Food Chemistry 211 (2016) 41–50

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Review

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

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A R T I C L E I N F O

Article history:
Received 24 January 2016
Received in revised form 22 March 2016
Accepted 4 May 2016
Available online 6 May 2016

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)

Keywords:
Allicin
Alliin
Allium sativum L.
Antioxidant activity
Bioactive compounds
Garlic extracts
Phenolics
S-allyl-cysteine sulfoxides

A B S T R A C T

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

Contents

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http://dx.doi.org/10.1016/j.foodchem.2016.05.029
0308-8146/

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). Commonly used for culinary purposes, garlic is also interestingly appreciated due to its therapeutic and medicinal properties, both in traditional and modern medicine. Being consumed either as raw vegetable (fresh leaves or dried cloves), or after processing in the form of oil, extract and even powder, pronounced differences in the chemical composition and, consequently, the content in bioactive compounds are observed between the various available garlic formulations (Lanzotti, Scala, & Bonanomi, 2014).

The main quality feature of garlic products is the distinct flavour of cloves, as a result of complex biochemical reactions (Randle & Lancaster, 2002). The main compounds responsible for that flavour are mostly sulfur-containing, non-volatile amino acids (thiosulfinates), among which alliin or S-allyl-cysteine sulfoxide (ACSO) is the most predominant garlic flavour precursor (Block, Naganathan, Putman, & Zhao, 1993; Horníčková et al., 2010; Fig. 1). Apart from their flavour attributes, these sulfur compounds are also responsible for the renowned medicinal properties of garlic, such as anticancer, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, cardioprotective and immunomodulatory activities (Alorainy, 2011; Banerjee, Mukherjee, & Maulik, 2003; Borlinghaus, Albrecht, Gruhlke, Nwachukwu, & Slusarenko, 2014; Capasso, 2013; Chen et al., 2013; Harris, Cottrell, Plummer, & Lloyd, 2001; Higuchi, Tateshita, & Nishimura, 2003; Khanum, Anilakumar, & Viswanathan, 2004; Kopeć, Piątkowska, Leszczyńska, & Elżbieta, 2013; Kumar et al., 2013; Lanzotti et al., 2014). Moreover, they may additionally increase the biosynthesis of glutathione, of which important antioxidant functions are known to use (Banerjee et al., 2003). Other significant volatile compounds (Fig. 1) with potent bioactive properties, are ajoenes (Block et al., 1993), as well as several sulfur-containing compounds other

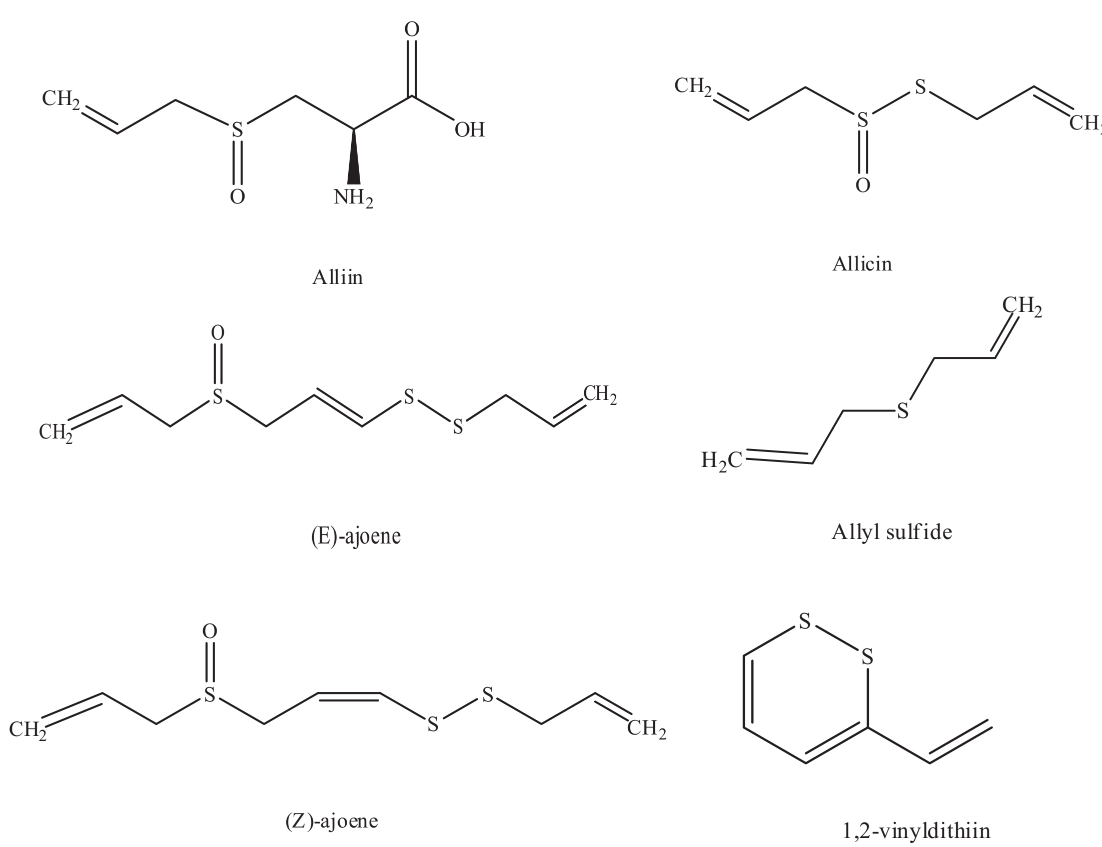

Fig. 1. Stereochemical structure of the most representative bioactive constituents from *Allium sativum* L.: alliin, allicin, allyl sulfide, (E)-ajoene, (Z)-ajoene and 1,2-vinyldithiin.

than alliin, such as allicin (**Fig. 1**, Table 1), 1,2-vinyldithiin, allixin and S-allyl-cysteine (Jabbes, Arnault, Auger, Dridi, & Hannachi, 2012; Kopeć et al., 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) (Fig. 2). The volatile nature of these bioactive compounds is highly involved with the defensive mechanisms of garlic plants against pests and several pathogens, since their release is 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, which are located at the cytoplasm level, by the enzyme alliinase, which is located at the vacuole (Bloem, Haneklaus, & Schnug, 2010).

However, apart from its volatile compounds, garlic is also rich in vitamins (especially vitamins of B complex and vitamin C), antioxidants, flavonoids and minerals (especially P, K and Se) (Pekowska & Skupień, 2009), whereas it is also considered a rich source of other non-volatile phytonutrients, with important medicinal and therapeutic properties, of which a particular emphasis is given to flavonoids, saponins and sapogenins, phenolic compounds, nitrogen oxides, amides and proteins (Lanzotti et al., 2014). 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 of ACSO's biosynthesis, and they are also considered as the storage pools of nitrogen and sulfur within the cell (Jabbes et al., 2012). Furthermore, garlic has been also suggested as one of the richest sources of total phenolic compounds among the usually consumed vegetables, whereas it is highly ranked regarding the *per capita* consumption of phenolics in the human diet (Lanzotti et al., 2014). However, there is a great variation in

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

Name	Biological potential	References
Alliin	Antioxidant Antimicrobial	Pârvu et al. (2011), Rabinkov et al. (1998), Rahman (2007)b)
Ajoenes	Anticancer Antimicrobial Antioxidant Cardioprotective	Capasso (2013), Harris et al. (2001), Rahman (2007)b), Yoshida et al. (1987)
Allyl sulfides	Anticancer Antimicrobial Antioxidant Antithrombotic	Khanum et al. (2004), Kopeć et al. (2013), Rahman (2007b), Rose, Whiteman, Moore, and Zhu (2005)
1,2-Vinyldithiin	Antimicrobial Antioxidant Antithrombotic	Higuchi et al. (2003)

Table 1
The most representative bioactive properties of allicin from *Allium sativum* L., including its related mechanisms of action.

Biological potential	Mode of action	References
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) Enhancement of the phosphorylation of ERK1/2 map kinases Inhibition of GSH dependent PGH2 to PGE2 isomerase	Borlinghaus et al. (2014), Kopeć et al. (2013), Rahman (2007b)
Anti-inflammatory	Activity in T-cell lymphocytes by inhibition of SDF1α-chemokine-induced chemotaxis Inhibition of transendothelial migration of neutrophils Enhancement of the phosphorylation of ERK1/2 kinase (via p21ras protein thioallylation) Inhibition of TNFα-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	Strengthening of the activity of immune cells Interaction with thiol-containing enzymes (such as, cysteine proteases and alcohol dehydrogenases) 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")	Alorainy (2011), Dini, Fabbri, and Geraci (2011), Pârvu et al. (2011), 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), Kopeć et al. (2013), Rabinkov et al. (1998), Rahman (2007b)
Cardioprotective	Inhibition of platelet aggregation Reduction of the blood pressure Alteration of the lipid profile Improvement of the vasodilatation Induction of the Nrf2/Keap1 system Suppression of cholesterol biosynthesis (i.e. through inhibition of the squalene-monooxygenase and acetyl-CoA synthetase enzymes)	Kopeć et al. (2013), Kumar et al. (2013), Rahman (2007a, 2007b)
Immunomodulatory	Strengthening of 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 chemokines	Borlinghaus et al. (2014), Kopeć et al. (2013), Rahman (2007b)

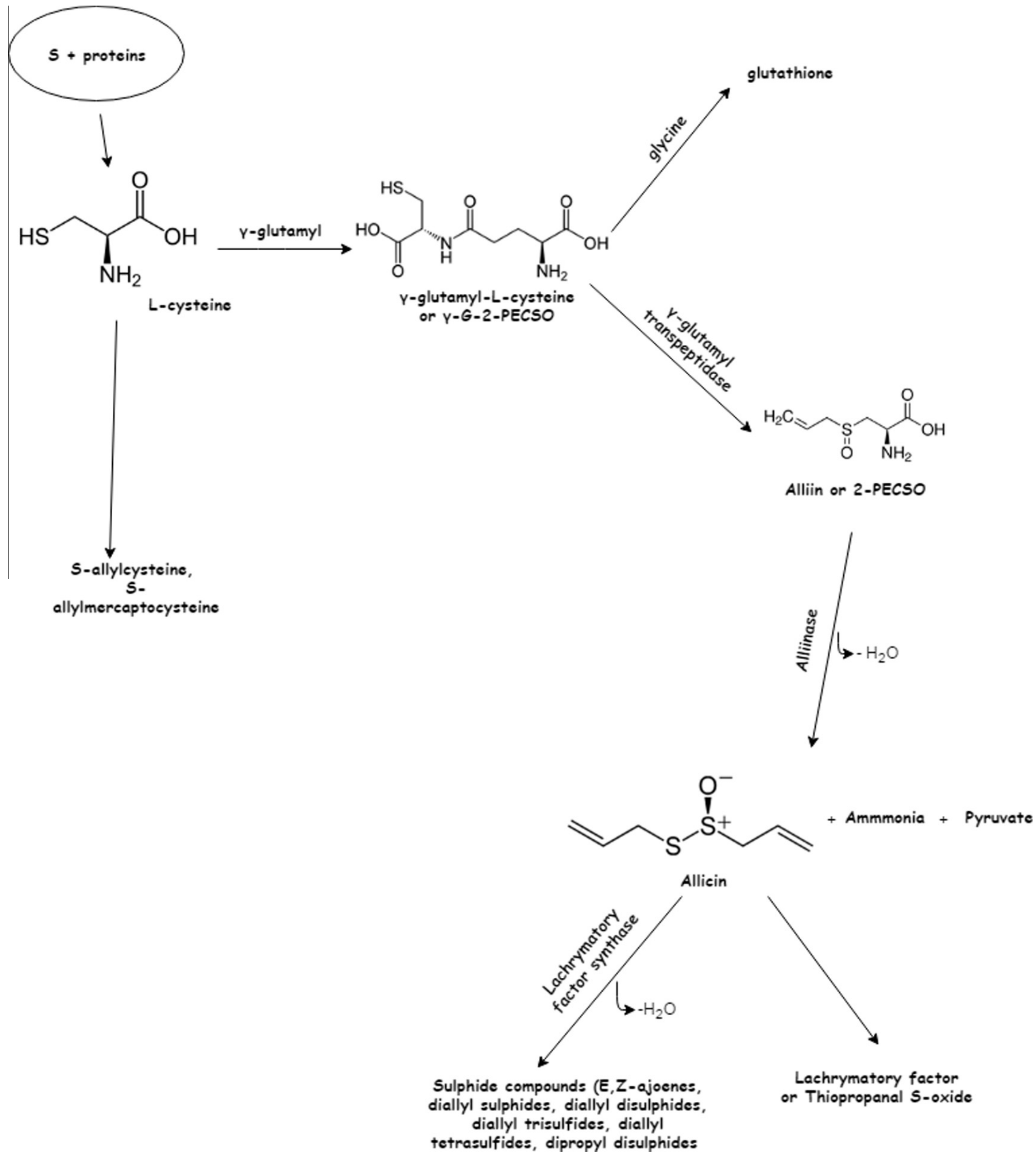

Fig. 2. Biosynthetic pathways of the main organosulfur compounds of *Allium sativum* L.

the total phenolic content, observed not only among the various genotypes and ecotypes, but also between the applied cultivation practices and growing conditions (Volk & Stern, 2009). Other quality traits related with the chemical composition, such as total soluble solids, pH and carbohydrate content, show also 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, focusing on how they can be affected by various pre-harvest factors and post-harvest conditions.

2. Pre-harvest factors

Various factors that affect the chemical composition of garlic are involved in the production process and, therefore could be a useful means 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 regime and fertilization schedule, are equally important, since they not only contribute to covering the crop requirements in water and nutrients, and therefore avoiding the nutrient deficit or water stress conditions, but also could beneficially affect the chemical composition and quality of the final product.

2.1. Genotype

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

According to Beato, Orgaz, Mansilla, and Montaño (2011), the selection of cultivar may be a useful means to increase the total phenolics and ferulic acid content, regardless of the growing conditions. Chen et al. (2013) evaluated 43 garlic cultivars for their phenolic compounds (total phenolics and flavonoids content) 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 they observed a great variation among the tested cultivars. They also identified three segregated groups of cultivars, with significant differences in their chemical composition (total phenolics and flavonoids content) and antioxidant activity. Similarly, Fanaei, Narouirad, Farzanjo, and Ghasemi (2014) reported significant differences among the tested garlic genotypes regarding their pungency and suitability for long storage and cooking, 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 related with bulb skin colour. For example, Gadel-Hak, Moustafa, Abdel-Naem, and Abdel-Wahab (2011) studied six garlic genotypes with different skin colour (three with white and three with purple colour), and found significant differences in terms of vitamin C and total fractionated oil content (higher in purple colour genotype), as well as in total phenolic compounds and total flavonoids content (higher in white colour genotype).

2.2. Growing conditions

Growing conditions may significantly affect chemical composition of garlic and, therefore, the cultivation in selected areas could be used for manipulating the bioactive compounds content and, consequently, the quality of the final product. Beato et al. (2011) studied the effect of 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 content, a significant effect on caffeic, *p*-coumaric, *p*-hydroxybenzoic and vanillic acids content was observed. Hong, Lee, and Moon (1997) also reported that growing conditions affect the alliin content, as well as that fructan content was the combined result of the interaction of location and genotype. Similarly, Montaño, Beato, Mansilla, and 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 the results, the authors suggested that although both genotype and location had a significant effect, the impact of genotype was higher to the γ-glutamyl peptides content and hardly significant to the alliin and isoalliin content. Thus, from these results it can be concluded that the organosulfur compounds profile can be used as a valuable tool to distinguish garlic ecotypes, grown at different locations.

2.3. Irrigation

Irrigation of garlic crop is essential to achieve not only the maximum potential of yield but also the highest quality of the final product. Despite the importance of irrigation for the quality of most 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 growth stage of 3–5 leaves, caused significant changes in antioxidants and the activities of antioxidant enzymes, such as catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), peroxidase (POD) and superoxide dismutase (SOD).

2.4. Fertilization

Garlic is a demanding crop in terms of nutrient requirements and, therefore, an intensive and complete fertilization regime must be carried out to ensure high yields and good quality. Diriba-Shiferaw, Nigussie-Dechassa, Kebede, Getachew, and 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 sulfur fertilization is positively correlated with alliin content in garlic bulbs, whereas the effect of nitrogen application rate on organosulfur compounds content is cultivar dependent, without, however, a higher content of alliin to be detected when the nitrogen rates increased.

Sulfur is the most important nutrient in terms of garlic quality (Fig. 3), since it is highly involved in the bioactive compounds biosynthesis, firstly through its incorporation in the amino acid

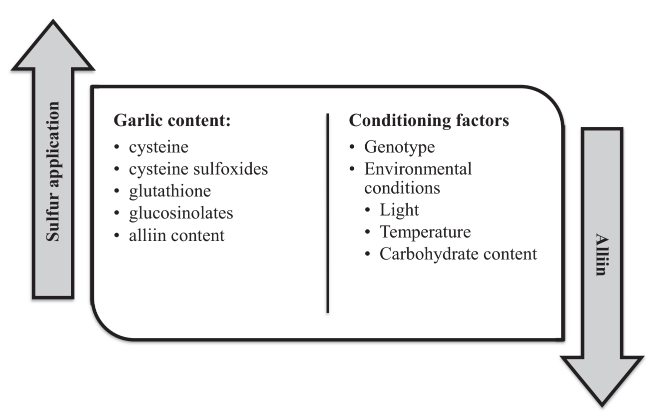

Fig. 3. The most important determinant factors to the garlic quality related with sulfur application.

cysteine, next with the addition of S-(2-propenyl) side chain and the oxidation of sulfur, and finally with the formation of alliin (Randle & 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 have no effect (Bloem, Haneklaus, & Schnug, 2011; Bloem et al., 2010; Huchette et al., 2007). In addition, leaf extracts from sulfur treated plants revealed to have a higher content of alliin than in 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 (Fig. 3), both in garlic bulbs and leaves (Bloem et al., 2010, 2011). Moreover, sulfur fertilization in rosy garlic plants (*Allium roseum* L.) has been reported to have a beneficial effect on the flavour and results in an increase of total polyphenols content and decrease of reduced carbohydrates (Imen, Najjaa, & Neffati, 2013). The effect of sulfur application on the alliin content in garlic bulbs is highly correlated with environmental conditions (light, temperature, carbohydrate content) and genotype though (Fig. 3). However, further tests should be carried out in order to confirm the beneficial effects of sulfur application and, subsequently to adapt the fertilization regimes to specific growing conditions and genotypes (Huchette et al., 2007).

According to Hatwal, Kavita, Choudhary, and Singh (2015), the application of sulfur, either in the form of $ZnSO_4$ (0.4%), or the elemental form (25 kg ha^{-1}), and vermicompost (15 t ha^{-1}) apart from increasing the yield, it also improved the garlic cloves quality in terms of total soluble solids, ascorbic acid, crude protein and sulfur content. Moreover, Ghasemi et al. (2015) showed that although the foliar application of Se, and humic acid via irrigation water, even at low rates (10 μg ml^{-1} and 10 kg ha^{-1} for Se and humic acid, respectively) resulted in an increase of the antioxidant activity, it had also a negative effect on the allicin content.

Additionally, the carbohydrates content is also related with garlic bulbs quality, whereas dry weight is mainly consists of scorodose, a fructan polysaccharide (Fenwick & Hanley, 1985). The application of vermicompost and soil as a growth substrate resulted in a higher scorodose accumulation, mostly because it induced bulbing at an earlier stage, and therefore allowed for a larger bulb filling period and consequently the translocation of more biosynthetic products from leaves to bulbs, comparing to soil with no vermicompost added (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 particular attention, since it 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 the 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 from leaves and stored in bulbs. Therefore, late harvesting could be used as a means to enhance the alliin content and, consequently to improve the bioactive potency of the final product. Montaño et al. (2011) reported that although the organosulfur compounds content is highly dependent on the genotype, the alliin and isoalliin content could be manipulated by adjusting the planting date, with late planting during December having a beneficial effect on their final content at harvest stage.

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

3. Post-harvest conditions

Natural products, especially those derived from plant sources, have been used over the years for multiple purposes, not only in their pure forms and crude extracts, but also as individual bioactive compounds (Santhosha, Jamuna, & Prabhavathi, 2013). In fact, there are increasing reports confirming the direct influence of maturation process, chemical composition, consumption forms (e.g. raw, cooked, dried, etc.), humidity, fractioning level and harvesting time on the final bioactive potency of numerous natural matrices (Medina & Garcia, 2007; Thomas, 1999). More specifically, processing and storage conditions are of major interest, since slight differences in the chemical composition and bioactive compounds content may result in profound variations in the bioavailability and final bioactivity of natural matrices (Cantwell, Hong, Kang, & Xie, 2001; Rahman, 2007a; Thomas, 1999). Therefore, and based on the latest scientific findings, different preparations have been preferably indicated as more beneficial 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). Therefore, considering that natural matrices possess a complex chemistry, it could be suggested that specific processing methods may be selected in order to prioritize one or the other bioactive ingredient, depending on the desirable result. Furthermore, the efficacy and the clinical recommendation of garlic supplements largely depends on the implemented processing methods, 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 soak bulbs in various solvents, such as alcohol, wine, milk and vinegar and extract several bioactive ingredients (Amagase, 2006). During the last few 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 in the final bioactive potential according to the type of garlic preparations.

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

Garlic can be consumed in the form of extracts, either from raw or dried powder garlic cloves. Lemar, Turner, and Lloyd (2002) observed that using fresh garlic extract preparations had a higher anti-*Candida* potential than using dried garlic powder extracts. The authors confirmed these conclusions after evaluating the effects of extracts 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 forms of 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 of cloves 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 antioxidants activity of garlic (Gorinstein et al., 2009; Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal, & Murcia, 2009). However, domestic cooking practices may significantly decrease garlic content in bioactive compounds and proteins, and consequently the antioxidant activity, especially after prolonged exposure (>20 min) at 100 °C (Gorinstein et al., 2009). In a recent study carried out by Locatelli, Altamirano, González, and 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 applied treatments, mostly due to different degrees of processing and exposure times to high temperatures. Therefore, it could be suggested that stir-frying of crushed or chopped cloves is the most preferable cooking treatment, since it allows the formation of OSCs (which are not detected in raw form), due to alliinase activation after processing, whereas the exposure to high temperatures is less detrimental comparing to the other cooking treatments. In contrast, de Queiroz et al. (2014) reported that a significant decrease in both bioactive compounds content and antioxidant activity of garlic occurs after boiling and frying; however, these different results could be attributed to the longer exposure to high temperatures, comparing to the study of Locatelli et al. (2015). Moreover, Cavagnaro, Camargo, Galmarini, and Simon (2007) suggested that crushing the garlic cloves prior to the cooking treatments (over-heating, boiling and microwaving) seems to alleviate the loss of antiplatelet activity and the reduction of thiosulfinates content. Similarly, Song and Milner (2001) confirmed that crushing the garlic cloves before cooking is essential in order to retain 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, and 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 to raw and heated garlic extracts (RGE and HGE, respectively). Moreover, the authors observed that despite the decrease in total phenolic content and antioxidant activity after the heating process, garlic extracts retained their antioxidant and health protective properties, regardless of the implemented processing method. Sato, Kohno, Hanano, and Niwano (2006) also found that garlic extracts, after subjecting them to a short-time fermentation, significantly increased both their antioxidant activity (SOD-like and radical scavenging activity) and their phenolic compounds content. Hong and Kim (1997) assessed the chemopreventive properties of diallyl sulfides (DAS), garlic extracts, and diallyl disulfides (DADS), and observed different protective effects on 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 of allyl-sulfides on chemically-induced carcinogenesis varied according to the used compound, the carcinogen, and the organ site investigated, whereas all these actions revealed to be more complex than previously widely assumed (Hong & Kim, 1997). Organosulfur compounds are among the most commonly and representative garlic constituents studied (Higuchi et al., 2003; Hong & Kim, 1997; Rahman, 2007a), because apart from their renowned bioactive properties, most of those odorous compounds are largely unstable and easily decomposed. So, after garlic cloves are subjected to 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 et al. (2008), allicin is very unstable when infused in vegetable oils; therefore, it rapidly loses its chemical and biological potency, when compared to ethanol and water infusions. Thus, it could be suggested that the most important bioactive properties of garlic oil are derived from other organosulfur compounds, apart from allicin. Furthermore, allicin instability is the major concern in garlic supplements, since the excipients and encapsulation agents, usually used to prevent allicin inactivation by gastric acids, markedly impair 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 (Fig. 4). However, despite the currently available wide variety of garlic supplements, they mainly fall into one of the following four general categories: dehydrated garlic powder, garlic oil, garlic oil macerate and aged garlic extract (AGE) (Amagase, 2006). Furthermore, among other requisites the 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 that several plant products (such as garlic), are considered as condiments or spices, and can be used for both culinary and medicinal purposes, ensuring proper and safe storage conditions is proved to be of major importance (Fig. 4). Proper storage conditions are crucial to retain the high quality of garlic bulbs and its by-products, taking into account the high instability of organosulfur compounds. The natural sensitivity of the most abundant bioactive components of garlic is mostly attributed to thermal degradation susceptibility, which impairs their potency.

Industrial advances have also been increasingly implemented towards improving 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 (Fig. 4).

3.2.1. Time

Storage duration is crucial for the bioactive properties of garlic. As previously reported, antioxidant capacity of garlic cloves was at a 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 have been also confirmed by Veríssimo et al. (2010), who reported that allicin content decreased over the stor-

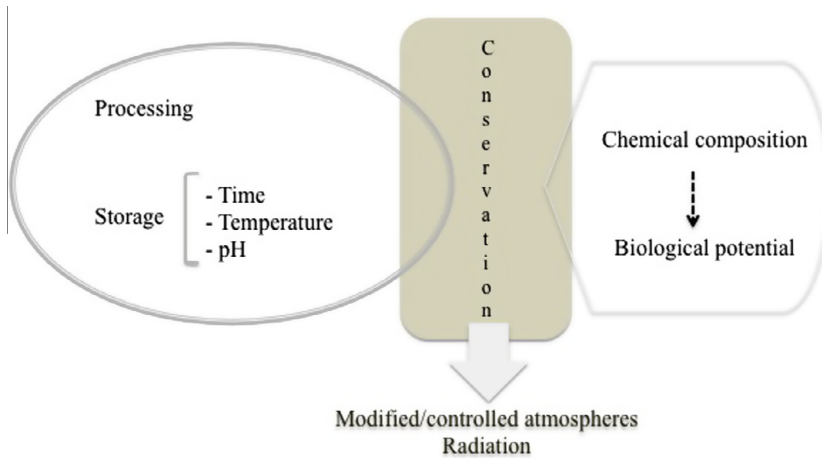

Fig. 4. Schematic presentation of post-harvest factors related with garlic quality.

age time, while the antioxidant activity and total phenolics content increased. On the other hand, Horníčková et al. (2010) investigated the total content of various garlic genotypes in regards of the three main sulfur-containing aminoacids, as well as the related changes of their sulfoxides content during storage, and observed a pronounced increase of the S-alk(en)ylcysteine sulfoxides content. This increase was attributed to the conversion of the corresponding γ-glutamyl dipeptides to sulfoxides, rather than the water loss (Horníčková et al., 2010). Despite isoalliin being a minor garlic constituent, its content has a crucial importance from a technological point of view: isoalliin is a triggering and precursor compound for the development 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 harvest. In spite of these undesirable effects for the food and pharmaceutical industries, on some occasions higher levels of free S-alk(en)ylcysteine sulfoxides are beneficial, since these amino acids are precursors of numerous biologically-active compounds (Horníčková et al., 2010). Therefore, depending on the final objective and further use of the garlic raw material, different procedures must be adopted, with storage for several weeks prior to processing being one of the mostly recommended treatments for garlic-based dietary supplements.

3.2.2. Temperature

Storage temperature is also an important factor since it can affect the chemical composition and consequently the 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 & Shiomi, 2006; Guevara-Figuero 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) of up to 30%, when garlic bulbs of 58 genotypes were stored at 5 °C. According to Ichikawa, Ide, and Ono (2006), the storage at low temperatures induces the conversion of γ-glutamyl peptides into sulfoxides, such as alliin and isoallin, whereas 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, and Mousavizadeh (2011) assessed the variations on the chlorophyll, carbohydrates, amylase and invertase enzymes content in garlic under low temperatures; qualitative and quantitative differences in the sugar content, which consequently stimulated sprouting process were observed. Additionally, the authors reported that during the chilling treatment days, chlorophyll, carotenoid, amylase and investase content 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, their chemical composition and, consequently, their biological potential. For example, Mattos, Silva, and 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 that the most acidic cultivar, i.e. cv. Peruano, presented the lower antioxidant activity. Furthermore, the authors observed that freeze-dried garlic presented lower content in bioactive compounds, especially in phenolic compounds (Mattos et al., 2010). On the other hand, several reports have shown that pH value can affect/determine the garlic volatile compounds formation (Khanum et al., 2004), especially thiosulfinates formation, and consequently their release after the rupture of the cloves (Rahman, 2007a). A representative example of such compounds is allicin, which is a very unstable compound. Kopeć et al. (2013) concluded that the optimum pH value that contributes to allicin stability ranges from 4 to 4.8, whereas at pH values lower than 3.5, or during thermal processing, the enzyme alliinase that is responsible for allicin degradation loses its activity.

3.2.4. 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, Li, Zhaojun, Xiuli, and Li (2010) evaluated the influence of different packaging conditions (15 days of storage at 4 °C) on fresh-cut garlic sprouts. The authors observed that fresh-cut garlic sprouts reached/showed the best quality when they were stored under modified atmospheres, in this case polyvinyl chloride (PVC) plastic bags containing a steady-state atmosphere of 5.8 kPa O_2 + 7.0 kPa CO_2 (Xihong et al., 2010). Similarly, Cantwell et al. (2001) studied the effects of different storage conditions (mainly varying O_2 and CO_2 content) on the final quality, sprout growth, decay and discoloration of garlic bulbs, and they concluded that atmospheres containing CO_2 have a beneficial effect, while those of 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. Therefore, the authors concluded that for fresh peeled garlic cloves, atmospheres containing CO_2 (5–15%) and/or low O_2 content (1–3%) revealed to be the most effective in retarding garlic discoloration and decay, at 5 °C and 10 °C and after 3 weeks of storage (Cantwell et al., 2001).

3.2.5. 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 confirmed by Pellegrini, Croci, and Orioli (2000). The authors observed that low gamma radiation doses showed no effects, while doses of 10 Gy reduced significantly garlic sprouting and stopped the mitosis process (Pellegrini et al., 2000). Pérez, Aveldaño, and Croci (2007), evaluated the effects of gamma rays on garlic bulbs, and they observed that a dose of 60 Gy, for 8 months, caused a considerable reduction in lipid and fatty acid content, 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 could be interpreted as the delaying or slowing down of this specific 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).

3.2.6. 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, however, affecting the acidity and colour index of foods. Thus, in the case of garlic, a proper curing treatment may have promissory benefits, especially by improving their shelf life and stability during the storage period. Matan, Matan, and Ketsa (2012) applied heat curing by subjecting garlic oil at 100 °C, and they observed a pronounced increase in 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 be a useful means in order to enhance the antifungal activity of garlic oil (Matan et al., 2012). Furthermore, it has been also pointed out that the best garlic flavour develops during the curing process, as well as that, improper temperatures can lead to unpleasant organoleptic changes (Medina & Garcia, 2007).

Thus, and despite the current advances, expanding the knowledge in 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. Special interest must be given on the objective of achieving maximum quality through cultivation practices, genotype selection and growing conditions. In addition, special attention must be given to 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 the various garlic populations and ecotypes must be further exploited in order to select germplasms with higher content in bioactive compounds which could contribute to improvement of 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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