

# Organosulfur Compounds from *Allium cepa*

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Species belonging to the genus *Allium* contain many organosulfur compounds (OSCs) that have been widely studied showing their biological properties and beneficial effects on intestinal health and gut microbiota (GM).

Keywords: *Allium cepa* ; dysbiosis ; gut microbiota (GM) ; intestinal health ; organosulfur compounds (OSCs) ; thiosulphonates

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## 1. Introduction

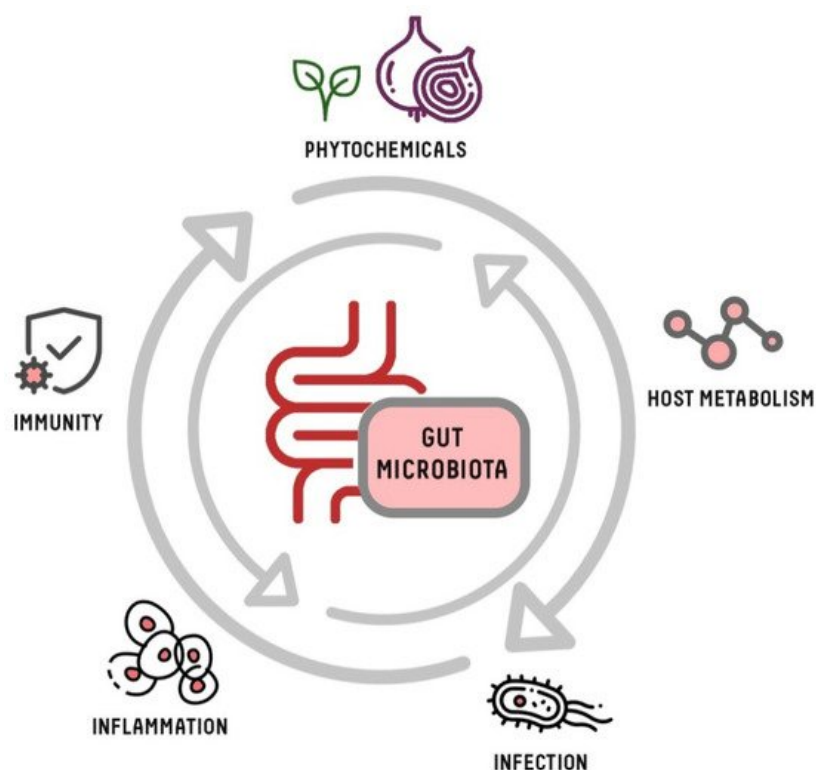
From the moment of their birth, humans are already colonised by microorganisms in the gastrointestinal tract (GT), oral cavity, vagina or skin. Gut microbiota (GM) includes the set of bacteria that colonise the GT and maintains the integrity of epithelium, establishing a physical barrier against pathogens. In addition, to ease digestion and absorption of nutrients, the GM helps metabolism by synthesis of essential nutrients <sup>[1]</sup> and also stimulates the production of antimicrobial compounds in the host. In recent years, plenty of assays have demonstrated that enteric bacteria play a fundamental role in aspects of human health such as the promotion of adaptive immunity, cognition pathologies, inflammatory bowel disease (IBD), metabolic syndrome or colorectal cancer <sup>[2][3][4][5][6][7][8]</sup>. Even host emotions and behaviour have been associated with changes in the ecology of their GM <sup>[9][10]</sup>. In fact, the gut microbiome is currently considered as an independent organ that shows both physiological and pathological effects <sup>[11][12]</sup>.

The composition and development of the newborn's GM are influenced by several aspects, including sex, the gestational age or drug use during the perinatal period <sup>[13]</sup>. Throughout the life of the individual, the symbiotic relationship of GM with the host can be commensal or mutualistic, including a range of factors affecting the composition of GM. There is evidence on the possible coevolution of the host and its autochthonous microbiota since this symbiotic association between host and microbiome could have played an important role in the process of human evolution <sup>[14][15]</sup>. The GM of mammals encompasses more than 100 billion microorganisms, including bacteria, eukaryotes cells such as certain fungi, archaeas and viruses. More than 90% of the bacteria in GM belong to the phylums *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia*, being the two most abundant *Firmicutes* (F) and *Bacteroidetes* (B). Most of the beneficial bacteria present in GM, such as *Lactobacillus* and *Bifidobacterium*, are included in *Firmicutes* phylum <sup>[10][16]</sup>. Imbalances in the composition of GM, also known as dysbiosis, can be caused by DNA mutations, antibiotic therapy, inflammatory processes or the lifestyle, in particular, due to unhealthy eating habits and sedentary behaviours. During dysbiosis, some genera or species of potentially harmful bacteria increase in the GM, leading to a disease-prone condition, such as respiratory, cardiovascular, neurological or gastroenterological disorders <sup>[1][17][18]</sup>. For instance, obese people have a higher F/B ratio compared to people with normal weight, which implies that also obesity could also be related to changes in GM <sup>[19]</sup>.

According to previous studies conducted in both humans and animals, changes in GM can occur rapidly, even within a few hours. These changes alter the functions of microorganisms and are usually characterized by a decrease in diversity and a higher F/B ratio <sup>[15]</sup>, which may lead to chronic metabolic diseases. A well-studied case is the relationship between GM of newborns and gestational diabetes mellitus <sup>[20]</sup>. Other clinical and preclinical studies have shown that the presence of some bacteria belonging to the *Proteobacteria* phylum, such as *Escherichia coli*, *Helicobacter pylori* and *Salmonella enterica*, can be associated with colon inflammation and colorectal cancer <sup>[1]</sup>. On the contrary, there is evidence that the depletion of some species in GM, such as *Faecalibacterium prausnitzii*, might be related to the development of IBD <sup>[21]</sup>.

Both the standard nutrient intake and severe dietary changes affect the composition and structure of GM. Diet-induced dysbiosis is a contributing factor to the development of diseases such as allergy, type 2 diabetes, cardiovascular disorders, IBD, Crohn's disease, ulcerative colitis, irritable bowel syndrome, nonalcoholic fatty liver disease and colorectal cancer <sup>[3][22][23][24][25][26][27][28]</sup>. However, as recent studies suggest, nutritional interventions can also modify the composition of GM in a positive manner, reducing the development of pathologies associated with inflammation such as intestinal diseases, rheumatoid arthritis, asthma and acne <sup>[29][30][31]</sup>.

Dietary strategies conducted with prebiotics and probiotics are able to modulate the composition and functionality of the human GM [32]. Other dietary habits, such as high consumption of fruits and vegetables, can also significantly improve the biodiversity of GM because of their high content in bioactive phytochemicals [11][33]. Phytochemicals are natural compounds produced by plants to help them to resist fungi, bacteria and plant virus infections and also to avoid their consumption by insects or other animals [34]. The most common found in foods are polyphenols, carotenoids, phytosterols, alkaloids, glucosinolates, terpenes and organosulfur compounds (OSCs) [35]. As shown in **Figure 1**, phytochemicals may also affect the composition and structure of GM, and consequently impact the host metabolism, inflammatory response or development of infections.

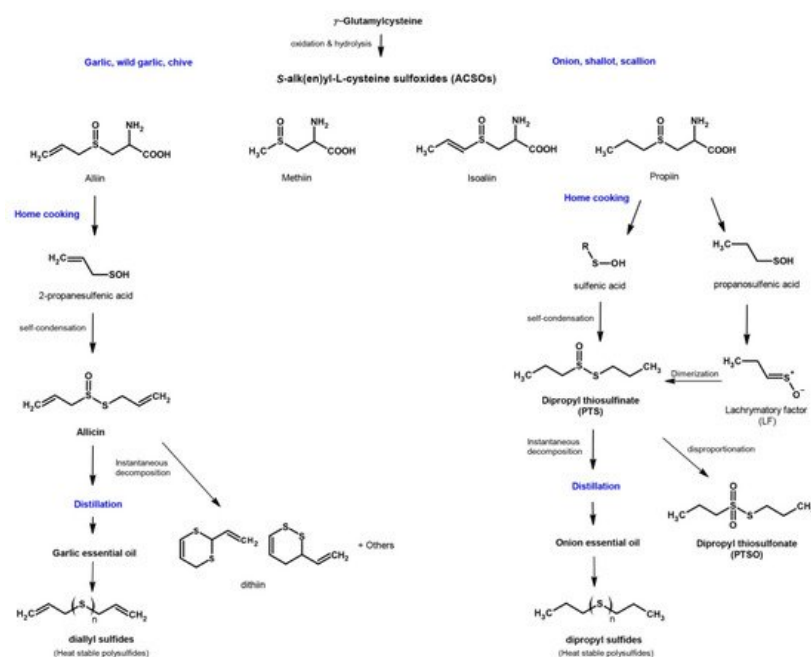


**Figure 1.** Interactions between host and gut microbiota. Phytochemicals influence the intestinal microbiota, which establishes mutual interactions and interdependencies with the host.

The GM plays a key role in the general health condition, having a symbiotic relationship with the host with a direct influence on numerous diseases [29][36] (**Figure 1**). The immune system influences the proliferation and colonisation of certain microorganisms in intestinal microbiota through the production of immunoglobulins or cytokines [37]. In addition, GM regulates host metabolism, immunity and stimulation of the central nervous system [38][39]. The microorganisms of GM transform the components of the food ingested through fermentative processes, producing metabolites that can be beneficial or harmful [18]. These metabolites, in combination with other compounds present in food, modulate the host's immune system. Reciprocally, some components of the diet can modulate the composition and functional capacity of GM, inducing the growth of probiotic bacteria [40]. In this way, diet can severely influence immunity and the progress of a possible host infection, altering the diversity, abundance and functions of GM [11][41] (**Figure 1**).

In recent literature, it can be found how prebiotics, probiotics or phytochemicals influence various pathologies, thus achieving an important therapeutic strategy for the prevention and treatment of human diseases [42][43]. In particular, dietary fibre and phytochemicals can change the composition of GM, inhibiting the growth of pathogens and increasing the population of beneficial bacteria. These bacteria produce some metabolites, such as short-chain fatty acids, which further modify the intestinal environment, improving glycaemic control, lipid profile and inflammation [15][44]. Phytochemicals have been shown to be capable of modulating GM in a few days after their inclusion in carefully controlled diets [1][34]. Although several studies have reported phytochemical-induced GM alterations, much remains to be investigated on the molecular mechanisms and interactions between the gut bacteria involved [34][45]. However, some clinically controlled dietary interventions have studied the effect of these compounds on the human GM, showing a positive influence on the health of the study subjects [17]. Although the most studied phytochemicals are polyphenols, other plant-derived compounds, such as OSCs from *Allium* vegetables, have also shown a significant potential effect to modify the composition of bacterial communities [46][47][48][49].

The genus *Allium* includes more than 900 species of plants, being the most studied species *Allium sativum* (garlic), *Allium cepa* (onion), *Allium ampeloprasum* var. *porrum* (leek) and *Allium ascalonicum* (shallot). The therapeutic properties of these plants are widely known since time immemorial [46][47][50]. OSCs are the most important class of bioactive compounds in *Alliaceae*, which are synthesized during tissue damage in *Allium* as part of the defence mechanism against foreign aggressions. OSCs confer biological properties that are beneficial for health, such as antibacterial, antifungal, antiviral, antiprotozoal, anti-inflammatory, antidiabetic, antioxidant, antimutagenic, hepatoprotective or neuroprotective activities, among others [51][52][53][54][55][56][57]. The main OSCs in the genus *Allium* include the precursors S-alk(en)yl-L-cysteine sulfoxides (ACSO), whose transformation leads to thiosulfonates, thiosulfonates and sulfides in variable amounts and types. The characteristic pungent aroma of each *Allium* species is associated with the different levels of ACSO precursors they contain, which are primarily transformed into thiosulfinate compounds. When *Allium* bulbs are crushed, an enzyme called alliinase, common to all *Allium* species, is released from the vacuoles of cells and catalyses the cleavage of ACSO into sulfenic acid intermediates [58]. These intermediates are highly reactive and produce thiosulfonates, as shown in **Figure 2**.



**Figure 2.** Route of formation of the main organosulfur compounds in garlic and onion.

Nevertheless, thiosulfonates are also highly reactive molecules and, under certain conditions, can decompose to form other sulfur compounds, including symmetric or asymmetric thiosulfonates and sulfides [55][56]. Depending on the *Allium* species, the pathways for the transformation of thiosulfonates and the derivatives formed may differ. In the case of garlic, alliin (S-allyl-L-cysteine sulfoxide) is the most important precursor. It is distributed into the cellular cytoplasm and, when the garlic is chopped or crushed, the contact of alliin and alliinase produces allyl sulfenic acid and dihydroallanine. Two molecules of allyl sulfenic acid are condensed, forming allicin (diallyl thiosulfinate), which is the most important OSC of the genus [55][56]. However, allicin is extremely unstable and, at room temperature, is rapidly transformed by different mechanisms in other compounds such as ajoene, dithiins or diallyl disulfide. In onion, the most common sulfur compounds are isoalliin (S-propenyl-L-cysteine sulfoxide) that changes into lachrymatory factor (Z-propanethial S-oxide); methiin (S-methyl-L-cysteine sulfoxide), also found to be present in garlic; and propiin (S-propyl-L-cysteine sulfoxide) that, due to the action of alliinase, leads to dipropyl thiosulfinate (PTS) [59]. Although PTS is more stable than allicin, it is also a labile compound that, as happens with other thiosulfonates, is transformed into dipropyl disulfide and propyl-propane thiosulfonate (PTSO) through dismutation or disproportionation reactions [55] (**Figure 2**).

## 2. Current Insights on Organosulfur Compounds from *Allium cepa*

The present work aims to discuss 17 articles that have studied the relationship between the consumption of *Allium* products, intestinal health and GM. This work is focused on the influence of the OSCs present in *Allium* species, although there are also some studies that describe beneficial effects on GM of non-sulfur compounds present in the genus *Allium*. For example, the oral administration of quercetin together with resveratrol was able to restore the dysbiosis of the GM induced by HFD in Wistar rats [60]. The results demonstrated that the treatment could modulate the GM of rats, decreasing the population of *Firmicutes* and inhibiting the relative abundance of obesity-related families such as *Coriobacteriaceae*, *Lachnospiraceae*, *Acidaminococcaceae*, *Desulfovibrionaceae* and *Bilophila*. In another recent study, dietary

supplementation with a polysaccharide from Jinxiang garlic alleviated colitis in mice. The treated animals showed an improvement in the structure of the intestinal mucosa, and, in addition, the blocking of certain pro-inflammatory cytokines was observed [61]. We have not found any other systematic review linking OSCs to gut health or GM, except a systematic review and meta-analysis that evaluated solely the use of allicin as a complementary therapy for *H. pylori* infection and whose efficacy was evaluated in randomized controlled trials [62].

Beyond allicin, other OSCs from *Allium* have shown antibacterial activity against Gram-positive and Gram-negative bacteria, including species of the genera *Escherichia*, *Salmonella*, *Bacteroidetes*, *Klebsiella*, *Streptococcus*, *Neisseria*, *Proteus*, *Clostridium*, *Mycobacterium*, *Staphylococcus*, *Micrococcus* and *Bacillus* [51]. Specifically, thiosulfinates and thiosulfonates have significant antimicrobial activity against *Escherichia*, *Salmonella*, *Clostridium*, *Campylobacter* and *Aspergillus* species [63], and also even against numerous strains of antibiotic-resistant bacteria isolated from human clinical samples [51].

OSCs from *Allium* have also shown antibacterial effects against other bacteria associated with severe gastrointestinal symptoms such as *C. difficile*. This bacterium can cause symptoms ranging from diarrhoea to life-threatening inflammation of the colon. Toxins are the main virulence factor that initiates *C. difficile* infection (CDI), causing inflammation and damaging the lining of the intestine. CDI most often affects older adults with long-term healthcare treatment and usually occurs after using antibiotics. For this reason, it could be important to find alternatives to antibiotics for the prevention and treatment of this disorder [64][65]. In a first study conducted by Roshan et al. [64], several natural products were tested against *C. difficile*, with the highest activity exerted by the juice extracted from fresh garlic cloves. The great advantage of using OSCs from *Allium* against CDI is that these compounds have a bactericidal effect against *C. difficile* at lower concentrations than those that affect beneficial bacteria such as *Lactobacillus* species. In a later study conducted by the same researcher team [65], fresh onion extract showed a reduction not only in *C. difficile* but also in the production of its pathogenic toxins, both in Vero (monkey kidney cells) and in HT-29 (human colorectal adenocarcinoma cells) cell lines.

Another species of the same genus, *Clostridium perfringens*, is part of the normal GM of livestock animals, mainly in poultry species such as broilers or turkeys. This bacterium grows under anaerobic conditions and produces toxic spores highly resistant to drying, heat, acids and other harsh environmental factors. Under normal health conditions, beneficial bacteria in the gut keep *C. perfringens* counts small in numbers, thus that it does not cause any disease in animals. However, when conditions change in the GT, the population of this bacteria increases, and the production of toxins may lead to the appearance of intestinal irritation and a disease known as necrotic enteritis, which severely affects the poultry industry [66]. The banning of the use of antibiotic growth promoters (AGP) in animal feed resurfaced this issue, increasing the number of flocks affected and, consequently, the economic losses [67]. Therefore, the inclusion of antimicrobial phytochemicals such as OSCs into feed has become a useful approach to reduce pathogenic bacteria populations in the GM and the reduction of losses caused by the aforementioned disease [68].

There is much evidence that the state of GM in poultry and swine contributes to their health and productivity [69][70]. The influence of the microbiome is particularly relevant in young animals since the composition of their GM is still under development [16]. According to Rubio et al. [71], *Bacteroidetes* family is involved in important metabolic activities in broilers, including the metabolism of carbohydrates and nitrogenous substances, as well as the prevention of the colonization of pathogens such as *Salmonella*, *Campylobacter jejuni* and *C. perfringens*. Other families involved in the composition of the cecal microbiota of broilers are *Lachnospiraceae*, *Ruminococcaceae* and *Micrococcaceae* [71]. Nevertheless, GM of swine is dominated by *Firmicutes*, followed by *Proteobacteria* in the large intestine and *Bacteroidetes* in the small intestine [72].

It has been reported that supplementation with *Allium hookeri* leaves produced modulation of GM in broilers [73]. In addition, the addition of onion powder to the diet of broilers caused a significant reduction in *E. coli* population and the increase of *Lactobacillus* and *Streptococcus* species, achieving an improvement in the gut histomorphology [74]. However, most of the research on the effects of OSCs on GM of birds has been conducted with the use of specific secondary metabolites from onion, as PTSO. For example, Ruiz et al. [75] reported that PTSO is able to modulate the GM composition associated with the ileal mucosa of *Bifidobacterium* species and the *Blautia coccoides*/*Eubacterium rectale* bacterial group of broiler chickens. Other positive data from this study were the increase of certain species of *Bifidobacterium* in the gut, such as *B. pseudolongum*, *B. longum* and *B. pseudocatenulatum*. In another recent article [76], the use of an onion extract containing PTSO was also able to modify GM of laying hens, reducing the number of *Enterobacteriaceae*, *Lactobacillus* and *Bifidobacterium* in faeces after one week of supplementation. In two studies conducted with broilers [63][77], the administration of an *Allium* product containing PTS and PTSO improved the gut health of the treated animals. In one of these assays, PTS and PTSO showed antimicrobial activity against different bacterial groups, reducing the fecal counts of *Enterobacteriaceae* and coliforms [63]. In the second study referred, PTSO

supplementation modulated the GM composition in broiler chickens and also improved the nutrient digestibility without affecting activity of mucosal enzymes [77]. Similar results were obtained in another assay with broilers [61], where a significant bactericidal effect of PTSO against enterobacteria, coliforms, *E. coli*, *C. jejuni* and *Salmonella*, both *in vitro* and *in vivo*, was revealed.

Results showing the benefits of OSCs have also been reported in experiments in swine. Sanchez et al. [78] have shown that the inclusion of PTSO in the growing-finishing pig diet was beneficial for their GM. The authors found a significant increase of *Lactobacillus* and a reduction in the counts of coliforms and enteropathogens (*Salmonella* and *Clostridium*) in faeces, compared to those animals that consumed a basal diet that included antibiotics but not PTSO. The analysis of microbiome in sows, reported in another recent article [79], showed the reduction of intestinal pathogenic species in those animals that ingested an extract composed of garlic and probiotics, compared to the control group. Moreover, in another assay conducted in swine [75], another extract containing PTS and PTSO was used as a feed additive. The results revealed an improvement in the intestinal health of the treated animals, increasing the digestibility of the nutrients.

Taking into account the results mentioned above, it could be concluded that PTSO, when added to the diet of the animals, is capable of reducing certain GM bacterial populations in a dose-dependent manner, being enterobacteria and coliforms the most affected. In another and more recent article, the effect of the intake of an onion and garlic extract including PTS and PTSO was evaluated on the GM of piglets [80]. In this trial, the bacteria diversity in different intestinal regions was analyzed. These data were compared to the gut composition of a group of piglets whose diet was supplemented with antibiotics (colistin and zinc oxide) and another control group. The microbiome of piglets belonging to the *Allium* group and the control group were very similar. On the contrary, the GM of piglets belonging to the antibiotic group showed a lower proportion of bacilli and a higher proportion of Clostridia and Bacteroidia. More specifically, the number of *Lactobacillus* decreased in these animals, especially in the colon, while the genus *Prevotella* increased [80].

Further experiments are necessary to deepen our understanding of the interactions between GM and the host, as well as the mechanisms of action involved. Future studies should try to homogenize, as far as possible, the characteristics of samples to be compared and the methods for detecting bacterial populations. However, given the numerous pieces of evidences discussed, both *in vitro* and in experimental models, the benefits for GM of treatments with OSCs seem clear. However, it would be interesting to perform dietary interventional assays with these products in humans since none has been made until the date with compounds derived from onion such as PTS or PTSO.

### 3. Conclusions

This is the first systematic review about the benefits on the GM and intestinal health by *Allium* products, specifically by secondary metabolites from onion. A total of 530 publications were found from the four different electronic databases used. This number was reduced to thirteen after removing duplicate publications and the application of the inclusion/exclusion criteria established by the reviewer team. In addition, two articles were also found to be eligible from the bibliography section of the pre-selected works, and two extra articles, not identified by the electronic databases, were added as the authors were aware of their existence. Taking into account all of the aforementioned, 17 original articles were included in this systematic review to describe the current scientific evidence of the modulating effect on GM exerted by *Allium* products and, in particular, by OSCs from *Allium cepa*. These compounds showed significant antibacterial activity against a broad spectrum of enteric pathogens, both *in vitro* and *in vivo*, including antibiotic-resistant bacteria isolated from clinical specimens. Numerous *in vivo* assays, conducted with different animal models, reported that the intake of OSCs from onion was able to modulate the composition of GM, increasing the beneficial bacterial populations. Moreover, the effects of these compounds in murine models with colitis and obesity suggested that they could be suitable candidates for the treatment of IBD or reverse the alteration of GM caused by HFDs. Despite the evidence found in different animal models, no article has been found reporting the effects of other OSCs, apart from allicin, in clinical trials or dietary interventions in humans. In this sense, it would be interesting to conduct new research to test the benefits of these compounds in human GM.

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