Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review

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ABSTRACT

Garlic (*Allium sativum* L.) is considered one of the twenty most important vegetables, with various uses throughout the world, either as raw vegetable for culinary purposes, as also an ingredient in traditional and modern medicine. Further, it has been also proposed as one of the richest sources of total phenolic compounds among the usually consumed vegetables, whereas highly ranked regarding its contribution of phenolic compounds in human diet. This review aims to examine all the aspects related with garlic chemical composition and quality, focusing on bioactive properties of the final produce. A particular emphasis is given on the organosulfur compounds contents, since they exert a great contribution to the effective bioactive properties of garlic, including its derived products. The determinant effects of pre-harvest (genotype and various cultivation practices) and post-harvest conditions (storage conditions and processing treatments) on chemical composition and, consequently bioactive potential of garlic are also discussed.

Chemical compounds studied in this article: (E)-ajoene (CID 5386591); (Z)-ajoene (CID 9881148); allicin (CID 65036); alliin (CID 87310); allixin (CID 86374); γ-glutamyl-S-2-propenyl cysteine (CID 11346811); diallyl disulfide (CID 16590); methyl allyl disulfide (CID 62434); S-allyl-cysteine (CID 97939050); 1,2-vinyldiithin (CID 90814902).

Key words: allicin; alliin; bioactive compounds; garlic extracts; phenolics; S-allyl-cysteine sulfoxides

1. Introduction

Garlic (*Allium sativum* L.) is one of the most important vegetables throughout the world with a total harvested area of 1.437.690 ha and an annual production of 24.255.303 tonnes of dry bulbs (FAO, 2013). The importance of garlic is due to its use not only for culinary but also for therapeutic and medicinal purposes in both traditional and modern medicine. It is consumed either as raw vegetable (fresh leaves or dried cloves), or after processing in the form of garlic oil, garlic extracts and garlic powder with differences in chemical composition and bioactive compounds content between the various forms (Lanzotti, Scala, & Bonanomi, 2014).

The main quality feature of garlic products is the distinct flavor of cloves, as the result of complex biochemical reactions (Randle and Lancaster, 2002). The main responsible compounds for that flavor are sulfur-containing non-volatile amino acids (thiosulfinates), among which alliin (**Figure 1**) or S-allyl-cysteine sulfoxide (ACSO) comprises the most predominant garlic flavor precursors (Block, Naganathan, Putman, & Zhao, 1993; Horníčková, Kubec, Cejpek, Velíšek, Ovesná, & Stavelíková, 2010). Apart from their flavor attributes, these sulfur compounds are also responsible for the renowned medicinal properties of garlic (**Figure 2**), and additionally may improve the biosynthesis of glutathione, from which important antioxidant functions are known (Banerjee, Mukherjee, & Maulik, 2003). Other important volatile compounds (**Figure 1**), with prominent bioactive properties are ajoenes (Block et al., 1993), as also several sulfur-containing compounds, such as allicin, 1,2-vinyldithiin, allixin and S-allyl-cysteine (Jabbes, Arnault, Auger, Dridi, & Hannachi, 2012; Kopeć, Piątkowska, Leszczyńska, & Elżbieta, 2013), and sulfides, such as diallyl-, methyl allyl-, and dipropyl mono-, di-, tri- and tetra-sulfides (**Table 2**), which are formed after the decomposition of thiosulfinates (Lanzotti et al., 2014). The volatile nature of these bioactive compounds is highly correlated with the defensive mechanisms of garlic plants against pests and several pathogens, being their release combined with cell damage and plant tissue lesions (Hile, Shan, & Block, 2004). The most characteristic volatile and odorous organo-sulfur compounds of garlic are released after the disruption of the cell membrane, causing the α,β-elimination of alliin and other sulfoxides, located at the cytoplasm level, by the enzyme alliinase, located at the vacuole (Bloem, Haneklaus, & Schnug, 2010).

However, apart from its volatile compounds, garlic is also highly rich in vitamins (especially vitamin B complex and vitamin C), antioxidants, flavonoids, minerals (especially P, K and Se) (Pekowska and Skupień, 2009), being even considered a rich source of other non-volatile phytonutrients with important medicinal and therapeutic properties, from which a particular emphasis is given to flavonoids, saponins and sapogenins, phenolic compounds, nitrogen oxides and amides and proteins (Lanzotti et al., 2014). Γ Γ-glutamyl peptides such as γ-glutamyl-S-2-propenyl cysteine (GluAlC), γ-glutamyl-S-trans-1-propenyl-cysteine (IsoGluAlC) and γ-glutamyl-S-methyl cysteine are important intermediates in the metabolic pathway for ACSO's biosynthesis, being even considered as the storage pools of nitrogen and sulfur (Jabbes et al., 2012). Further, garlic has been also suggested as one of the richest sources of total phenolic compounds among the usually consumed vegetables, whereas highly ranked regarding the *per capita* consumption in human diet (Lanzotti et al., 2014). However, there is a great variation in the total phenolic contents, observed not only among the various genotypes and ecotypes, but also dependent to the cultivation practices and growing conditions (Volk and Stern, 2009). Other quality traits related with the chemical composition, such as total soluble solids, pH and carbohydrate contents, have also shown a great variation among genotypes, regardless of the growing conditions (Pardo, Escribano, Gómez, Alvarruiz, 2007).

On this basis, the present review aims to present all the recent studies regarding the chemical composition and the bioactive properties of garlic, including a particular focus in how they can be affected by various pre-harvest factors and post-harvest conditions.

2. Pre-harvest factors

Various factors that determine the chemical composition of garlic are involved in the production process and, therefore could be a useful mean to enhance the quality and the bioactive properties of the final product. Among these factors, the selection of the genotype is pivotal, with a great variety of cultivars to choose from according to soil and climate requirements, thus allowing for high quality products without compromising the total yield. Cultivation practices, particularly the irrigation regimes and fertilization schedules are also equally important, once they not only contribute to covering the crop requirements in water and nutrients, and to avoiding the stress conditions, but also play a strong inference that could beneficially affect the chemical composition and quality of the produce.

2.1 Genotype

Genotype exerts a significant effect on chemical composition of garlic; therefore the choice of cultivar, according to the climate requirements and market needs, must be essential aiming the quality of the final product. The fact that garlic is propagated asexually in many areas around the world, making farmers use of cloves from the previous growing season, as well as that the existence of various ecotypes comprises different cytotypes, being cultivated in certain areas for many decades, may raise the quality issues in terms of uniformity and content in bioactive compounds (Figliuolo, Candido, Logozzo, Miccolis, & Spagnoletti, 2001). The existent biodiversity and variation related with the biochemical properties of garlic has been increasingly reported. For example, Hirata, Abdelrahman, Yamauchi, & Shigyo (2014) assessed the content of 103 garlic clones collected from various regions throughout the world in S-allyl-cysteine sulfoxide and total phenolics, and observed a wide variation on the chemical composition. Based on these results, the authors concluded that this variation might be attributed to adaptability of these species to various environmental conditions during the expansion and diffusion processes of cultivation throughout the world, as a means of survival under unfavourable environmental conditions. Khar, Banerjee, Jadhav, & Lawande (2011), also observed the existence of variations in sulfoxides contents among 93 garlic ecotypes, and suggested that breeding status, morphological features (bulb colour) and place of origin have lower significant effect on chemical composition than comparing to the genetic background of the studied cultivars.

According to Beato, Orgaz, Mansilla, & Montaño (2011), the selection of cultivar may be a useful means to increase the total phenolics and ferulic acid contents, regardless of the growing conditions. Chen et al. (2013 evaluated 43 garlic cultivars for their phenolic compounds (total phenolics and flavonoids contents) and antioxidant activity through various assays [(DPPH [2, 2-diphenyl-1-picrylhydrazyl] radical scavenging activity, HRSC (hydroxyl radical scavenging capacity), FRAP (ferric ion reducing antioxidant power), CUPRAC (cupric ion reducing antioxidant capacity), and MCA (metal chelating activity)], and to the measured parameters, the authors observed a great variation among the tested cultivars. They also identified three segregated groups of cultivars, with significant differences on the chemical composition, regarding total phenolics and flavonoids contents, and also antioxidant activity. In a similar manner, Fanaei, Narouirad, Farzanjo, & Ghasemi (2014), stated that significant differences occurs in garlic pungency and suitability for long storage and cooking among various genotypes, whereas Jabbes et al. (2012) observed a significant variation in organosulfur compounds of Tunisian garlic landraces. On the other hand, differences on the chemical composition and quality of garlic genotypes may be associated with bulb skin color. For example, Gadel-Hak, Moustafa, Abdel-Naem, & Abdel-Wahab (2011) studying six garlic genotypes with different skin color (three with white and three with purple color), founded significant differences in terms of vitamin C and total fractionated oil contents (higher in purple color genotype), as also in the total phenolic compounds and total flavonoids contents (higher in white color genotype).

2.2 Growing conditions

Growing conditions may significantly affect chemical composition of garlic and, therefore the cultivation in selected areas could be used to ensure a proper manipulation of the bioactive compounds contents and, consequently, the quality of final products. Beato et al. (2011), studied the effect of various growing conditions by cultivating ten garlic genotypes at four different locations, and reported that although growing location did not affect total phenolics and ferulic acid contents, a significant effect on caffeic, *p*-coumaric, *p*-hydroxybenzoic and vanillic acids contents occurred.

Hong, Lee, & Moon (1997), also reported that growing conditions affects the alliin content, as also that fructan content was the combined result from the interaction of location and genotype. Similarly, Montaño, Beato, Mansilla, & Orgaz (2011) studied the organosulfur compounds content (three γ-glutamyl peptides and four cysteine sulfoxides) derived from various garlic cultivars and ecotypes, grown at four different locations of Spain. Based on their findings, the authors suggested that although both genotype and location had a significant effect, the impact of genotype was higher to one of the γ-glutamyl peptides and hardly significant to alliin and isoalliin contents. Thus, from their results it was possible to conclude that the organosulfur compounds profile can be used as a valuable tool to distinguish garlic ecotypes, growing at different locations.

2.3 Irrigation

Irrigation of garlic is essential not only to achieve the maximum potential of yield but also the highest quality of the final product. Despite the importance of irrigation for the quality of most of the vegetables, to our knowledge, so far no reported studies are available regarding the effect of irrigation on chemical composition and quality of garlic. However, Csiszár et al. (2007) have reported that mild water deficit (a decrease in soil water content by 40%) during the growing season, as implemented by water holding for one week at the stage of 3 to 5 leaves, cause significant changes in antioxidants and in the activities of antioxidant enzymes, such as catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), peroxidase (POD) and superoxide dismutase (SOD).

2.4 Fertlization

Garlic is a demanding crop in terms of nutrient requirements and, therefore an intensive and thorough fertilization regime must be carried out to ensure high yields and a good quality of the final product. Diriba-Sshiferaw, Nigussie-Dechassa, Kebede, Getachew, & Sharma (2014) have suggested the application of 92, 40 and 30 kg ha^{-1} of N, P and K respectively, in order to achieve the maximum yield and quality of garlic bulbs. In fact, high amounts of nitrogen (300 Kg ha^{-1}) in two different forms (ammonium sulphate and urea), resulted in an increase of garlic pungency, as expressed by high pyruvic content, and nitrate accumulation in plant tissues (640 mg g^{-1} dry weight), respectively (Ershadi, Noori, Dashti, & Bayat, 2010). In addition, Huchette et al. (2007), suggested that although sulfur fertilization is positively correlated with alliin content in garlic bulbs, the effect of nitrogen in organosulphur compounds content is cultivar dependent, without however a higher content of alliin to be detected, even increasing the nitrogen rates.

Sulfur is the most important nutrient in terms of garlic quality (**Figure 3**), once is highly involved in the bioactive compounds biosynthesis, through its incorporation in the amino acid cysteine, next to the addition of S-(2-propenyl) side chain and oxidation of S, and consequently formation of alliin (Randle and Lancaster, 2002). The application of sulfur during the growing season may significantly increase the alliin content in garlic bulbs, whereas high nitrogen rates exert an adverse effect, or even no effect (Bloem et al., 2010; Bloem et al., 2011; Huchette et al., 2007). In addition, leaf extracts from sulfur treated plants revealed to be a higher content in alliin than non-treated plants, and therefore a more pronounced therapeutic potential (Nasim, Dhir, Samar, Rashmi, & Mujib, 2009).

Apart from alliin, sulfur application has been reported to increase the content of other sulfur containing metabolites, such as cysteine, cysteine sulfoxides, glutathione and glucosinolates (**Figure 3**), both in garlic bulbs and leaves (Bloem et al., 2010; Bloem, Haneklaus, & Schnug, 2011). Moreover, sulfur fertilization in rosy garlic plants (*Allium roesum* L.) has been reported to have a beneficial effect on the flavor and result in an increase of total polyphenols content and in a decrease of reduced carbohydrates (Imen, Najjaa, & Neffati, 2013). However, the effect of sulfur application on the alliin content in garlic bulbs is highly correlated with environmental conditions (light, temperature, carbohydrate content) and genotype (**Figure 3**); however, further tests should be carried out in order to confirm the beneficial effects of the sulfur application and, subsequently to adapt the fertilization regime under specific environments and genotypes (Huchette et al., 2007).

According to Hatwal, Kavita, Choudhary, & Singh (2015), the application of sulfur, either in the form of $ZnSO_4$ (0,4%) or in the elemental form (25 kg ha^{-1}), and vermicompost (15 t ha^{-1}) apart from increase the yield, also improves the garlic cloves quality, in terms of total soluble solids, ascorbic acid, crude protein and sulfur contents. Moreover, Ghasemi et al. (2015) showed that the foliar application of Se, and humic acid via irrigation water, even at low dosage (10 μg mL^{-1} and 10 kg ha^{-1} for Se and humic acid respectively), although resulted in an improvement of the antioxidant activity, exerted a negative effect on the allicin content.

Additionally, the carbohydrates content is also related with garlic bulbs quality, whereas dry weight is mainly accounted by scorodose, a fructan polysaccharide (Fenwick and Hanley, 1985). The application of vermicompost as a growth subtrate resulted in a higher scorodose accumulation, since it induces bulbing, at an earlier stage, allowing for a larger bulb filling period and, then translocation of biosynthetic products from leaves to bulbs (Argüello, Ledesma, Núñez, Rodríguez, & Goldfarb, 2006).

2.5 Other factors

Alliin content in garlic bulbs can be affected by many factors during the cultivation period, as previously described. However another important factor that deserves a particular attention, once is essential for the quality of the final product, is the harvesting stage. According to Bloem et al. (2010), alliin is being translocated from leaves (where its biosynthesis takes place) to bulbs, with an increasing trend during growing season. Although at the earlier growth stages, alliin is accumulated in leaves, as bulb formation initiates and plant approaches harvesting stage alliin and its precursors are translocated and stored in bulbs. Therefore, late harvesting could be used to enhance the alliin content and, consequently, to improve the bioactive potency of the final product. Montaño et al. (2011) reported, for the first time, that although the organosulfur compounds content is highly dependent of the genotype, the alliin and isoalliin contents might be manipulated by adjusting the planting date, with late planting during December having a beneficial effect on their content.

Although farming systems (intensive, conventional and organic farming) have been pointed to affect not only garlic plant growth and yield, but also the quality of the final product from various crops, in terms of chemical composition, no effect on quality features, such as allicin content has been observed so far (Mizraei, Liaghati, & Mahdavi, 2007).

3. Post-harvest conditions

Natural products, in particular those derived from plant sources, have been used over the years, for multiple purposes, not only on their pure forms and crude extracts, but also their individual bioactive compounds (Santhosha, Jamuna, & Prabhavathi, 2013). In fact, there are increasing reports evidencing the direct influence of the maturation process, chemical composition, consumption forms (such as, fresh, cooked, dried, etc.), humidity, fractioning level and harvesting time on the final bioactive potential of numerous natural matrices (Medina and Garcia, 2007; Thomas, 1999). Particularly, processing and storage steps have deserved a pivotal relevance, since slight differences on the chemical composition (particularly, on bioactive components) may cause profound variations on the bioavailability and final bioactivity of natural matrices (Cantwell, Hong, Kang, & Xie, 2001; Rahman, 2007a; Thomas, 1999). Thus, and based on the latest scientific findings, different preparations have been preferably indicated rather than other, depending on the type of clinical affection occurred.

In the case of garlic, its health benefits arise from a wide variety of chemical compounds, and probably from their synergistic interactions (Amagase, 2006). Thus, and since natural matrices possess a complex chemistry, it is feasible to suppose that processing methods may be selected in order to prioritize one or other bioactive ingredient, depending on the desirable result. Furthermore, the efficacy and the clinical recommendation of garlic supplements largely depends from the processing methods employed, while their safety mainly depends on the storage conditions (Amagase, 2006; Veríssimo et al., 2010).

3.1 Processing

Agro-industrial, alimentary and other biotechnological processing techniques are widely used to improve the efficacy and bioavailability of numerous matrices, as well as to reduce some unpleasant characteristics, when present. Especially for garlic, it is not new that some civilizations widely used to use several solvents, such as alcohol, wine, milk and vinegar to soak and, then extract several bioactive ingredients (Amagase, 2006). Recently, and over to the last years, numerous studies have shown that several biochemical modifications and inter-conversions occur during the processing steps. In fact, several reports have shown considerable variations on the final bioactive potential according to the type of garlic preparations used.

Among to the most commonly used processing methods, used in food and pharmaceutical industries, blanching comprises the one of the major importance, and usually applied to garlic; as part of this procedure, peeled garlic cloves are exposed to high temperatures, using water, steam, microwaves, radio frequencies and infrared irradiation (Szymanek, 2011). This technique aims to retain colour and texture, to avoid microbial infections and to hinder enzyme activity after peeling (Jaiswal, Gupta, & Ghannam, 2012). Kinalski and Noreña (2014) have reported that blanching lead to a significant decrease of the thiosulfinates content and antioxidant activity, being these detrimental effects improved with the increase of time and temperature applied.

Garlic can be consumed in the form of extracts, either from raw or dried powder garlic cloves. Lemar, Turner, & Lloyd (2002) observed that using fresh garlic extract preparations exerted a higher anti-*Candida* potential than using dried garlic powder extracts. The authors stated those conclusions through evaluation of the effects on the *Candida* cells morphology and growth inhibition (Lemar et al., 2002). Moreover, other authors have reported that fresh garlic extracts should be preferably used and widely recommended in case of microbial infections (Chudzik, Malm, Rajtar, Kolodziej, & Polz-Dacewicz, 2010), as well as those derived from aqueous extracts (Belguith et al., 2010), since the most prominent effects were achieved by using these garlic preparations. Apart from garlic extracts, Yamazaki and Okuno (2008) have reported that warming cloves at 55 °C, for a period of up to two weeks, was more effective and less time consuming for alliin accumulation comparing to soaking in aqueous ethanol.

Other processing methods usually applied in biotechnological and food industries, such as blanching, boiling, frying and microwaving, did not significantly affect the content in bioactive compounds (anthocyanins, ascorbic acid, flavonoids, flavanols, polyphenols and tannins) and antioxidant activities of garlic (Gorinstein et al., 2009; Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009). However, domestic cooking practices may significantly decrease their contents in bioactive compounds and proteins, and consequently antioxidant activity, especially under prolonged exposure (>20 min) at 100 °C (Gorinstein et al. 2009). In a recent study, carried out by Locatelli, Altamirano, González, & Camargo (2015), the effect of various pre-cooking (chopped, crushed and whole cloves) and cooking treatments (raw, rolling-boil, simmering and stir-frying) on the main organosulfur compounds (OSCs) from garlic cloves was examined. The obtained results revealed significant differences between the various treatments applied, mostly due to different degrees of processing and exposure times to high temperatures. So, and based on these findings, it can be suggested that stir-frying of crushed or chopped cloved is the most preferable cooking treatment, since it allows the formation of OSCs which are not detected in raw form, due to alliinase activation. In contrast, de Queiroz et al. (2013) reported that a significant decrease in both bioactive compounds content and antioxidant activity of garlic occurs after boiling and frying, in spite these differences appears to be attributed to the longer exposure to high temperatures, comparing to the study of Locatelli et al. (2015). Moreover, Cavagnaro, Camargo, Galmarini, & Simon (2007) suggested that the crushing of garlic cloves prior to the cooking treatments (over-heating, boiling and microwaving) seems to alleviate the lost of antiplatelet activity and reduction of the thiosulfinates content. In the same line, Song and Milner (2001) confirmed that crushing of garlic cloves before cooking is essential for retaining their anticancer properties.

On the other hand, the chemopreventive effects of garlic extracts, including its individual constituents, mainly organosulfur compounds, have been also increasingly investigated. Park, Park, & Park (2009) studied the antioxidant and antigenotoxic effects of several extracts derived from garlic and detected a higher content of total phenolic compounds in aged-garlic extracts (AGE), when compared with raw and heated garlic extracts (RGE and HGE, respectively). Moreover, the authors observed that despite the occurrence of a decrease in total phenolic contents and antioxidant activity, after the heating process, garlic extracts retain their antioxidant and protective effects, regardless of the implemented processing method. Sato, Kohno, Hanano, & Niwano (2006) also founded that garlic extracts, after suffers a short-time fermentation significantly increased, both their antioxidant activity (SOD-like and radical scavenging activity) as also content in phenolic compounds. Hong and Kim (1997) after assessing the chemopreventive properties of diallyl sulfides (DAS), garlic extracts, and diallyl disulfides (DADS), they observed different protective effects in rat livers and lungs, which were directly dependent on the garlic preparation used (DAS, garlic extracts or DADS). The authors also stated that the chemopreventive effects provided by allyl-sulfides on chemically-induced carcinogenesis varied according to the used compound, the carcinogen, and the organ site investigated, apart from that these actions revealed to be more complex than previously widely assumed (Hong and Kim, 1997). In fact, organosufur compounds are among the most commonly and representative garlic constituents studied (Higuchi, Tateshita, & Nishimura, 2003; Hong and Kim, 1997; Rahman, 2007a), because apart from their renowned bioactive properties, most of those odorous compounds and largely unstable and easily decomposed. So, after garlic suffers minor processing techniques, some of these compounds are transformed or even disappear.

Moreover, and although garlic oil preparation is a well established technique used in the garlic processing, according to Fujisawa, Suma, Origuchi, Kumagai, Seki, & Ariga (2008), allicin is very unstable when infused in vegetable oil; so, it quickly loses their chemical and biological potential, comparing to ethanol and water infusions. Thus, it is possible to infer that the most important bioactive properties of garlic oil derived from other organosulfur compounds, which not allicin. Further, their instability is the major concern in garlic supplements, since the excipients and encapsulation agents, used to prevent its inactivation by gastric acids, markedly impairs the alliinase activity (Amagase, 2006).

In this sense, it is clearly evident that manufacturing processes markedly affect the final composition and consequently the bioactivity of garlic by-products (**Figure 4**). But, despite of the currently available wide variety of garlic supplements, they mainly fall into one of the four general categories: dehydrated garlic powder, garlic oil, garlic oil macerate and aged garlic extract (AGE) (Amagase, 2006). Furthermore, and not least important, is the fact that among other requisites, manufacturers must ensure the safety, stability and efficiency of garlic supplements, and all the garlic by-products must be properly accompanied by relevant documentation and certification.

3.2 Storage

Taking into account the pivotal importance of several plant products, being some of them included in the condiments and spices group, and used both for culinary and medicinal purposes (such as garlic), assuring proper and safe storage conditions is proved to be of the major importance (**Figure 4**). Proper storage conditions are crucial to retain the high quality of garlic and its derived by-products, taking into account the level of unstability of organosulfur compounds. The natural sensibility of the most abundant bioactive components of garlic is mostly attributed to thermal degradation, which impairs their potency.

3.2.1. Time

Storage time is an involved and pivotal functional determinant for the garlic bioactive properties. As previously highlighted, antioxidant capacity of garlic cloves was maximum after 8 weeks of storage at 20±2 °C, whereas for organosulfur compounds and polyphenols, the maximum content was observed between 6 and 8 weeks of storage, followed by a significant decrease after that time period (Fei, Tong, Wei, & De Yang, 2015). These results has been confirmed by Veríssimo et al. (2010), who reported that allicin content decreased over the storage time, while the antioxidant activity and total phenolics content increased. On the other hand, Horníčková et al. (2010) aiming to investigate the total content of the three main sulfur-containing aminoacids in cloves derived from different garlic genotypes, and moreover to determine the related changes on their sulfoxides contents during storage, they observed a pronounced increase of the S-alk(en)ylcysteine sulfoxides contents. This increase was attributed to the conversion of the corresponding γ-glutamyl dipeptides to sulfoxides, rather than to the loss of water (Horníčková et al., 2010). However, the occurrence of this phenomenon has important implications. Despite isoalliin is the minor garlic constituent, its content has a crucial importance from a technological point of view: isoalliin is a triggering and precursor compound of the occurrence of undesirable blue or blue-green discoloration of several commercial garlic preparations (Horníčková et al., 2010). Thus, in order to avoid the occurrence of this process, garlic should be processed as soon as possible, after harvested. But, aside from these undesirable effects for the food and pharmaceutical industries, in some situations higher levels of free S-alk(en)ylcysteine sulfoxides are beneficial, once these amino acids are precursors of numerous biologically-active compounds (Horníčková et al., 2010). Thus, depending on the final objective and utilization of the garlic raw material, different procedures must be adopted, being its storage for several weeks prior to processing, one of the mostly recommended treatment for garlic-based dietary supplements.

3.2.2. Temperature

Storage temperature is also an important factor that displays a pivotal influence on the chemical composition and consequently final bioactivity potency of garlic. Storage of garlic cloves at low temperatures (5 °C), also known as conditioning, has been reported to affect the expression of 1-SST gene which is related with fructan metabolism and consequently with carbohydrate and total soluble solids content (Bekenblia and Shiomi, 2006; Guevara-Figueroa et al., 2015). In addition, Horníčková et al. (2010) observed an increase in the total amount of the major S-alk(en)ylcysteine sulfoxides (e.g. alliin, methiin and isoalliin) by up to 30%, when garlic bulbs of 58 genotypes were stored at 5 °C. According to Ichikawa, Ide, & Ono (2006), the storage at low temperatures induces the conversion of γ-glutamyl peptides into sulfoxides, such as allin and isoallin. Moreover, when storage at high temperatures (23 °C) is implemented, the conversion of γ-glutamyl peptides is still observed. However, isoalliin is further converted into cycloalliin, thus affecting the quality of the final product.

Veríssimo et al. (2010) observed that the antioxidant potential of garlic decreases, with the increasing temperature. Furthermore, Atashi, Akbarpour, Mashayekhi, & Mousavizadeh (2011) by assessing the variations on the chlorophyll, carbohydrates, amylase and invertase enzymes contents in garlic under low temperatures, they observed qualitative and quantitative differences on the sugar contents of garlic, which consequently stimulated its sprouting. Additionally, the authors reported that during the chilling treatment days, chlorophyll, carotenoid, amylase and investase contents increased significantly, reaching the highest levels on the 30th day after treatment initiation (Atashi et al., 2011).

3.2.3. pH

Physicochemical conditions, such as pH value, which directly reflects the acidity and alkalinity of multiple products, also has an important effect on the final quality of harvested products, the chemical composition and, consequently, the biological potential. For example, Mattos, Silva, & Moretti (2010) studied the physicochemical and functional characteristics of fresh and processed garlic, derived from different origins/cultivars, which showed significant variations. The authors observed the the most acidic cultivar, i.e. cv. Peruano, presented the lower antioxidant activity. Furthermore, the authors observed that freeze-dried garlic presented lower contents in bioactive compounds, among them phenolic compounds (Mattos et al., 2010). On the other hand, several reports have shown that pH value largely affects/determines the garlic volatile compounds formation (Khanum, Anilakumar, & Viswanathan, 2004), among them thiosulfinates formation, and consequently their release after the rupture of cloves (Rahman, 2007a). A representative example, and along the same line with the previously highlighted unstable compound, is allicin, which is destroyed by alliinase. Kopec et al. (2013) concluded that the optimum pH value that contributes to its stability ranges from 4-4.8, whereas at pH values lower than 3.5 or during thermal processing, it loses its activity.

3.3. Conservation

Industrial advances have been also increasingly exploited towards to improve the shelf life of numerous food products. Modified/controlled atmospheres and irradiation are among the most commonly used biotechnological techniques, aiming to ensure the stability and, furthermore to maintain the general microbiological, physicochemical and organoleptic characteristics of garlic, during the post-harvest period (**Figure 4**).

3.3.1. Modified/controlled atmosphere conditions

The potential of modified/controlled to improve and retain the quality of garlic and its derived by-products has been thoroughly studied. Xihong, Zhaojun, Xiuli, & Li

(2010) evaluated the influence of different packaging conditions (15 days of storage at 4ºC) on fresh-cut garlic sprouts. The authors observed that modified atmospheres, in this case polyvinyl chloride (PVC) plastic bags containing a steady-state atmosphere of 5.8 kPa O2 + 7.0 kPa CO_2, were the bags in which fresh-cut garlic sprouts reached/showed the best quality (Xihong et al., 2010). In the same line, Cantwell et al. (2001) studying the effects of different storage conditions (mainly variyng O_2 and CO_2 contents) on the final quality, sprout growth, decay and discoloration of garlic bulbs, they concluded that atmospheres containing CO_2 provide beneficial effects, while low O_2-containing atmospheres alone presented a weak effect. Additionally, when the CO_2 percentage was >15%, several injuries appeared after 4-6 months of storage. Thus, the authors conclued that for fresh peeled garlic, atmospheres containing CO_2 (5-15%) or low O_2 content (1-3%) revealed to be the most effective in retarding garlic discoloration and decay, at 5 ºC and 10 ºC after 3 weeks of storage (Cantwell et al., 2001).

3.3.2. Irradiation

Irradiation techniques have been also increasingly studied, mainly by assessing their ability to improve the garlic shelf life. For example, their efficiency to inhibit the garlic sprouting and mitosis has already been evidenced by Pellegrini, Croci, & Orioli (2000). The authors observed that low gamma radiation doses showed no effects, while doses of 10 Gy significantly reduced garlic sprouting and stopped mitosis process (Pellegrini et al., 2000). Pérez, Aveldaño, & Croci (2007), evaluated the effects of gamma rays in garlic bulbs, and they observed that a dose of 60 Gy, for 8 months, provoked a considerable reduction in lipid and fatty acid contents, with a concomitant reduction of garlic bulbs sprouting occurrence. Finally, the authors concluded that lipids and fatty acids are deeply involved in the normal biosynthetic process of sprout growth and, therefore, the long-term effects of irradiation must be interpreted as the delay or slowing down of this process (Pérez et al., 2007).

However, the radiation process has been also recommended and even applied to avoid microbial contamination during the storage period, as well as to replace chemical fungicides application during the post-harvesting period (Thomas, 1999).

3.3.3. Curing process

The curing process is a non-recent and widespread practice among industries that involves a heat treatment of products prior to other processes. In fact, several reports have confirmed that curing has positive effects on fruit quality and reduces storage losses, without affecting the acidity and color index of numerous foods. Thus, in the case of garlic, a proper curing treatment may confer promissory benefits, namely contributing to improving their shelf life and stability during the storage period. Matan, Matan, & Ketsa (2012) applied heat curing by subjecting garlic oil at 100 ºC and they observed a pronounced increase on the proportion of diallyl disulfide (a major constituent of garlic oil), and at the same time a slight induction of diallyl sulfide decomposition. Therefore, they concluded that heat curing could possess a key role on the enhancement of the antifungal activity of garlic oil (Matan et al., 2012). Furthermore, it has been also pointed that the best garlic flavor develops during the curing process, as well as that inappropriate temperatures can lead to unpleasant organoleptic changes (Medina and Garcia, 2007).

Thus, and despite the current advances, the deepening knowledge on this field should be incited and further exploited, particularly by using different temperatures, exposure times and relative humidity indices.

4. Conclusions

Quality of garlic, as expressed by chemical composition and bioactive compounds content is highly dependent of both pre- and post-harvest conditions. Of particular concern must be the objective of achieving a maximum quality through cultivation practices, genotype selection and growing conditions. However, special care must be also taken through the processing chain, since the organosulfur compounds responsible for the bioactive properties of garlic are very unstable and highly susceptible to the various processing treatments. Moreover, the genetic variability among different garlic populations and ecotypes must be valorised in order to select germplasms with higher content in bioactive compounds and, therefore contribute to improve the garlic and its derived-products quality. The latest aspect is particularly important in temperate regions, where garlic is asexually propagated and genetic preservation is easier.

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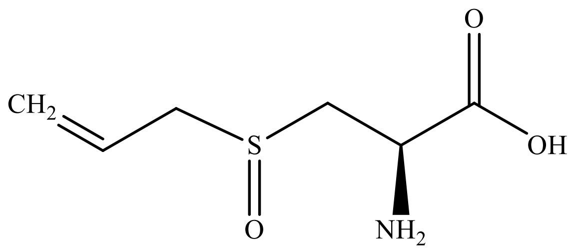
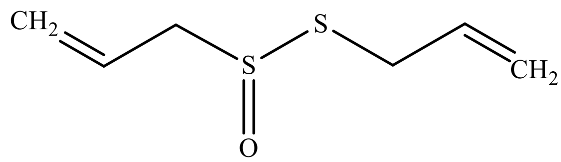
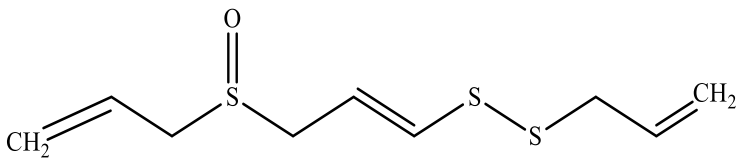


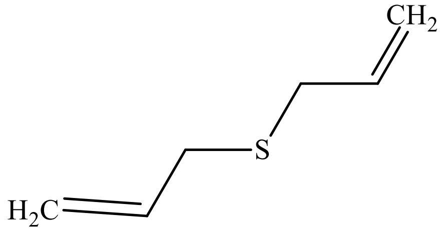
Alliin



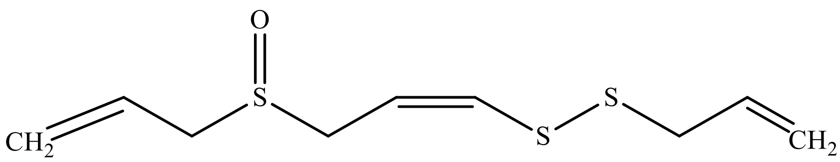
Allicin



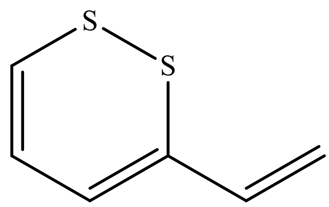
(E)-ajoene



Allyl sulfide



(Z)-ajoene



1,2-vinyldithiin

Figure 1. Stereochemical structure of the most representative bioactive constituents from *Allium sativum* L.: alliin, allicin, allyl sulfide, (E)-ajoene, (Z)-ajoene and 1,2-vinyldithiin.

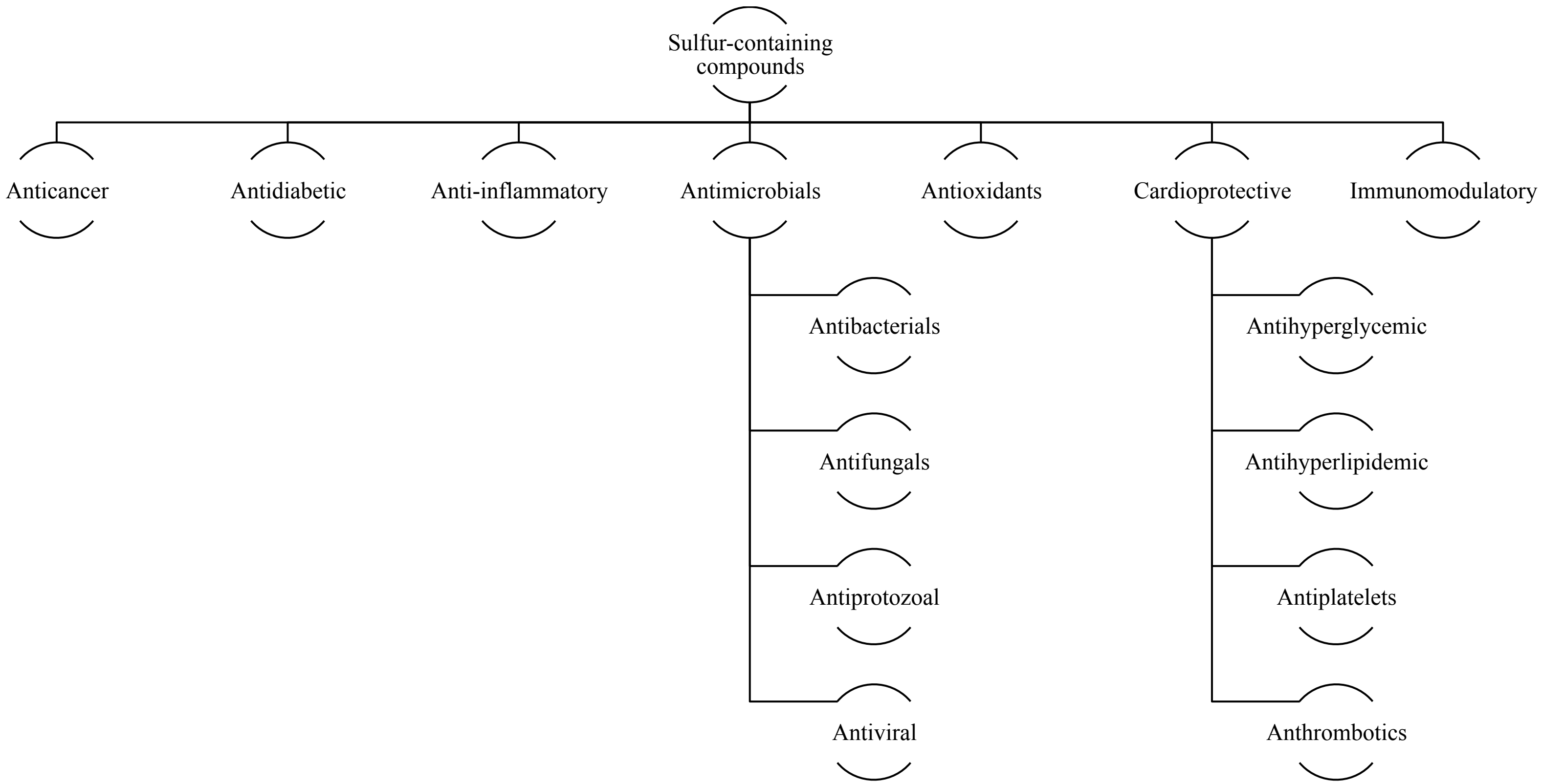


Figure 2. Bioactive properties of the most abundant garlic constituents, sulfur-containing compounds.

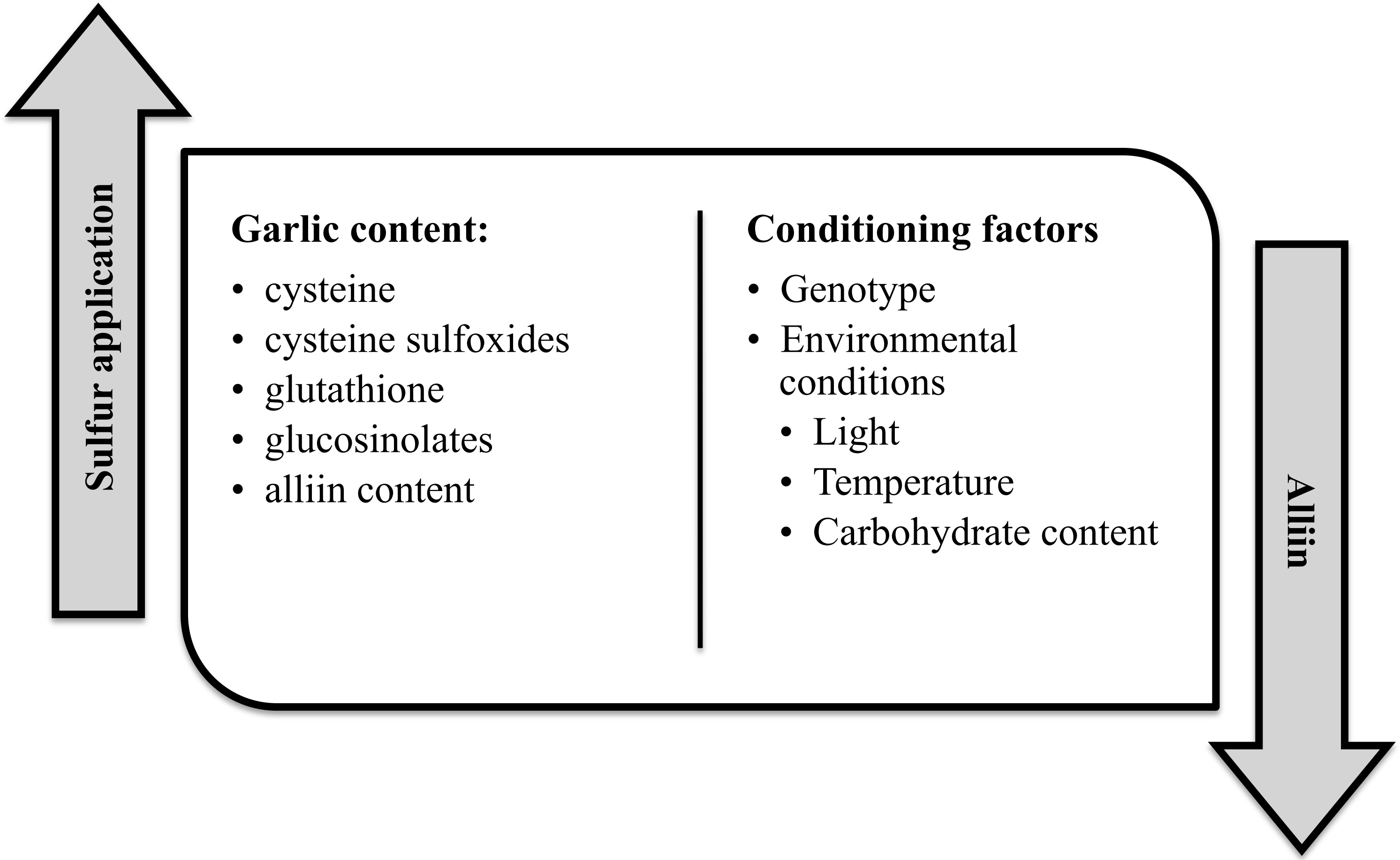


Figure 3. Most important determinant factors to the garlic quality during to the fertilization process

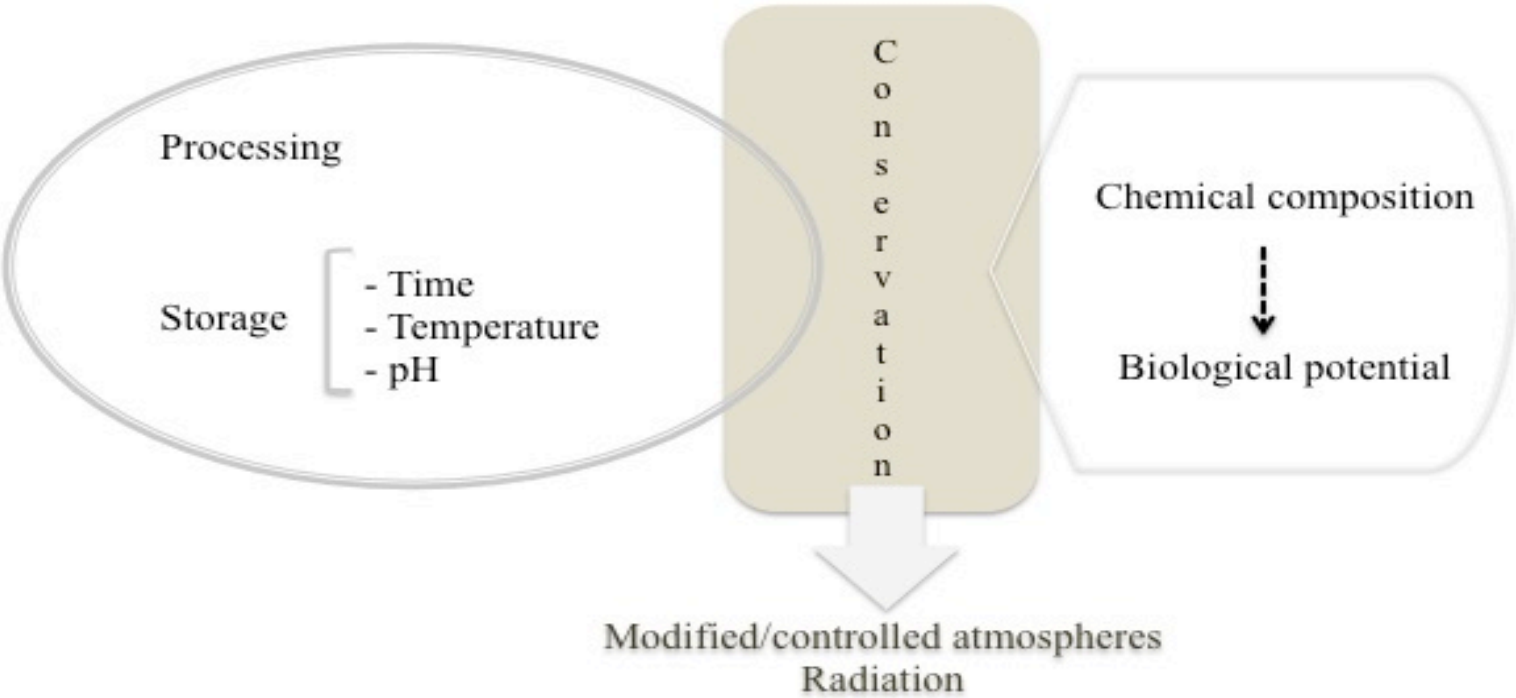


Figure 4. Schematic presentation of post-harvest factors related with garlic quality.

Table 1. Most representative bioactive properties of allicin from *Allium sativum* L., including it related mechanisms of action.

Biological potential	Mode of action	Reference
Anticancer	Blockage of nitrosamines formation and bioactivation Induction of the cytochrome C release by mitochondria Inhibition of cancer cells proliferation Induction of apoptosis (i.e. both caspase-independent and dependent pathways) Enhance the phosphorylation of ERK1/2 map kinases Contribute to the apoptotic DNA-laddering Inhibition of GSH dependent PGH2 to PGE2 isomerase	(Borlinghaus et al., 2014; Kopec et al., 2013; Rahman, 2007b)
Anti-inflammatory	Acts in T-cell lymphocytes by inhibition of SDF1 α -chemokine-induced chemotaxis Inhibition of transendothelial migration of neutrophils Enhance the phosphorylation of ERK1/2 kinase (via p21ras protein thioallylation) Inhibition of TNF α -dependent pro-inflammatory cytokines release Inhibition of phosphatase-activity (directly related with ERK1/2 phosphorylation) Suppression of reactive nitrogen species release	(Borlinghaus et al., 2014; Capasso, 2013; Rahman, 2007b)
Antimicrobial	Strength the activity of immune cells Interaction with thiol-containing enzymes (such as, cysteine proteases and alcohol dehydrogenases)	(Alorainy, 2011; Dini et al., 2011; Ghannoum, 1988; Pârvu et al., 2011;

	Inhibition of acetyl-CoA synthetases Inhibition of spore germination and hyphae growth Induction of glutathione oxidation, leading to a shift of the cellular redox-potential Induction of apoptosis ("oxidative route")	Rahman, 2007b)
Antioxidant	Radicals trapping Interaction with thiol containing proteins Scavenging of hydroxyl radicals Inhibition of superoxide and NO production Modification of SH-dependent activities	(Borlinghaus et al., 2014; Capasso, 2013; Kopec et al., 2013; Rabinkov et al., 1998; Rahman, 2007b)
Cardioprotective	Inhibition of platelet aggregation Reduction of the blood pressure Alteration of the lipid profile Improves the vasodilatation Induction of the Nrf2/Keap1 system Ability to suppress cholesterol biosynthesis (i.e. through inhibition of the squalene-monooxygenase and acetyl-CoA synthetase enzymes)	(Kopec et al., 2013; Kumar et al., 2013; Rahman, 2007a,b)
Imunomodulatory	Strength the activity of immune cells Modulation of macrophage secretory and cellular activities Inhibition of spontaneous and induced TNF-α secretion of pro-inflammatory cytokines and	(Borlinghaus et al., 2014; Kopec et al., 2013; M. S. Rahman, 2007)

chemokines

Table 2. Widely renowned biological functions of the most abundant bioactive compounds of garlic

Name	Biological potential	Reference
Alliin	Antioxidant Antimicrobial	(Pârvu et al., 2011; Rabinkov et al., 1998; M. S. Rahman, 2007)
Ajoenes	Anticancer Antimicrobials Antioxidant Cardioprotective	(Capasso, 2013; Harris et al., 2001; K. Rahman, 2007; Yoshida et al., 1987)
Allyl sulfides	Anticancer Antimicrobials Antioxidant Antithrombotic	(Khanum et al., 2004; Kopec et al., 2013; M. S. Rahman, 2007; Rose et al., 2005)
1,2-vinyldithiin	Antimicrobials Antioxidants Antithrombotic	(Higuchi et al., 2003)