et al., 2015). Furthermore, the cooking preparation method can also determine the phytochemical digestive behavior (Alminger et al., 2014; Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010; McClements, Decker, Park, & Weiss, 2008; Minekus et al., 2014).

On those mentioned above, phytochemical health benefits depend not only on their intake amounts but also on their bioaccessibility (Cilla et a., l2018). Among all known phytochemical groups, organosulfur compounds (OSCs) have been widely studied since they are associated with numerous bioactive properties (Putnik et al., 2019; Quesada et al., 2020). Due to OSCs bioactive nature, numerous studies have investigated their quali-quantitative levels in different food preparations (Locatelli, Altamirano, González, & Camargo, 2015; Ramírez, Locatelli, González, Cavagnaro, & Camargo, 2017). *Allium sativum* L. (garlic)

Abbreviations: **2VD**, 2-vinyl-4H-1,3-dithiin; **DAS**, diallyl sulfide; **DADS**, diallyl disulfide; **DATS**, diallyl trisulfide; **DLLME**, dispersive liquid-liquid microextraction; **HPLC**, high performance liquid chromatography; **OSCs**, organosulfur compounds.

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excels from other vegetables due to their high OSCs content (Corzo--Martínez, Corzo, & Villamiel, 2007). Regarding OSCs bioaccessibility in garlic preparations, a recent work (Torres-Palazzolo et al., 2018) from our research group addressed their behavior during gastrointestinal digestion. There, OSCs showed bioaccessibility percentages from 52 to 100, although these results were obtained by studying only one garlic preparation. After that, our group was motivated to evaluate OSCs behavior in different garlic preparations subjected to *in vitro* gastrointestinal digestion. Therefore, the present work is focused on the impact of different garlic preparations on the digestive stability and bioaccessibility of OSCs. Also, we compared the bioaccessibility and digestive stability among naturally occurring OSCs and added-OSCs. To our knowledge, this is the first study that explores the role of different garlic matrices on OSCs digestive behavior.

2. Materials and methods

2.1. Reagents and analytical standards

Diallyl sulfide (DAS; 97%), diallyl disulfide (DADS; 80%), porcine pepsin (P7000), porcine pancreatin (P7545) and porcine bile salts (B8756) were purchased from Sigma-Aldrich (Saint Louis, USA). Diallyl trisulfide (DATS; 98%) was purchased from LKT Laboratories, Inc (St. Paul, MN, USA). Acetonitrile (ACN) chromatographic grade was purchased from J.T.Baker (USA). Methanol (MeOH), and dichloromethane (DCM) chromatographic grade were 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 DADS (Ramírez, Locatelli, Torres-Palazzolo, Altamirano, & Camargo, 2017). E/Z ajoene was obtained by heating and stirring of allicin in acetone/water (40:60, v/v) (Ramírez, Locatelli, Torres-Palazzolo et al., 2017). 2-vinyl-4H-1,3-dithiine was synthesized by heating allicin in acetone/methanol (60:40, v/v) following the procedure described by Iberl, Winkler, & Knobloch, 1990 with slight modifications. All synthesized compounds were properly purified and quantified as described in previous work (Ramírez, Locatelli, Torres-Palazzolo et al., 2017).

2.2. Sample preparation and conditioning

Five of the most consumed garlic preparations (González, Vidoni, Locatelli, & Camargo, 2017; Locatelli et al., 2015; Suleria et al., 2015) were selected for evaluating their matrix impact on OSCs bioaccessibility. These preparations (Fig. 1) were made under controlled conditions using '*Sureño*' garlic cultivar belonging to garlic germplasm from the Instituto Nacional de Tecnología Agropecuaria (INTA).

2.2.1. Garlic preparations containing allicin

Powdered garlic preparation was obtained by freeze-drying whole garlic cloves at −58 °C for 72 h in a vacuum freeze-drying system (LabConco Model Freezone 2.5, Kansas, MO, USA). The resulting lyophilized material was ground and stored at −20 °C. Before the analysis, they were reconstituted with water (1:1 w/w) and kept standing for 5 min to ensure allicin formation. Homogenized garlic was prepared by blending fresh vegetal material with water (1:10 w/w) for 5 min. Chopped garlic samples were prepared by cutting cloves into 2 mm cubes. Samples rested 5 min after tissue disruption to allow allicin formation.

2.2.2. Garlic preparations containing ajoene, 2-vinyldithiine and polysulfides

Stir-fried garlic samples were prepared the same day that the *in-vitro* digestion protocol was carried out. 50 g of sunflower oil were added to a pan and fire-heated and until the oil temperature reached 180 °C, then 50 g of chopped garlic were added and cooked for 2 min. Subsequently, stir-fried garlic was drained on absorbent paper and kept at −20 °C until be subjected to digestion protocol. Previously an aliquot was taken to OSCs determination by HPLC.

Garlic oil macerate was obtained following the protocol suggested by González et al., 2017. Fifty grams of raw chopped garlic were added to 500 mL of sunflower oil and stored at room temperature for 30 days in a closed amber glass bottle. After maceration, the oil was filtered and reserved in a clean bottle.

2.2.3. Samples added with OSCs

OSCs addition was made on lyophilized stir-fried garlic, prepared as was described in section 2.2.2. Then, it was lyophilized at −58 °C for 72 h in a vacuum (Freeze Dry Systems LabConco Model Freezone 2.5, Kansas, MO, USA). The lyophilized material was ground in a mortar and stored under a nitrogen atmosphere at −18 °C. This preparation was chosen because it assures the better quali-quantitative level of ajoenes, vinyldithiines and polysulfides (Locatelli et al., 2015). Allicin did not be studied with these compounds because it was previously demonstrated that partially decomposes into other OSCs during digestion

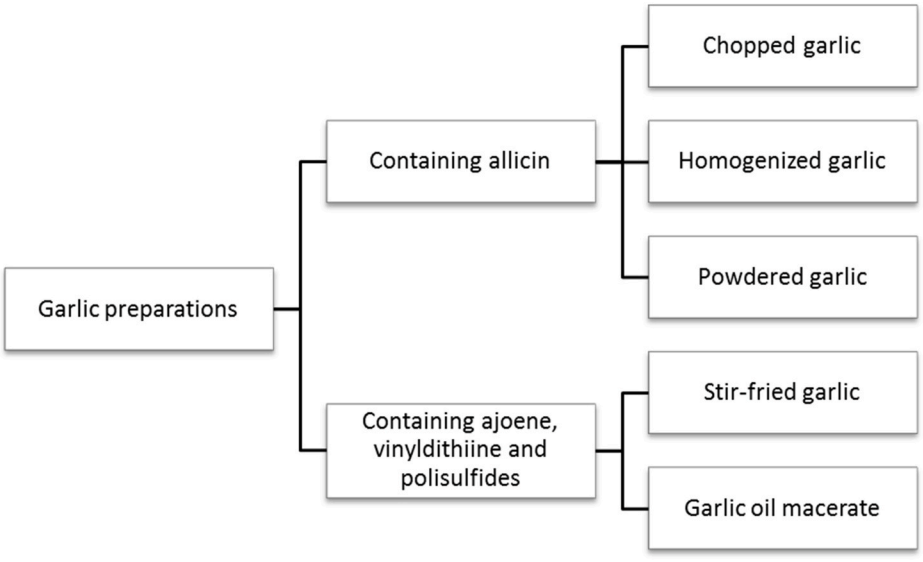

Fig. 1. Schematic summary of prepared garlic samples.

(Torres-Palazzolo et al., 2018).

The addition was made by directly spiking OSCs standards into the sample, reaching final concentrations of around 700–800 μg g-1 (see Table S1 in supplementary material). The initial OSCs concentration in supplemented freeze-dried stir-fried garlic was measured right before *in vitro* digestion as it is explained in section 2.4. The exact concentrations of each preparation with and without OSCs were calculated by parallel quantification of both samples. After that by subtraction of concentrations measured in added preparation by non-added preparations"

2.3. OSCs extraction

Garlic samples containing allicin were extracted by dispersive liquid-liquid microextraction following the procedure described in a previous work from our research group (Ramírez, Locatelli, Torres-Palazzolo et al., 2017). One milliliter of acetonitrile containing 600 μL of chloroform was injected rapidly into samples. Subsequently, the mixture was centrifuged for 3 min at 425 g (Eppendorf MiniSpin plus, Ham-burg, Germany). Finally, the chloroform phase was recovered and dried under a nitrogen stream, and then it was dissolved in 500 μL of MeOH, filtered and injected into HPLC-UV for analysis.

Meanwhile, garlic samples containing more lipophilic compounds were extracted with 2 mL of ACN and ultrasonicated for 1 min (González et al., 2017; Locatelli et al., 2015). Then, samples were centrifuged at 10, 000 *g* for 15 min, and ACN layer was recovered and filtered for OSCs quantification.

2.4. In vitro digestion

All the samples aforementioned were subjected to an *in vitro* gastrointestinal digestion protocol as described by Torres-Palazzolo et al., 2018. First, 5 mL of simulated gastric fluid (porcine pepsin 1600 U mL^{-1}; HCl 0.1 mol L^{-1}; $CaCl_2{\cdot}2H_2O$ 3.6 mmol L^{-1}; $MgCl_2{\cdot}6H_2O$ 1.5 mmol L^{-1}; NaCl 49 mmol L^{-1}; KCl 12 mmol L^{-1}; KH_2PO_4 6.4 mmol L^{-1}) were added to 500 mg of samples, and then pH was adjusted to 2. Subsequently, samples were incubated at 37 °C with orbital agitation at 250 rpm for 60 min in a closed flask. After gastric digestion was completed, pH was adjusted to 6.8 and 3 mL of simulated intestinal fluid (pancreatin 16,000 USP L^{-1}; $NaHCO_3$ 0.1 mol L^{-1}; bile salts 25 g L^{-1}) were added. Samples were incubated for 120 min at 37 °C with orbital agitation at 250 rpm. Finally, the samples were immediately centrifuged for 30 min at 10,600 g. Thus, OSCs remaining amount from both supernatants (accessible fraction) and pellets (no-accessible fraction) were extracted and quantified.

The behavior of different garlic preparations under *in vitro* digestion conditions was compared by two parameters: digestive stability and bioaccessibility. On the one hand, the digestive stability was calculated as the percentage between the OSCs remaining amounts in both accessible and no-accessible fractions and the OSCs concentration determined in 1 g of initial sample before digestion (Eq. (1)). On the other hand, the bioaccessibility was calculated as the percentage of the phytochemical amount released from 1 g of sample to the gastrointestinal fluid (Eq. (2)) (Alminger et al., 2014; Barba & Orlien, 2017; Poojary et al., 2017). In contrast, the no-bioaccessible percentage referred to the amount located in the matrix sediment after digestion with respect to the initial concentration in 1 g of sample.

$$Stability\ [\%] = \frac{Remaining\ amount\ [\mu g\ g^{-1}]}{Initial\ amount\ [\mu g\ g^{-1}]} \cdot 100 \quad (1)$$

$$Bioaccessibility\ [\%] = \frac{Accessible\ amount\ [\mu g\ g^{-1}]}{Initial\ amount\ [\mu g\ g^{-1}]} \cdot 100 \quad (2)$$

2.5. HPLC analysis: instrumentation and operating conditions

Chromatographic analyses were performed using a Shimadzu High Performance Liquid Chromatograph (LC-20AT) with a diode array detector (DAD SPD-M20A), (Shimadzu, Japan). The HPLC column used was an ODS Spherisorb (254 × 4.6 mm I:D. 5 μm particle size) (Waters, USA). HPLC data was processed using the LabSolutions 5.82 software.

Operating conditions were adapted from those previously reported in Ramírez, Locatelli, Torres-Palazzolo et al., 2017 as follows: isocratic elution using as mobile phase ACN/water/MeOH 50:41:9 (v/v/v) at 1 mL min^{-1}; and wavelengths of 210 and 254 nm for detection. Peak identification was carried out by comparing the retention times and UV-spectra with those of reference standards.

2.6. Statistical analysis

INFOSTAT (Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. InfoStat versión 2016. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar) was the statistical software employed for data analysis. Results were compared by ANOVA and Tukey's tests. *P*-values <0.05 were considered to be significant. The effect of garlic treatments in OSCs stability and bioaccessibility were analyzed by MANOVA and Hotelling test. Also, a conglomerate analysis was performed.

3. Results and discussion

Results are presented in Figs. 2–4. Numerical data are also presented in the supplementary material section.

3.1. Garlic preparations containing allicin

Fig. 2 shows digestive stability, bioaccessible and no-bioaccessible percentages of allicin. Regarding digestive stability, garlic powder stood out because it showed the highest values (78%). While decreasing allicin stability was observed in chopped (28%) and homogenized garlic (25%). Garlic preparations had significantly higher stability percentages compared to the allicin standard (17%). The behavior of allicin standard could be explained, at least partially, by the matrix absence. In this case, allicin is physically unprotected against the digestive environment conditions, which could lead to further degradation. Similar results were previously reported for other isolated phytochemicals (Alminger et al., 2014; Courraud, Berger, Cristol, & Avallone, 2013; Failla, Huo, & Thakkar, 2008; Palafox-Carlos, Ayala-zavala, & González-Aguilar, 2011). On the other hand, several works have claimed that interactions between phytochemicals and food matrix components, such as carbohydrates, could exert a protective effect on the compounds, enhancing their digestive stability (Alminger et al., 2014; Courraud et al., 2013; McClements et al., 2008; Mcclements et al., 2015; Parada & Aguilera,

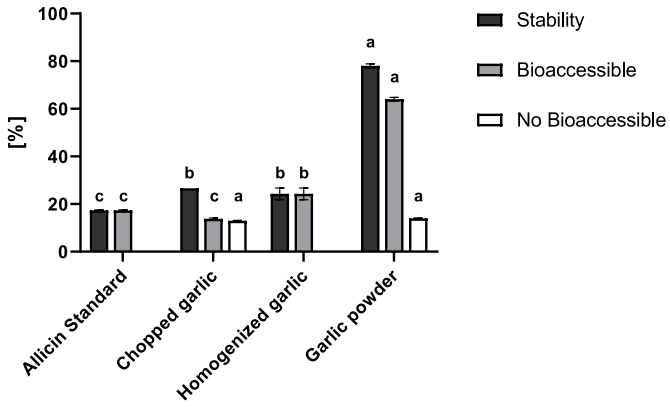

Fig. 2. Allicin digestive behavior in different garlic matrices.
Bars represent the mean ± standard deviation of two replications. Significant differences between analogous bars of the samples are represented with different letters.

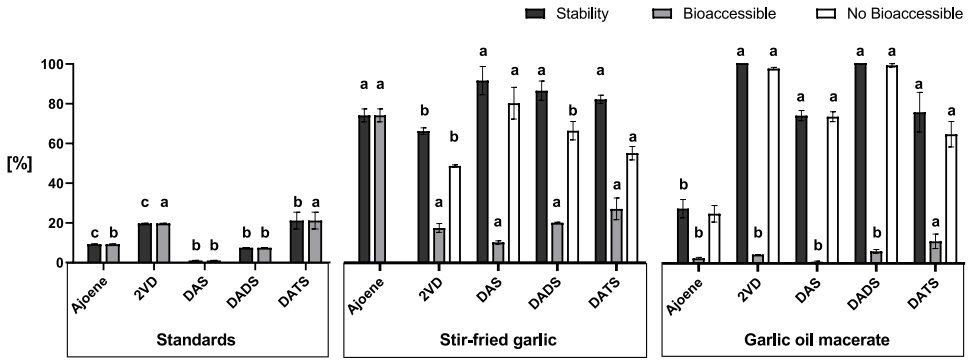

Fig. 3. Organosulfur compounds digestive behavior in stir-fried garlic and garlic oil macerate.
2VD: 2-vinyl-4H-1,3-dithiine; **DAS:** diallyl sulfide; **DADS:** diallyl disulfide; **DATS:** diallyl trisulfide. Bars represent the mean ± standard deviation of two replications. Significant differences between analogous bars of the samples are represented with different letters.

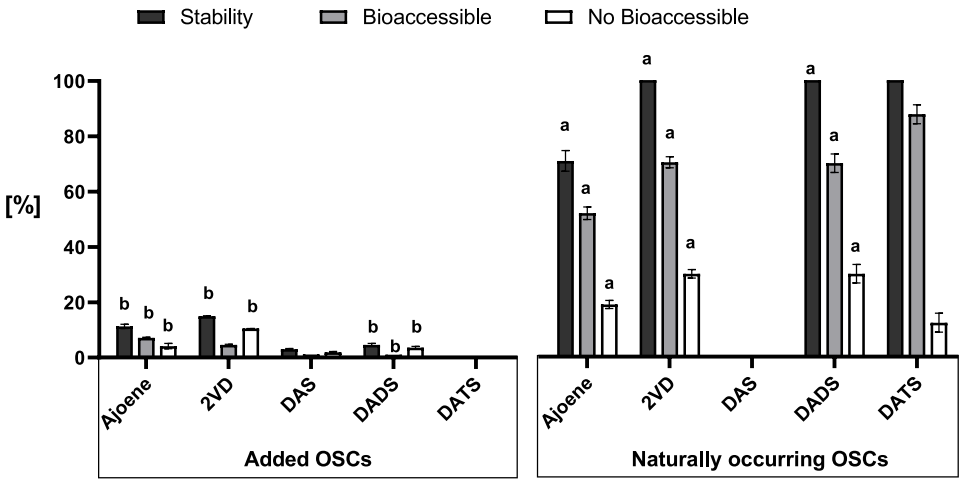

Fig. 4. Comparison of organosulfur compounds (OSCs) digestive behavior between added OSCs and naturally occurring OSCs.
2VD: 2-vinyl-4H-1,3-dithiine; **DAS:** diallyl sulfide; **DADS:** diallyl disulfide; **DATS:** diallyl trisulfide. Bars represent the mean ± standard deviation of two replications. Significant differences between analogous bars of the samples are represented with different letters.

2007). Noteworthy, garlic as well as other *Allium* species, contains high fructooligosaccharides (FOS) amounts (Losso & Nakai, 1997; Putnik et al., 2019). On account of the above, our results allow hypothesizing that FOS could stabilize and protect suspended OSCs into the digestive fluids (Palafox-Carlos et al., 2011). Particularly, the superior performance of garlic powder could be explained by a decreased particle size that enlarges the surface area available for the overall digestion extractability of both phytochemicals and matrix components (Parada & Aguilera, 2007).

Regarding allicin bioaccessible percentage (grey bars in Fig. 2), which refers to the compound released amount from the vegetable matrix to digestive fluids, it was observed that garlic powder presented the highest value (64%). As discussed in the preceding paragraph, decreasing the particle size of the food increases the overall extractability. Meanwhile, the released matrix components protected suspended phytochemicals within the digestive fluids (Alminger et al., 2014; Lemmens et al., 2010; Palafox-Carlos et al., 2011). On the other hand, bioaccessibility results for the other samples were significantly lower, 24% for garlic homogenate, 17% for allicin standard and 13% for chopped garlic. The last three samples mentioned above had in common limited solids amounts suspended in the digestive media. In this particular case, once allicin is suspended within the digestive media and there is no protective effect provided by suspended matrix components, it becomes more susceptible to degradation (Alminger et al., 2014). Then, allicin, a highly unstable compound, can rapidly transform into other metabolites such as allyl methyl sulfide, allyl mercaptan (Rosen et al., 2000) and hydrogen sulfide (Tocmo, Liang, Lin, & Huang, 2015). These metabolites may originate from allicin reaction with other biomolecules of the digestive media, *i.e.* sulfhydryl residues of proteins, glutathione or cysteine molecules (Tocmo et al., 2015; Torres-Palazzolo et al., 2018; Zhang, Li, Lee, & Parkin, 2010).

On the other hand, chopped garlic and garlic powder had around 12–13% of no-bioaccessible allicin. We hypothesize these allicin molecules were trapped within the cellular structure of vegetable tissue; whereas homogenized garlic did not present tissue sediment after digestion.

3.2. *Garlic samples containing E/Z-ajoene, 2-vinyldithiine and polysulphides*

Fig. 3, shows digestive stability, bioaccessible and no-bioaccessible percentages of ajoene, 2VD and polysulfides present in a standard solution, stir-fried garlic and garlic oil macerate. Regarding OSCs stability, garlic oil macerate presented the highest stability values (27–100%), followed by stir-fried garlic (66–86%). Lastly, OSCs standards presented the lowest values (1–21%). As observed for samples with allicin, the presence of matrix was associated with enhanced OSCs stability during digestion (Alminger et al., 2014; Mcclements et al., 2015; Parada &

Aguilera, 2007). However, in these cases, the protective effect could be primarily due to OSCs retention in the no-accessible fraction, which would lead to the physical isolation from the digestive environment (white bars of Fig. 3). For stir-fried garlic, the protective effect could be caused by the natural entrapment of phytochemicals within the vegetable tissues (Parada & Aguilera, 2007), added to an increased affinity for the matrix due to the partial absorption of oil during stir-frying. On the other hand, in garlic oil macerate OSCs were retained within the no-emulsified oil phase due to their lipophilicity (Singh & Singh, 2010). This circumstance resulted in diminished digestive degradation because OSCs are more protected in the oily environment than in the aqueous fluids (Mcclements et al., 2015; Poojary et al., 2017).

Regarding OSCs bioaccessibility (grey bars in Fig. 3), stir-fried garlic showed the highest levels (10–74%), while OSCs standards and garlic oil macerate presented lower values, 1–21% and 0.9–11% respectively. Remarkably, garlic oil macerate had limited OSCs bioaccessibility values despite having high stability values. This fact could be explained by inefficient emulsification of oil droplets, causing in turn micelle coalescence in the colloidal digestive phase (Courraud et al., 2013; Salvia-Trujillo et al., 2017). This result should be carefully considered because, under *in-vivo* conditions, lipophilic phytochemical bioaccessibility could increase when they are co-ingested with fatty meals because digested monoglycerides upregulate bile salts secretion enhancing phytochemical emulsification (Courraud et al., 2013; Custodio, Wu, & Benet, 2008). For this reason, our results could underestimate the real OSCs bioaccessibility in this sample. On another note, a higher OSCs bioaccessibility was observed in stir-fried garlic; this fact could be due to its lower oil content. Other researchers observed improved bioaccessibility when lipophilic phytochemicals were co-digested with slightly oily matrices. This phenomenon was attributed to enhanced emulsification of the mixed micelles. The smaller lipid droplets, with the increased superficial area, modulate the ability of lipase to interact with the lipid phase giving faster and more complete digestion (Mcclements et al., 2015). Additionally, other components of the garlic matrix could contribute to the overall OSCs bioaccessibility. Polysaccharides released during digestion can avoid the flocculation state of lipid droplets and prevent the precipitation of mixed micelles within the gastrointestinal fluids (Mcclements et al., 2015). As a result, the rates of lipid digestion and bioactive emulsification rise.

3.3. Garlic samples added with OSCs

Stability and bioaccessibility of added and naturally occurring OSCs were compared. With this aim, samples of freeze-dried stir-fried garlic were digested with and without the addition of OSCs standards. Fig. 4 shows that naturally occurring OSCs were significantly more stable (71–100%) than those added to the sample (1–15%). Noteworthy, OSCs standards stability (Fig. 3) was not enhanced when they were directly added to a garlic matrix (Fig. 4).

It has been reported that contrary to the isolated compounds, naturally occurring compounds are protected against degradation given that they are entrapped within the vegetable matrix, whether attached to membranes, occluded inside cell compartments, or bound to cell walls. This interaction could explain the different stability levels between added OSCs and naturally occurring OSCs.

Accordingly, naturally occurring OSCs were also the most bioaccessible (52–88%). In the case of added OSCs, their poor bioaccessibility (1–7%) could be explained by their digestive instability along with slight retention inside the garlic matrix. Thus, added OSCs could be rapidly suspended into digestive fluids, even before the garlic matrix components could be released and protect them in the liquid phase.

On the other hand, an improved OSCs extractability was observed in freeze-dried stir-fried garlic compared to OSCs in stir-fried garlic (see in Fig. 3). This result could be because chopped stir-fried garlic was freeze-dried and grounded before the digestion procedure. The latter reinforces the hypothesis that decreasing the vegetable particle sizes, and reducing the oil content, the overall OSCs bioaccessibility increases. The bioaccessibility could not be evaluated for DAS because the sample of freeze-dried stir-fried garlic did not present quantifiable amounts of this compound. Probably, DAS could not be detected due to its high polarity and vapour pressure that contributed whit its losses. Besides, the component was found in lower proportion compared to the other sulfides, so that only traces remain below the LOD of the determination of method.

Lastly, for the development of functional foods added with isolated OSCs it should be considered the prior protection of OSCs from the digestive environment, e.g., encapsulating them (Gleeson, Ryan, & Brayden, 2016; Mcclements et al., 2015; Parada & Aguilera, 2007).

3.4. Multivariate comparison of the digestive behavior of garlic preparations

To sum up, Fig. 5 shows a dendrogram which presents a hierarchy among all the garlic preparations under study. Additionally, a MANOVA with Hotelling analysis was made to discriminate the digestive behavior of samples, according to their OSCs bioaccessibility and stability values. The first node was composed of powdered samples of raw and stir-fried garlic. This group had the best digestive performance, probably caused by a smaller particle size of the food matrix. Thus, increasing the overall extractability efficiency of both OSCs and matrix components, as previously discussed. The next node was composed of stir-fried garlic and garlic oil macerate. These matrices protected OSCs during digestion but had lower bioaccessibility than the previous samples. The last group consisted of allicin and the other OSCs standards (ajoene, 2VD, DAS, DADS and DATS), homogenized garlic and chopped raw garlic. The samples mentioned above showed the lowest OSCs stability, probably due to insufficient matrix protection. As can be seen, this multivariate analysis agrees and summarizes our previously discussed results, and enables us to identify the most suitable forms to introduce OSCs to the diet.

4. Conclusions

Different garlic preparations were digested to evaluate the matrix influence on OSCs bioaccessibility and stability. Results allowed us to conclude that garlic processing plays a fundamental role in OSCs bioaccessibility. The naturally occurring OSCs are more stable than added or isolated OSCs. Chiefly, the matrix protective effect on naturally occurring OSCs can result from both the interaction between the compounds and matrix components in the digestive fluids, as well as the phytochemical disposition within the vegetable tissue. According to our results, raw and stir-fried garlic powders are effective forms to incorporate organosulfur compounds to the diet. However, further studies adapting the digestive conditions for garlic oil macerate and stir-fried garlic should be carried out, since these preparations remain as a promising dietary source of 2VD and DATS.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

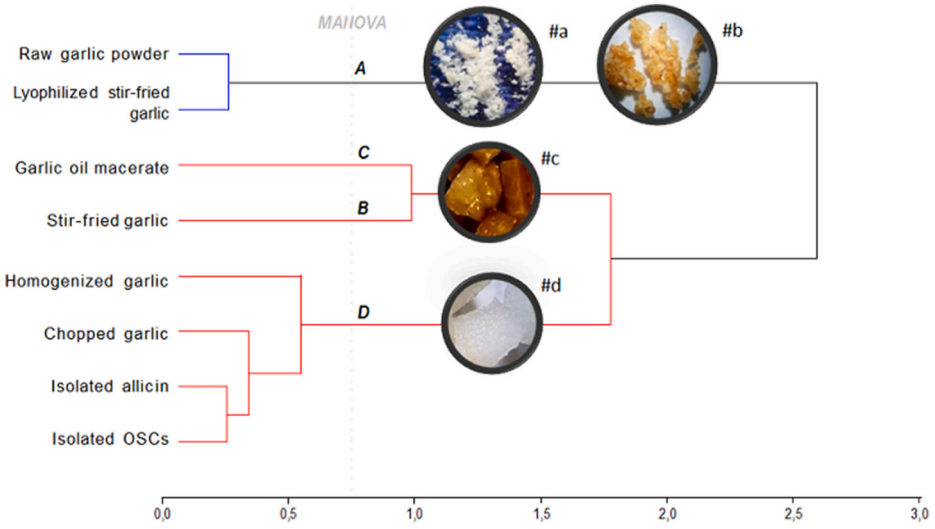

Fig. 5. Conglomerate analysis of garlic samples based on organosulfur compounds stability and bioaccessibility.
Average linkage classification method, cophenetic correlation factor 0.92. $^{A/B/C/D}$ - Significantly different digestive behavior corresponds to different capital letters (MANOVA plus Hotelling analysis, $p < 0.05$). $^{\#a}$ Raw garlic powder. $^{\#b}$ Lyophilized stir-fried garlic. $^{\#c}$ Stir-fried garlic. $^{\#d}$ Chopped garlic. Photographic images were captured using a magnifying glass (5×).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110301.

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