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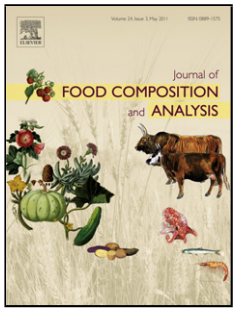

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Original Research Article

Bioaccessibility and permeability of bioactive compounds in raw and cooked garlic

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Highlights

- Bioactive compounds of raw and cooked garlic could be absorbed at a moderate rate
- Raw garlic digestion showed a greater organosulfur compounds quantitative yield
- Garlic compound 2-vinyldithiin evidenced the best permeability and blood stability

Abstract

Numerous studies have shown that organosulfur compounds (OSCs) from raw and cooked garlic act as bioactive phytochemicals. Nevertheless, data related to OSCs bioaccessibility is scarce. This information would be useful to establish how garlic should be consumed for maximize its beneficial effects. In the present work, cooking influence on OSCs bioaccessibility, intestinal permeability and blood stability was assayed. Target analytes were allicin, (*E*)/(*Z*)-ajoene, 2-vinyl-4H-1,3-dithiin (2-VD), diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS). The results showed that all OSCs studied were bioaccessible in raw and cooked garlic. In contrast, allicin, DAS and 2VD would be able to permeate less than 5% through the intestine. Furthermore, OSCs reacted rapidly with blood; less than 20% of 2VD, DAS and DADS could remain unreacted at the half hour. In conclusion, raw and cooked garlic intake provides bioactive OSCs that could be absorbed at a moderate rate. Raw garlic digestion showed a greater OSCs quantitative yield. 2-Vinyldithiin was the garlic compound that showed better permeability and stability.

Keywords

Garlic; cooked garlic; organosulfur compounds; bioaccessibility; permeability; blood stability; Food analysis; Food composition**.**

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.

1 Introduction

Since ancient times, garlic (*Allium sativum* L.) has been used either for its culinary properties or its quality as a medicinal plant. Several recent studies have associated a moderate consumption of garlic with improvements in health (Amagase, 2006; Block, 2010; Iciek et al., 2009). Evidence suggests that its medicinal activities are mainly due to its organosulfur compounds (OSCs) content (Corzo-Martínez et al., 2007; Iciek et al., 2009). Some of the biological activities attributed to these OSCs are antitumoral, antimicrobial, antifungal, antiviral, antiplatelet aggregating, blood pressure and blood glucose modulator, and immune enhancement (Block, 2010; Corzo-Martínez et al., 2007).

When garlic tissue is disrupted, the enzyme alliinase rapidly lyses the cytosolic alkenyl-cysteine sulfoxides (mainly alliin) to form thiosulfinates, mostly allicin. In lyophilized garlic, allicin formation starts when water is added (de Diego et al., 2007). This compound is chemically unstable, thermolabile and it can be transformed upon different culinary processes, producing diverse OSC profiles. Its transformation products are polysulfides, ajoenes and vinyldithiins (Block, 2010; Locatelli et al., 2015). Consequently, due to the variability of OSCs profiles in garlic products, medicinal effects may vary accordingly.

On the other hand it has been reported that OSCs could also be metabolized after being consumed, generating metabolites that could be bioactive (Gao et al., 2013; Iciek et al., 2009; Miron et al., 2010; Rabinkov et al., 1998). Therefore, the claim that consumption of certain products improves health must be supported by studies addressed from different perspectives, such as phytochemical bioactivity, bioaccessibility and bioavailability (Amagase, 2006; Food and Drug Administration (FDA), 2002) . Bioavailability refers to the rate and extent to which the active ingredient or active moiety is absorbed from a product and becomes available at the site of action (Food and Drug Administration (FDA),

2002). While, the bioaccessibility has been defined as the fraction of a compound that is released from its matrix in the gastrointestinal tract and thus becomes available for intestinal absorption (i.e., enters the blood stream) (Fernández-García et al., 2009). The information acquired through bioaccessibility studies can be applied directly to food design and it would contribute to ensure or improve its nutritional efficacy. Bioactive compounds accessibility will be influenced by the composition of the digested food matrix, the synergisms and antagonisms of the different components, physicochemical properties, such as pH, temperature and texture of the matrix. Additionally, processing of vegetable foods can influence the bioaccessibility of nutrients (Carbonell-Capella et al., 2014; Fernández-García et al., 2009; Rein et al., 2013).

Currently, there are several methods to study availability issues; such as *in-silico* predictions, *in-vitro* methods (simulated gastrointestinal digestion, artificial membranes, Caco-2 cell cultures, isolated/reconstituted cell membranes, *ussing* chambers), *ex-vivo* techniques (gastrointestinal organs in laboratory conditions), *in-situ* assays (intestinal perfusion in animals), and *in-vivo* models (animal or human studies) (Carbonell-Capella et al., 2014).

At present, only few studies relate OSC stability with digestive fluids (Freeman and Kodera, 1995; Ramirez et al., 2017; Rosen et al., 2001). However, no information was found about OSCs behavior during cooked garlic gastrointestinal digestion under physiological conditions. Neither were data found regarding the OSC intestinal permeability. Only Lawson hypothesized that OSCs would undergo reactions with cysteine in the intestine, reducing their availability (Lawson, 1993). Concerning OSC stability in blood, only a few works have been found. All of them resulted in lower analytes

concentrations (Freeman and Kodera, 1995; Lawson, 1993; Ramirez et al., 2017; Sun et al., 2006).

Recent research from our group has shown that, after cooking, garlic still contains bioactive compounds and it shows antioxidant activities (Locatelli et al., 2015, 2017). In addition, a specific methodology was developed to analyze OSCs from biological fluids (Ramirez et al., 2017).

Although OSC bioactivities have been extensively studied, little attention has been paid to their bioaccessibility issues. Therefore, the present paper presents information about OSC bioaccessibility and assimilation in raw and cooked garlic. With this objective, an *in-vitro* gastrointestinal digestion, *ex-vivo* intestinal permeation and OSC incubation in blood were performed. Subsequent, OSC analysis was performed by a validated dispersive liquid-liquid microextraction (DLLME) coupled with HPLC-UV analysis (Ramirez et al., 2017). To the authors' knowledge, this is the first time that a study has been carried out to evaluate OSC bioaccessibility and assimilation in raw and cooked garlic.

2 **Materials and methods**

2.1 Reagents and analytical standards

Diallyl sulfide (DAS) (A35801; 97 %), diallyl disulfide (DADS) (32621; 80 %), dimethyl sulfoxide (DMSO) (276855; anhydrous ≥99.9 %), porcine pepsin (P7000), porcine pancreatin (P7545) and porcine bile salts (B8756) were purchased from Sigma-Aldrich (Saint Louis, MO). Diallyl trisulfide (DATS) (98 %) was purchased from LKT Laboratories, Inc. (St. Paul, MN). Chromatography grade acetonitrile (ACN), methanol (MeOH), and dichloromethane (DCM) were purchased from Merck (Kenilworth, NJ). Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Molsheim, France). Allicin was synthesized by oxidation of DADS (González et al., 2007). *E/Z*-4,5,9-

trithiadodeca-1,6,11-triene-9-oxide (ajoene) was obtained by heating and stirring allicin in acetone/water (40:60, *v/v*) (Locatelli et al., 2014). 2-Vinyl-4H-1,3-dithiin (2VD) was synthesized by heating allicin in acetone/methanol (60:40, *v/v*) (Iberl et al., 1990; Locatelli et al., 2014).

2.2 Preparation, conditioning and cooking of garlic samples

Garlic bulbs from the germplasm collection of Instituto Nacional de Tecnología Agropecuaria (INTA) La Consulta, Mendoza, were used in all the experiments. Raw garlic was peeled and then chopped using a garlic press. Then, garlic was cooked by stir frying (sautéed); this process was selected because our group previously reported that best quali-quantitative OSC profile was obtained in this way (Locatelli et al., 2015). After treatments, raw and cooked 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 City, MO). The resulting freeze-dried material was stored until analysis.

2.3 *In-vitro* bioaccessibility analysis

Three individual freeze-dried garlic samples (500 mg) were reconstituted with 1.5 mL distilled water and allowed to stand for 15 min before being subjected to further treatment.

In-vitro gastrointestinal digestion was carried out according to Hedrén et al. (Hedrén et al., 2002) with slight modifications. First, 5 mL of simulated gastric fluid (containing porcine pepsin 1600 U/ml) were added to hydrated garlic samples and pH was adjusted to 2 by 2M HCl addition. Then, samples were incubated at 37 °C with orbital agitation at 250 rpm for 60 min in a closed flask. When gastric digestion was completed, pH was adjusted to 6.8 by 2M NaOH addition and 3 mL of simulated intestinal fluid were added. Samples were incubated for 120 min at 37 °C with orbital agitation at 250 rpm; at the end point, they

were immediately centrifuged for 30 min at 10,600 *g*. The supernatant was subjected to extraction and OSCs quantification by HPLC according to Ramirez et al. (Ramirez et al., 2017) using a liquid chromatograph (Konik KNK- 500-series) with a UV/Vis detector (Konik, Barcelona, Spain).

Bioaccessibility (%) was determined as the ratio between OSCs amount in supernatant (or accessible fraction, $A_{fraction}$) and the initial OSCs amount in garlic (Dahan and Hoffman, 2007).

$$Bioaccessibility\ (\%) = \frac{A_{fraction}}{Initial\ content}.100 \quad (1)$$

2.4- Ex-vivo procedures

All the animals used in these work were cared in accordance with the Guiding Principles in the Care and Use of Animals of the US National Institute of Health. All procedures were approved by the Institutional Animal Care and Use Committee of the School of Medical Sciences, UNCuyo (Protocol approval N° 66/2015).

Adult male Wistar Kyoto rats, weighing between 230–310 g were provided by the *Bioterio de Fisiopatología* (Physiopathology animal facility) of the School of Medical Sciences, UNCuyo. Animals, fasted overnight with free access to water, were sedated and anesthetized by an intraperitoneal injection of xylazine hydrochloride (0.1 mg kg^{-1}) and ketamine (60 mg kg^{-1}). Upon verifying the loss of the pain reflex, a midline longitudinal incision of 4 cm was made. Whole blood was obtained by puncture of the abdominal vena cava and was collected in heparinized tubes. Rats died by exsanguination. Subsequently, the duodenum was located, removed and washed with Tyrode buffer (136.9 mM NaCl, 2.7 mM KCl, 11.9 mM $NaHCO_3$, 4.2 mM NaH_2PO4, 1.2 mM $CaCl_2{\cdot}2H_2O$, 0.5 mM $MgCl_2{\cdot}6H_2O$, 15 mM glucose) for immediate use.

2.4.1- Intestinal absorption of OSCs

The non-everted rat intestinal sac has proved to be a simple and rapid technique; it is influenced by different factors, such as passive and active diffusion, efflux and first-past metabolism (Ruan et al., 2006). The protocol followed was according to Ruan et al. (Ruan et al., 2006) with slight modifications. The clean intestines were prepared into sacs with a length of about 5 cm, and they were filled with 800 µl of the compound and suspended in oxygenated Tyrode buffer. Each intestinal sac contained 100–200 µg mL^{-1} of target analyte suspended in Tyrode buffer and DMSO (final concentration ≤ 1%). Final fill volume, length and diameter of the sacs were recorded for obtaining precise calculations. Then, each non-everted intestinal sac was placed in a conic tube containing oxygenated Tyrode buffer (15 ml). The sacs were maintained in an incubator with an orbital shaker operating at 100 strokes per min at 37 °C. The solution outside the sacs was sampled at 30, 60 and 120 min and replaced with the same volume of fresh oxygenated Tyrode buffer each time. In addition, OSCs mixture permeability was tested; sacs were filled with a solution containing 60 µg mL^{-1} of each compound. The collected volumes were subjected to DLLME and subsequent HPLC analysis (Ramirez et al., 2017). The experiment was carried out within the first two hours of duodenum extraction, by duplicate. The apparent permeability coefficient (P_{app}) was calculated by the following equation:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{C_0 \ A} \qquad (2)$$

Where *dQ/dt* is the steady-state rate of appearance on the acceptor solution, *A* is the surface area of the intestinal sacs, and C_0 is the initial concentration inside the sacs. This coefficient value can be extrapolated to the fraction absorbed in humans (Ruan et al., 2006).

2.4.2- OSC stability in whole blood

E/Z-Ajoene, vinyldithiin, DAS, DADS and DATS were suspended with DMSO (≤ 0.5% final) and added to 1 mL whole blood at 100 µg mL^{-1} final concentration. Samples were incubated at 37 °C for 30, 60 and 240 min. Plasma and packed cells were obtained by centrifugation, 5 min at 6.700 *g* and they immediately underwent extraction. Plasma fraction was extracted by DLLME according to Ramirez et al. (Ramirez et al., 2017) with slight modifications; 800 µL chloroform-acetonitrile mixture (62:38, *v/v*) were quickly added to plasma and centrifuged for 3 min at 425 *g*. Chloroform drop was dried under nitrogen stream and resuspended in 500 µL MeOH. Simultaneously, packed cells were extracted by solid-liquid extraction; 750 µL ACN were added to samples and they were ultrasonicated for 1 min and centrifuged for 10 min at 16,600 *g*. Subsequently, supernatants were filtered and analyzed by HPLC.

Regarding allicin stability, assays were made separately because shorter incubation periods were considered: 1, 3 and 5 min at 37 °C. Its final concentration in whole blood was 100 µg mL^{-1}. After that, samples were centrifuged and extracted as previously described.

2.5- Statistical analysis

For statistical treatment of data INFOSTAT software was employed. All values are expressed as the mean ± standard deviation. *In-vitro* digestion results were compared using Student´s *t*-test ($p < 0.05$). Blood stability results were analyzed by ANOVA and differentiated by Tukey test ($p < 0.05$).

1- Results and discussion

3.1- OSC bioaccessibility in raw and cooked garlic

In order to estimate OCS bioaccessibility, an *in-vitro* gastrointestinal digestion of raw and stir fried garlic was performed. The analytes amounts were measured in the samples

initially hydrated and, then after digestion in the gastrointestinal fluid. Table 1, shows how the qualitative-quantitative OSC profile of raw and cooked garlic samples differs significantly. Only allicin was quantified in raw garlic whereas ajoene, 2-vinyldithiin, DADS and DATS were measured in cooked garlic. This results is consistent with those obtained by other authors (Iberl et al., 1990; Locatelli et al., 2015; Ramirez et al., 2017). In the same table, OSC bioaccessibility results are also shown; amounts found in the simulated gastrointestinal fluid after *in-vitro* digestion represent the fraction that could be absorbed *in vivo* (Dahan and Hoffman, 2007).

In freeze-dried raw garlic samples, allicin could be quantified after digestion; this implies that allicin remains accessible. In this regard, the previous sample hydration was an important step, which allowed allicin formation by allinase enzyme, before it was inactivated in gastric pH (Lawson et al., 2001). In a previous work, Freeman and Kodera (Freeman and Kodera, 1995) studied allicin accessibility of commercial dehydrated garlic tablets; they observed that without a previous hydration only 1% of declared potential allicin was produced under simulated digestive conditions. Furthermore, it was demonstrated that once allicin is formed, gastric or intestinal pH would not be a significant factor affecting allicin availability in the body during digestive periods. Previously, it was estimated that around 90% allicin remained after incubation at 37 °C for 5 hours in water at pH 1.2 and 7.5, in agreement with our results (Freeman and Kodera, 1995; Lawson et al., 2001; Rahman, 2007; Wang et al., 2015).

Our results provide convincing evidence about allicin stability in simulated digestive conditions. This fact triggers the controversy over the use of dehydrated garlic tablets as phytopharmaceuticals, since allinase is inhibited and allicin formation is limited at gastric pH. Therefore, pharmaceutical efficacy of these tablets, consumed without prior

hydration, should not be attributed to the potential allicin, but to other garlic compounds, such as alkenyl cysteine sulfoxides or derivatives thereof, present in the dehydrated garlic powder.

However, despite allicin stability at digestive pH, it is necessary to take into account that a minor proportion (up to 10%) decomposed into new volatile compounds, such as polysulfides, vinyldithiins and ajoene (Block, 2010; Mishra et al., 2001; Rahman, 2007; Wang et al., 2015). Remarkably, these allicin transformation products amounts were higher than those found in cooked garlic, which contains these OSCs naturally. With the advantage in this case, that formed OSCs were located in the digestive fluids and would be accessible to intestinal absorption.

On the other hand, OSCs were 52–87% accessible in cooked garlic samples. To our best knowledge, it was not possible finding data to compare these results, so this is the first time that OSC bioaccessibility has been evaluated in cooked garlic.

DAS amounts could not be detected in stir-fried garlic matrix; although, our previous work had revealed that it could be present in the remaining oil used for stir frying (517 ± 88 μg g^{-1} of DAS) (Locatelli et al., 2015).

Additionally, the sedimented garlic matrix was analyzed to evaluate if quantities of inaccessible OSCs were present. It was possible to detect amounts of ajoene 5.4 ± 0.5, 2VD 7 ± 1, DADS 14 ± 1, DATS 18 ± 4 μg g^{-1}. The levels found were directly related with their liposolubility indexes (corroborated with log S values in ALOGPS 2.1 software); this could be explained because garlic was stir fried. It should be noted that the sum of accessible and non-accessible quantities, compared to the initial OSC amounts in samples, denote degradation of one third of total ajoene, maintenance of 2VD and a slight increase in DADS and DATS. The latter fact may be due to degradation of non-volatile flavor

precursors and more unstable OSCs (Block, 2010; Yu et al., 1994), and possible improved phytochemical extraction from vegetable matrix after digestion (Bhatt and Patel, 2013). According to our results, 13–48% of OSCs from cooked garlic could remain intact without being absorbed in the intestine, acting as *in-situ* antioxidants (Locatelli et al., 2017) and playing an important role by protecting the gastrointestinal tract itself from oxidative damage, and by delaying the origin or development of inflammation (gastritis, Crohn's disease and ulcerative colitis among others) and cancer (especially colon and rectum) (Halliwell et al., 2000).

In general, digestion was not a limiting step for OSC bioaccessibility, given that all target OSCs were found to be accessible to varying degrees (~100% allicin in raw garlic and 52% ajoene, 58% 2VD, 66% DADS, 87% DATS in cooked garlic).

3.2- Intestinal permeability

For OSC intestinal permeability assessment a non-everted rat intestinal sac technique was employed (Ruan et al., 2006). Evidence supports that rat intestine is a suitable model to predict intestinal absorption in humans (Lennernäs, 2014). It is noteworthy that, OSC concentrations were chosen taking into account previous data on cellular cytotoxicity (Cho and Xu, 2000) and considering the final quantities after habitual garlic intake.

Table 2 shows the permeability study results after two hours of incubation. As can be seen from the table, only allicin, 2VD and DAS could pass the intestinal barrier when their intraluminal concentrations were 100–200 μg mL^{-1}. The most permeable compound was 2VD with a 4.6% permeated amount, followed in a decreasing rate by allicin and DADS. In addition, only 2VD could be detected when the intestinal sacs were filled with an OSC mixture at 60 μg mL^{-1} each. Moreover, although 2VD intraluminal amount changed, its

permeation percentage remained around 5%. This could indicate a linear relation between permeated amount and intraluminal OSC concentration.

Based on reported P_{app} coefficients, we can say that OSCs will present low intestinal absorption; previously, a validated *in-vitro/in-vivo* correlation determined that these P_{app} values ($< 1 \times 10^{-6}$) corresponded to an absorption rate of less than 33% in humans (Ruan et al., 2006). However, considering phytochemicals, this is not surprising, since several of them generally show poor absorption, especially since small intestinal metabolism and efflux could significantly limit the uptake of several natural compounds (Holst and Williamson, 2008; Ruan et al., 2006). OSCs with low permeability could be introduced in the blood stream by delivery systems that improve their bioavailability.

Also it should be considered that OSCs could be absorbed through alternative routes after their intake, such as oral mucosa (Squier, 1991), colon (Kinget et al., 1998), and lymphatic system (Porter and Charman, 1997); this would increase their *in-vivo* bioavailability. Nevertheless, remaining OSC intraluminal amounts could act as *in-situ* antioxidants, with consequent beneficial effects on health (Halliwell et al., 2000).

Summarizing, intestinal absorption proved to be a limiting step for OSC availability, as only three compounds got through the enterocytes, at less than 5% of the intraluminal amount under study conditions.

3.3 Blood stability

To study OSC behavior in blood, 100 µg mL^{-1} of target analytes were incubated in whole blood and then, spiked samples were immediately centrifuged and analyzed. Figure 1 shows the OSC amounts remaining at different times, expressed as percentages. These were calculated by relating OSC amount in each fraction with the amount initially added.

After half-hour incubation, ajoene and DATS were not detectable. These results are consistent with previous studies (Lawson, 1993; Sun et al., 2006). On the other hand, 2VD, DAS and DADS were quantified in plasma and packed cells, only 20% of spiked amounts remained detectable. This could be explained by OSC reaction with sulfhydryl residues of plasma proteins (Ramirez et al., 2017) and conjugation with erythrocyte cytoplasmic compounds, such as glutathione (Miron et al., 2000; Zhang et al., 2010).

Regarding OSC distribution among the blood components, a multiple comparisons Tukey test was performed for the compounds in each blood fraction. The results are observed in Figure 1 (and Table 3 of supplementary material); different superscript letters indicate a significant change in the remaining OSC amounts for that incubation time. OSCs were located in a greater proportion in the packed cells fraction, where 2VD and DAS amounts remained statistically constant from half an hour until the fourth hour. On the other hand, all plasma amounts showed a significant decreasing trend between the first and last time. This point would be essential since 15% of accessible 2VD and 13% of DAS could remain stable into the bloodstream. In this regard, Highley and De Brujin (Highley and De Bruijn, 1996) proposed that compounds accumulated in erythrocytes could be exchanged directly with the tissue during their passage through capillaries.

Considering data from a previous study which reported allicin half-life time in blood was less than one minute (Lawson, 1993), incubation times of allicin stability test (in the present study) were shorter than the other analyzed OSCs. Hence, its results were considered separately. Despite this, allicin could not be detected at the first minute. This fact was consistent with Freeman and Kodera, who did not detect allicin in blood after 5 min of incubation; it was also consistent with Lawson, who claimed that allicin half-life was less than 1 min (Freeman and Kodera, 1995; Lawson and Wang, 1993). The complete

and rapid degradation of allicin in blood would suggest that its systemic bioavailability is very limited. Thus, allicin would be an intermediate on the pathway towards other biologically important sulfur compounds (Amagase, 2006; Chan et al., 2013; Iciek et al., 2009; Rahman, 2007). These considerations highlight the need for further research on garlic compounds metabolism, to identify true systemic bioactive compounds.

4 Conclusions

Summarizing, digestion would not considerably affect OSC bioaccessibility, given that the analytes were 52–100% accessible. Raw garlic digestion allowed ajoene, 2VD and DADS formation. On the other hand, intestinal absorption proved to be a limiting step, since only allicin, DAS and 2VD would be able to pass through the intestine, in amounts less than 5%. Finally, there was observed a rapid OSC transformation in blood; less than 20% of 2VD, DAS and DADS could remain. Remarkably, these analytes were found in a greater proportion in blood packet cells.

From the above it could be concluded that raw or stir fried garlic consumption provides OSCs that degrade throughout the assimilation process. Notwithstanding, 2VD would be the most stable compound in the early assimilation stages. Moreover, the importance of OSCs acting as *in-situ* bioactive compounds in the gastrointestinal tract and generating novel bioavailable metabolites with beneficial health properties should not be ruled out.

Conflicts of interest: none.

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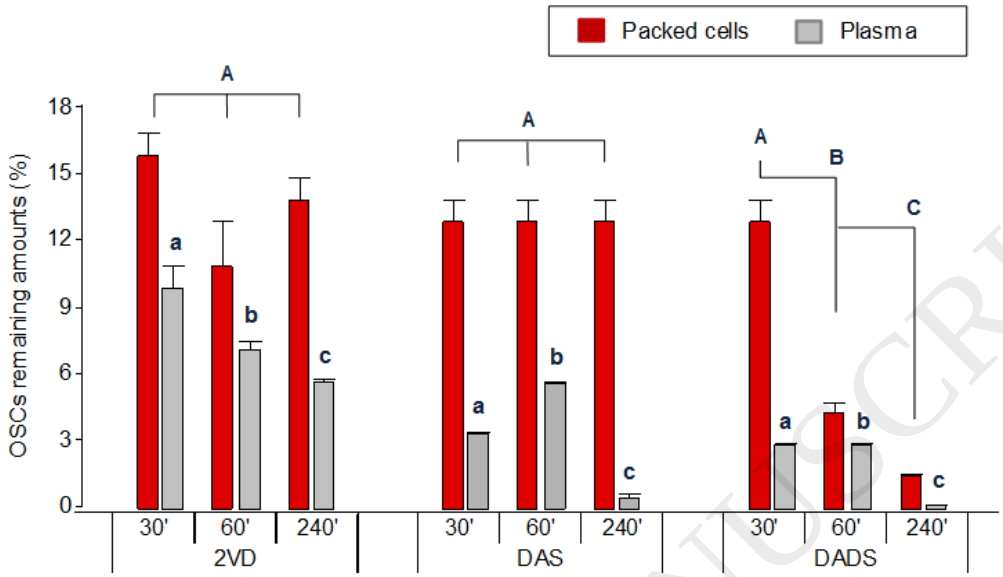
Figure caption

Figure 1 – OSCs remaining amounts in blood at different times [%].
Remainder values in each blood fraction followed by different superscript letters are significantly different ($p \leq 0.05$).



Tables

Table 1 – Organosulfur compounds bioaccessibility in raw and cooked garlic

Treatment		OSCs					
		Allicin	**Ajoene**	**2VD**	**DAS**	**DADS**	**DATS**
Raw garlic	**Initial content** [a]	5870 ± 42 [A c]	n.d. [b]	n.d	n.d.	n.d.	n.d.
	Accessible amount [a]	5906 ± 25 [A]	43 ± 4	41 ± 5	n.d	123 ± 1	n.d.
	Bioaccessibility [%]	100	-	-	-	-	-
Cooked garlic	**Initial content**	n.d.	29 ± 1 [A]	12 ± 3 [A]	n.d.	7.9 ± 0.8 [A]	80 ± 2 [A]
	Accessible amount	n.d.	15 ± 1 [B]	6.8 ± 0.2 [B]	n.d.	5.2 ± 0.3 [B]	70 ± 1 [B]
	Bioaccessibility [%]	-	52	57	-	66	87

a) OSCs content is expressed in μg g^{-1} of freeze-dried garlic powder
b) n.d. not detected (detection limits according to Ramirez et al.)[16]
c) Compound values followed by different superscript capital letters are significantly different ($p \leq 0.05$)

Table 2 – OSCs intestinal permeability parameters

OSCs		Initial lumen amounts [μg ml^{-1}]	Permeated amount [%]	Permeated amount [μg cm^{-2}]	P_{app} [a] [cm s^{-1}]
Allicin		100	1.3 ± 0.1	0.08 ± 0.01	1.1×10^{-7}
Ajoene		118	n.d. [b]	n.d.	n.d.
2VD		200	4.6 ± 0.3	0.50 ± 0.03	3.5×10^{-7}
DAS		200	0.5 ± 0.1	0.08 ± 0.01	5.6×10^{-8}
DADS		210	n.d.	n.d.	n.d.
DATS		200	n.d.	n.d.	n.d.
MIX	**2VD**	60	5.5 ± 0.1	0.16 ± 0.01	3.7×10^{-7}
	Ajoene DAS DADS DATS	60	n.d.	n.d.	n.d.

All compounds were quantified after two hours experiment
a) apparent permeability coefficient
b) n.d. not detected (detection limits according to Ramirez et al.) [16]