

Review

Selenized garlic: a future prospect or already a current functional food?

Ajo selenizado: ¿un alimento funcional futuro o actual?

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ABSTRACT

In the last years, functional foods have awakened consumer, scientific and business interest. A commonly found vegetable in such kind of foods includes garlic (*Allium sativum*). By its ability for selenium (Se) bio-accumulation, garlic can turn into an attractive option of selenized food. Selenium is an essential micronutrient for many organisms including plants, animals, and humans. It is an important trace element due to its antioxidant properties and plays a main role in prevention of cancer and cardiovascular diseases. Nowadays, there is an increasing interest in the study of Se speciation due to the different roles that each species manifests in toxicological and nutrition fields. However, Se exhibits a narrow interval between toxicity and essentiality, which is puzzling toxicologists and alarming nutritionists and legislators. In the present review, an overview on the development of selenized garlic studies and its potential implementation in Argentine production is exposed. The development of novel foods with added value such as selenized garlic could be an attractive alternative for local market. Moreover, it becomes a good offering for factory owners, considering that Mendoza represents about 85% of total garlic production in the country.

Keywords

garlic • selenium • functional food • selenized foods • speciation

RESUMEN

En los últimos años, los alimentos funcionales han despertado el interés de consumidores, científicos y empresarios. El ajo (*Allium sativum*) puede ser un ejemplo de ello. Gracias a su capacidad de bioacumular selenio (Se), dicha hortaliza puede convertirse en una atractiva opción de alimento enriquecido con Se. El Se es un micronutriente esencial para numerosos organismos, incluyendo plantas, animales y humanos. Este micronutriente es de gran importancia debido a sus propiedades antioxidantes, y actúa, en consecuencia, como protector de numerosas enfermedades, tales como cáncer y problemas cardiovasculares. Actualmente, existe un mayor interés en el estudio de especiación de Se debido a los distintos roles que cada especie manifiesta en los campos nutricionales y toxicológicos. Sin embargo, las concentraciones de Se poseen un estrecho intervalo entre toxicidad y esencialidad, desconcertando a toxicólogos y alarmando a nutricionistas y legisladores. En este trabajo, se presenta un panorama del grado de avance sobre el estudio de fortificación de ajo con Se y sus perspectivas de aplicación en el sistema productivo de la República Argentina. Este tipo de producción, con un significativo valor agregado, representa una atractiva oportunidad de mercado para la provincia de Mendoza, considerando además que la misma posee cerca del 85% de la producción nacional.

Palabras clave:

ajo • selenio • alimento funcional • alimentos selenizados • especiación

INTRODUCTION

Selenium (Se) is an essential ultratrace element for numerous species, including human beings. It is a metalloid that can be present in soil, water, and living organisms (69). A deficient intake of Se in humans is associated with health disorders, including fertility reduction, cardiovascular diseases and higher risk of cancer (oxidative stress) (4). Several studies have shown that supplementation with Se reduced incidence of cancer in humans (14, 17). Nevertheless, the narrow margin between Se essentiality and toxicity should not be forgotten (47).

Nowadays, studies related to Se accumulation or fortification in different plant species have been reported. Thus, lettuce (*Lactuca sativa*) (56), potato (*Solanum tuberosum*) (52), broccoli (*Brassica oleracea* var. *botrytis*) (72), chicory (*Cichorium intybus*) (23), rice (*Oryza sativa*) (11), carrot (*Daucus carota*) (36), onion (*Allium cepa*) (25), and garlic (*Allium sativum*) (62, 66) have been some of the considered vegetables. Garlic belongs to the *Liliaceae* family and it has been widely grown and used as a spice since ancient times. El-Bayoumy et al. have emphasized on the beneficial effects of consumption of garlic on health due, in part, to the seleno- compounds (Se-compounds) present in this vegetable (21). Thus, the study of bioavailability and distribution of Se species in the aforementioned botanical species acquires great importance from nutritional and toxicological standpoints.

In recent years, consumers have shown interest in the use of so-called "functional foods". These foods are developed specifically to improve health and reduce risk of diseases (42). They present biologically active components that provide health benefits (73, 74). This type of food has taken significance not only for consumers, but also for scientists and entrepreneurs. Thus scientists are focusing on generation of greater amounts of functional foods, in addition to studying its positive or negative healthy and environmental effects. On the other hand, functional foods can be considered as a business strategy because of its high economic value (42). Therefore, garlic manifests a great potential to become into a functional food as it is used daily and it has the natural ability to accumulate Se, generating hence Se-compounds with potential health benefits. This topic is of great relevance in vegetable producing areas, such as Argentina (second largest exporter of garlic worldwide) (7).

Selenium: an essential or toxic element?

The essentiality of Selenium (Se) was first discovered in 1957 through its vital role in prevention of liver necrosis in animals deficient in vitamin E. It was the exact moment from which Se researches began to significantly expand (60). Using biochemistry, Se was identified in 1973 as an important component of glutathione peroxidase, a tetrameric protein that contains four atoms of Se per molecule (58). This enzyme helps intracellular defense mechanisms against oxidative damage by preventing production of active oxygen species and hence inhibiting the toxicity of some metals such as lead, mercury, etc. (34, 71). Soon afterward, in 1980, studies of Se were increasing and, consequently, seleno-proteins (Se-proteins) were discovered. This revelation showed that the element was involved not only in an antioxidant activity, but also in multiple aspects of mammalian metabolism (3, 39, 40). More recently, it has proven to be an important component of the iodothyronine deiodinase and thioredoxin reductase (1).

Nevertheless, Se manifests a singular dual nature. Thus, it is an essential nutrient at low concentrations, whereas high concentrations of it cause toxicity. In turn, it has been observed that the daily requirement of Se for an adult man is approximately 1 mg per kg⁻¹ of body weight. In contrast, the intake of a daily dose of Se higher than 1 µg g⁻¹ is considered toxic (20, 55).

An element was originally considered "essential" when a dietary deficiency of it caused adverse health effects. Currently, trace elements are considered "essential" when they express an explicit biochemical function.

Particularly, Se excels because of its antioxidant properties, which work to protect the body against cardiovascular disease and many different types of cancers (14). Several studies have been referred to the aforementioned aspect. Thus, it has been found that Se compounds have the tendency to inhibit or retard the process of tumor initiation and promotion. Some clinical trials have shown that treatment with Se is associated with lower incidence of certain kind of cancers (13).

However, experimental antitumor activity could be associated with supranutritional doses of Se only. Furthermore, many studies have focused on the effectiveness of various compounds of Se in the fight against cancer (28, 76). According to a study by Ip et al., selenobetaine, its methyl ester and selenomethyl-selenocysteine (Se-methyl-Se-Cys) were the most effective agents in inhibiting tumor formation (33).

Two endemic diseases are related to an inadequate intake of Se. One of them is designated as Keshan disease, which affects mostly children and young women. It is manifested by chronic heart failure, enlarged heart and heart rhythm disorder. In order to study this disease, several works have been developed. Nowadays, it is well known that Se supplementation reduces the incidence of Keshan disease (10). Furthermore, there is a pathology named Kashin-Beck disease, which is characterized by degenerative osteoarthritis. It is usually fatal and affects about two million children in China, North Korea, and several regions of Russia (50).

Additionally, it is important to note that the range between the essentiality of Se and its toxicity is very narrow, i.e., the difference between toxic and required Se concentration is minimal. It has been informed that Se has caused poisoning problems in cattle, with subsequent effects such as malformed hooves and bones, gastrointestinal disorders and dermatitis. Selenium can accumulate as selenocysteine (Se-Cys), selenomethionine (Se-Met), selenogluthione, etc. It usually replaces the sulphur amino acids. In advanced cases, cirrhosis, atrophy of the heart and anemia are manifested (43, 75).

Combs Jr. et al. suggest that Se has two principal roles in cancer prevention: on the one hand, as essential component of antioxidant enzymes and, on the other hand, as anticarcinogenic metabolite. A model for these two apparent behaviors of Se in cancer prevention is exhibit in figure 1 (page 305).

According to this model, different types of anticarcinogenic activities are affected by different dosage of Se. These activities are mediated through any of the specific functions, which are interceded by distinct enzymes or certain Se-dependent metabolites that are produced when the dose is relatively high (13).

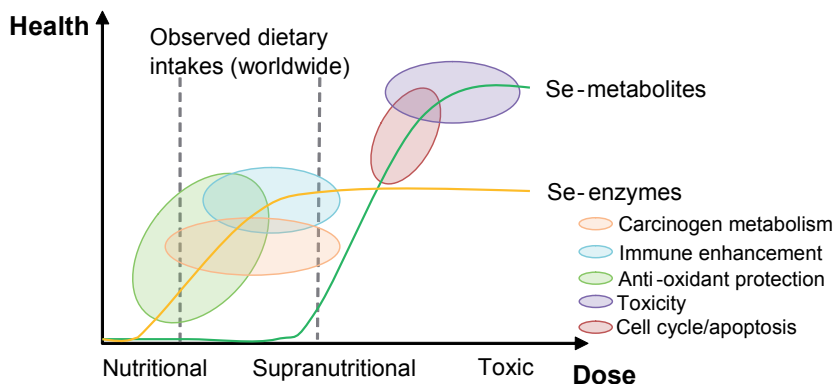


Figure 1. Two-stage model for the roles of Se in cancer prevention.

Adapted from Combs & Gray (13).

Figura 1. Modelo en dos etapas para los roles de Se en la prevención de cáncer.

Adaptado de Combs & Gray (13).

It is well known that the exposure phase of the element is determined by not only natural, but also anthropogenic sources, such as soil, water, food (cereals, fish, vegetables), fertilizers (20), and steel industry (54). Regarding to the toxicokinetic stage, absorption occurs primarily through the digestive tract, but may also occur by pulmonary or contact route (24, 61). The distribution of Se between different organs has also been documented and it is clear that the highest amount of Se is stored in kidney, followed by liver, spleen, testes, heart, lungs, muscles and brain, in decreasing order (12, 59).

Finally, excretion occurs predominantly via the kidneys, and to a lesser extent via the faeces. Elimination through exhaled air (via the lungs) only is manifested in the presence of extremely high levels of Se in the body (30). The toxicodynamics involves the study of mechanism of poison action. Unfortunately, nowadays the full knowledge of selenosis remains yet obscure. It is likely that Se manifests its toxic effect by inhibiting enzymatic or redox systems in the organism. When Se is administered as selenate (SeO_4^{2-}), it is reduced to selenite (SeO_3^{-2}) and carried by the bloodstream to the liver and spleen, where it is reduced to elemental Se, by glucose. The elemental Se is not toxic. When the body is unable to reduce selenite by excess of it or glucose deficiency, an attack to the cells is produced. Consequently, the cell results completely destroyed (57).

The symptoms that occur in affected individuals vary according to type of poisoning. In the case of an acute intoxication, respiratory disorders, dyspnea, bronchitis, pulmonary edema, gastrointestinal, cardiovascular and eye irritation are frequently observed. When the poisoning is chronic, discoloration of the skin, hair loss, deformed nails, nervous system disorders, gastrointestinal problems and garlic breath odor are revealed (35, 49).

Selenium metabolism and accumulation in plants

In plants, selenate and sulfate compete in the absorption stage and it has been proposed that both species are picked up by a sulfate conveyor in the plasma membrane of the root (79). The preference for selenate absorption varies according to the plant and, moreover, it is affected by several factors, including soil salinity (68). After absorption, translocation of Se from the root is highly dependent on the supplemented species. Selenate is more easily transported than both selenite and Se in the form of an organic compound (77). The distribution of Se in the different compartments of the plants depends on the species, developmental stage and physiological condition. Furthermore, the form and concentration in which Se is supplemented and the presence of other substances, such as sulphates, affects Se distribution (4, 79). It has been shown that Se accumulator plants accumulated Se in young leaves during the first stage vegetative growth. In contrast, in the reproductive stage, the greatest accumulation of Se is found in the seeds (68). Another route of Se input is manifested from the atmosphere through the surface of the leaves (41).

As explained in figure 2 selenate is adsorbed from the soil, and then reduced to selenite, which, in turn, is reduced to selenide (Se_2^-), with the participation of reduced glutathione. Selenide is subsequently transformed into Se-Cys, in a directly comparable form with the metabolism of sulphur (79). It has been postulated that Se-Cys is metabolized to Se-Met in the same way as their analogues of S (75).

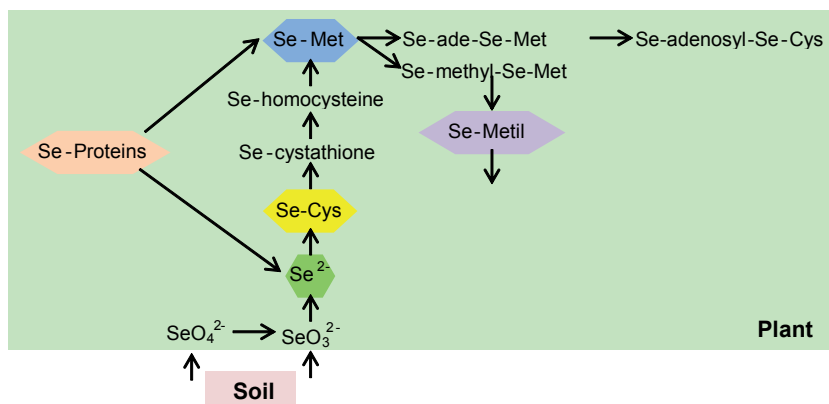


Figure 2. Metabolic pathway of Se in plants (28).

Figura 2. Vía metabólica del Se en las plantas (28).

Selenium is introduced into the food chain through the incorporation of vegetable proteins, mainly as Se-Cys or Se-Met (15). Se-Met may be then metabolized to selenoadenosyl-Se-Met (Se-ade-Se-Met), selenomethyl-selenomethionine (Se- methyl- Se-Met), which in turn is converted into Se-methyl-Se-Cys y γ -glutamyl-selenomethyl-selenocysteine (γ -glutamyl-Se-metil-Se-Cys). At high levels of Se, Se-methyl-Se-Cys becomes the predominant Se-compound, although some other compounds may also be present in lower level (75). Finally, the Se vegetables compounds will be used by animals in the synthesis of their own Se-proteins.

In the schematic representation of figure 2 (page 306) are excluded volatile species of Se, which will be shown below (figure 3). The removal of various volatile compounds of Se (dimethylselenide (DMSE)) by plants is important because some plants can take a huge amount of Se from the soil and transform it through biochemical pathways in many species volatiles. This process is called "phytovolatilization" and it may be part of the mechanism governing phytoremediation, important for its potential in cleaning up highly contaminated sites (41, 79). Biogenic volatilization of Se from soil and plants is recognized as a detoxification process (20).

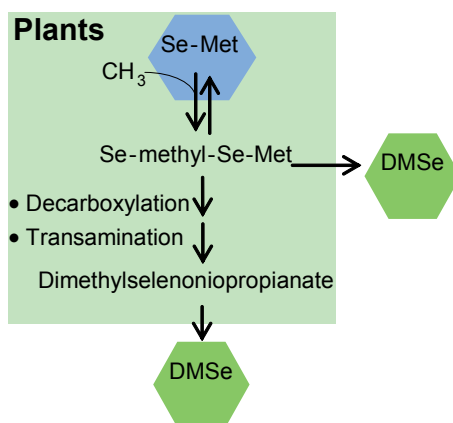


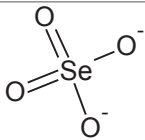
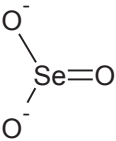
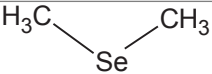
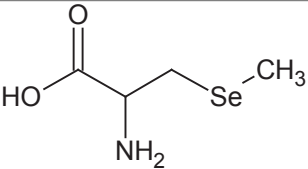
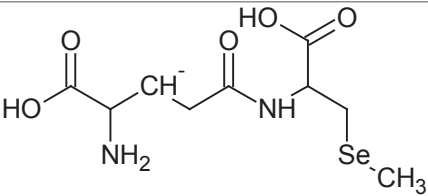
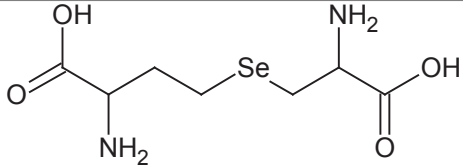
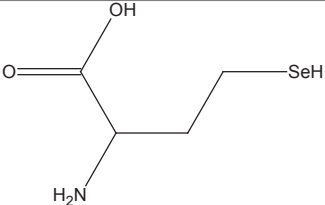
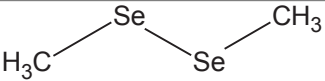
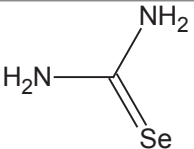
Figure 3. Volatilization of Se-Met to DMSe in plants (20).

Figura 3. Volatilización de Se-Met a DMSe en plantas (20).

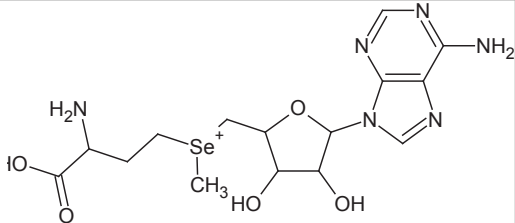
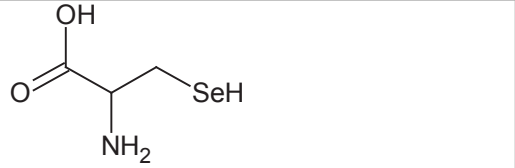
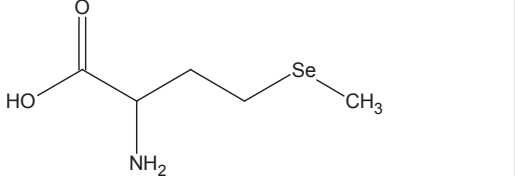
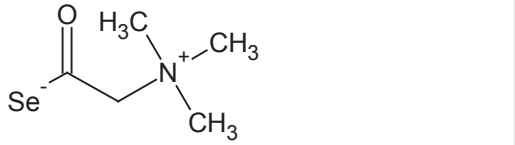
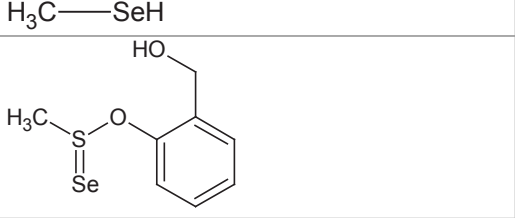
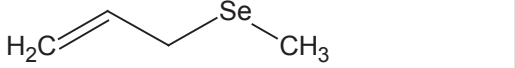
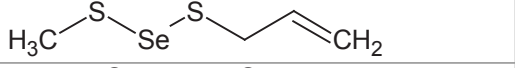
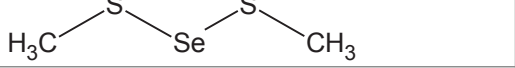
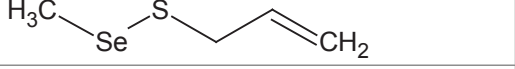
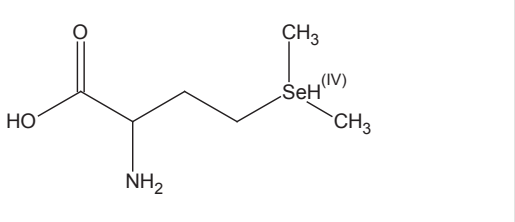
The majority of plants do not have the ability to accumulate a large amount of Se and, even for some species, small amounts of Se can be toxic, leading to a decreased growth or plant death (66). Regarding to the aforementioned storage capacity, plants species can be divided into three groups: non-accumulators, indicators and accumulators. Non-accumulators plants rarely contain $> 100 \text{ mg Se g}^{-1}$ dry material, indicators contain more than $1000 \text{ mg Se g}^{-1}$ dry material, and accumulator plants may contain more than $40,000 \text{ mg}^{-1} \text{ Se g}^{-1}$ dry material. Last situation can occur when samples belongs to environments rich in Se (4). The accumulation rate of Se is highly dependent on not only the plant, but also the chemical species involved in Se supplementation (79).

The biological significance of a metal can be understood on the basis of chemical reactions that occur in a system involving the metal, in which case it is very important both total concentration and chemical form (metallome). Metallomics and metalloproteomics are emerging fields dedicated to evaluate the role, uptake, transport and storage of trace metals that are essential for life. Metallomics is defined as the analysis of all species of metals and metalloids within a cell or tissue type, while metalloproteomics focuses on studying the function of metals associated with proteins (27, 63, 64). Currently, there are some studies that could be framed within the fields of metallomics and metalloproteomics (12, 16, 22). However, that much remains to be studied in depth. A list of the compounds found in garlic is detailed in table (pages 308, 309). Se-methyl-Se-Cys is the main species found in this vegetable, exhibiting significant beneficial effects to human health, followed by Se-Cys and Se-Met.

Table. Structural formula of some Se-compounds.
Tabla. Fórmula estructural de algunos seleno-compuestos.

Compound	Structure
Selenate (SeO_4^{2-})	
Selenite (SeO_3^{2-})	
Dimethylselenide (DMSe)	
Selenomethyl-selenocysteine (Se-metil-Se-Cys)	
γ -glutamyl-seleno-methyl-selenocysteine (γ -glutamyl-Se-metil-Se-Cys)	
Selenocystathionine (Se-Cystathionine)	
Selenohomocysteine	
Dimethyldiselenide	
Selenourea	

Selenized garlic: a future prospect or already a current functional food?

Selenoadenosyl-selenomethionine (Se-ade-Se-Met)	
Selenocysteine (Se-Cys)	
Selenomethionine (Se-Met)	
Selenobetaine	
Methylselenol	$\text{H}_3\text{C}-\text{SeH}$
Methanosulphenoselenoic acid	
Allyl methyl selenide	
Allylthio methyl selenide	
Bis(methylthio)selenide	
2-propenesulfenoselenoic acid methyl ester	
Selenomethyl-selenomethionine (Se-metil-Se-Met)	

Selenium metabolism and accumulation in garlic

Recently, garlic has acquired an important role in human diet, being a common food in some parts of the world. This vegetable belongs to *Allium* genus, in conjunction with onion (*Allium cepa*), leek (*Allium porrum*), chive (*Allium schoenoprasum*), and shallot (*Allium ascalonicum*).

Curiously, it has been stated that Se accumulation rate in plants depends on the type of plant and soil. In alkaline soils, selenate is the predominant form, whereas in well-drained soils, with an acidic or neutral pH, Se exists primarily under selenite form (78). Most studies referred to Se accumulation have been developed with garlic (*Allium sativum*), broccoli (*Brassica oleracea*), Indian mustard (*Brassica juncea*), canola (*Brassica napus*), and some mushrooms (2, 22, 44). Generally, Se-Cys is metabolized into various non-proteinogenic amino acids (46). Thus, Se-Cys can be turned into three different species: Se-methyl-Se-Cys, Se-cystathionine and γ -glutamyl-Se-methyl-Se-Cys. This mechanism takes place through a methyltransferase, which methylates the Cys to form Se-methyl-Se-Cys. On the other hand, Se-methyl-Se-Cys may also be formed as a result of the formation of DMSe, which is consequently volatilized. Finally, Se-Methyl-Se-Cys may in turn be metabolized to γ -glutamyl-Se-methyl-Se-Cys or DMSe (48).

As mentioned above, garlic is one of the accumulator plants of Se. Under certain conditions, concentrations may be higher than 1 g of Se per kg of dry plant material (48). Regrettably, specific information about the chemical forms of Se in garlic is still very limited. However, available data suggests that Se is able to transform inorganic Se in Se-methyl-Se-Cys, γ -glutamyl-Se-methyl-Se-Cys, Se-cystathionine, Se-homocysteine, γ -glutamyl-Se-cystathionine, and methylselenol (38). It is important to point out that anticancer properties are attributed to monomethyl forms present in garlic (49). Additionally, it has been extensively studied the effectiveness of garlic containing high concentrations of Se in the reduction of mammary tumors in laboratory animals (31, 32).

Furthermore, it has been observed that anticancer effect is verified in the first stages of mammary carcinogenesis but not in the final periods (33). *In vitro* studies have been developed in order to evaluate a dipeptide present in garlic extracts named γ -glutamyl-Se-methyl-Se-Cys. Thus, there is a strong evidence for the hypothesis that γ -glutamyl-Se-methyl-Se-Cys is an effective anticancer agent and that their action is very similar to that of Se-methyl-Se-Cys (18).

Selenium speciation in garlic

In recent years, determination of trace Se levels has acquired relevance due to its aforementioned dual nature. Izgi et al. assessed the Se levels in different garlic samples using electrothermal atomic absorption spectrometry (ETAAS), after a preconcentration step. For these kinds of samples, Se levels of $0.015 \mu\text{g g}^{-1}$ were found. Nevertheless, as time was going on, it has been an increasing interest in the study of Se speciation, because of the different roles that each specie manifests in biology, toxicology, clinical chemistry and nutrition fields (20).

Considering that garlic presents many volatile species of Se, gas chromatography (GC) in combination with atomic emission detection (AED) and mass spectrometry (MS) has been widely used in several studies. Thus, GC- AED has been applied to the detection of volatile Se species in highly enriched garlic samples. As a result, eight compounds have been detected, including DMSE, methanesulfenosenoic acid and its methyl ester, dimethyl diselenide, bis (methylthio) selenide, allyl methyl selenide, 2-propenesulfenosenoic acid methyl ester and (allylthio)-(methylthio) selenide. The structures have been established using mass spectrometry by comparison with synthesized standards (8). The same method has been used for Se speciation in human breath after consumption of garlic enriched with the element. The main compounds found were: DMSE, allyl methyl selenide, methanesulfenosenoic acid methyl ester, 2-propenesulfenosenoic acid methyl ester and dimethyl diselenide (9).

Different species of Se present in garlic have been separated by liquid chromatography coupled to mass spectrometry with inductively coupled plasma (LC-ICP-MS). The forms Se-Me-Se-Cys and γ -glutamyl-Se-methyl-Se-Cys have been determined. In addition, garlic was treated with saliva, allowing hence detection and analysis of the extracted species during mastication. It was found that, although the major species in garlic is the dipeptide γ -glutamyl-Se-methyl-Se-Cys, Se-Methyl-Se-Cys is the primary compound present in extracts after treatment with gastrointestinal fluids (19). Furthermore, it has been observed that total Se content affects the Se distribution in samples of the same type. Thus, it has been considered that garlic samples with concentrations of less than $333 \mu\text{g g}^{-1}$ contain more γ -glutamyl-Se-methyl-Se-Cys than Se-methyl-Se-Cys. Conversely, if the concentrations of garlic are higher than $333 \mu\text{g g}^{-1}$, the situation is totally the opposite (37).

Using HPLC-ICP-MS, different species in garlic samples have been detected, such as selenate, selenite, Se-methyl-Se-Cys, Se-Met, γ -glutamyl-Se-methyl-Se-Cys and glutamyl-Se-methyl-Se-Met. However, selenite, selenate and Se-Met were undoubtedly the dominant species in the vegetables under study (45).

Selenized garlic and its potential as a functional food

Currently, numerous studies have been based on Se fortifications in garlic plants. Fortifications have been made by three different ways: via the leaves, through irrigation (mixed with other fertilizers) and through hydroponics (62, 66, 69). Although foliar applications are less effective with respect to the final concentration obtained in the bulb, they are less harmful to the soil, avoiding hence distribution of large amounts of Se in the soil. Likewise, foliar application would avoid the competition with sulfate for the absorption sites in the garlic plant, allowing hence the effective Se fortification of plants even in areas with sulphated soils. On the other hand, the hydroponic alternative is a closed system that enables not only a good control of fertilization without environmental consequences, but also an exceptional Se accumulation efficiency in the bulb. However, numerous cares have to be considered and large-scale production is not viable.

In most studies of fortification that use any of the routes previously mentioned, organic and inorganic Se species are applied. The results have shown that inorganic

Se supplementation produces better results in fortification. Selenate, in turn, is the inorganic species that has achieved better results (62, 66, 69). These fortification studies have not yet been used in crops on large scales. Consequently, significant amount of garlic fortified have not still been produced. Therefore, the production of this kind of food, which could be categorized as functional, has not yet been developed.

As mentioned above, functional foods are a category of food products that are marketed as having certain compounds that produce health benefits. It has been demonstrate their beneficial effects on one or more body functions, providing thus a better health status. Furthermore, this kind of foods display a preventive role through the reduction of risk factors that cause the onset of a disease (70). In recent decades, dietary habits have changed. Currently, consumers not only seek to reduce the consumption of injurious health food, but also to find those foods that have health benefits and delay the inception of a disease process. Functional foods emerged in the 1980s as a measure of the government of Japan to extend the life expectancy of the population. Nevertheless, only a few decades later, this new concept began to be adopted by the rest of the world (74). As can be observed in figure 4, the number of publications was reduced from the 1980 to mid 1995. From this year, a resounding growth in the number of publications was perceived. The interest of scientists, government, and producers was, of course, the main cause that promoted the growth. However, in case of garlic, it does not seem to be widely studied as a type of functional food. Thus, it has been only studied in two of these papers published in the year 2000 and 2012 (26).

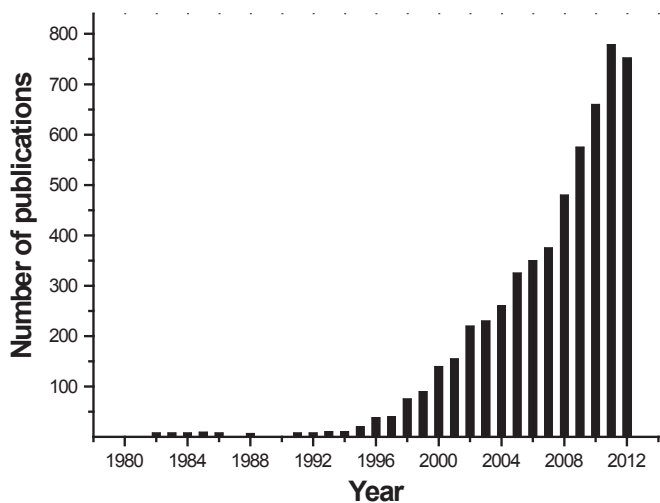


Figure 4. Number of publications according to year of publication. Data from the Scopus search (<http://www.scopus.com>) by entering the term "functional food" as a single search filter.

Figura 4. Número de publicaciones de acuerdo al año de publicación. Datos obtenidos de búsqueda en Scopus ingresando el término "alimento funcional" como un filtro de búsqueda simple.

Selenized garlic: a future prospect or already a current functional food?

Garlic manifests a significant potential to become into a functional food due to its daily consumption. Thus, garlic fortified is sold in U. S. A. as dietary supplement. It means that garlic is fortified in hydroponics growing, dried, ground and processed into capsules for direct consumption.

Finally, a promising challenge could be the development of fortified fresh vegetables. These new products will undoubtedly capture the attention and interest of the majority of consumers.

Future perspective: garlic importance and production in Mendoza province

The main producers of garlic worldwide can be divided into five groups: Asia, Europe, North America, South America and around the world. Considering the area under cultivation and harvesting, Asia, led by China, is the world's largest producer (5, 51).

Within this market, Argentina is a net supplier with respect to the global market, due to its exportations (more than two thirds of their production) are outweighed by imports (6). In Argentina, the main production areas belong to Mendoza and San Juan provinces, which made up 95% of the garlic production in the country (85% and 10%, respectively) (7). The Andean desert, in which these provinces are located, has growing conditions that offers to garlicks differential characteristics compared to other areas (53).

From 1989, the situation of garlic producing sector has changed because of the Garlic/INTA Project, which promoted the development of a national strategy in this field. As a result of this project, several monoclonal cultivars of garlic were obtained (67).

In Argentina, more than 15 "pure" garlic varieties (high genetic homogeneity) are registered, each of which with distinct characteristics. These varieties (varietals) are grouped into the following types: Rosados, Colorado, Morado, Blancos, Violetas and Castaños. *Alpa Suquia* is the most important variety belong to Rosado type, while *Morado* and *Serrano* are distinguished in the Morado type.

Finally, *Norteño*, *Lican*, *Gostoso* and *Castaño* are the most relevant Blancos, Violetas, Colorados and Castaños varietiestypes, respectively.

Figure 5 (page 314) shows the geographical distribution of the surface with garlic plantations in Mendoza province. According to the period 2009/2010, the proportions of production of commercial types of garlic exhibit a 36.8% for Colorado garlic, 18.8% for Blanco garlic, and 44.4% for Violeta garlic (29).

These varieties have different planting dates and, consequently, harvesting occurs with the same temporal sequence that planting, since all cultivars are characterized by similar growth cycles (about 8 months) (65).

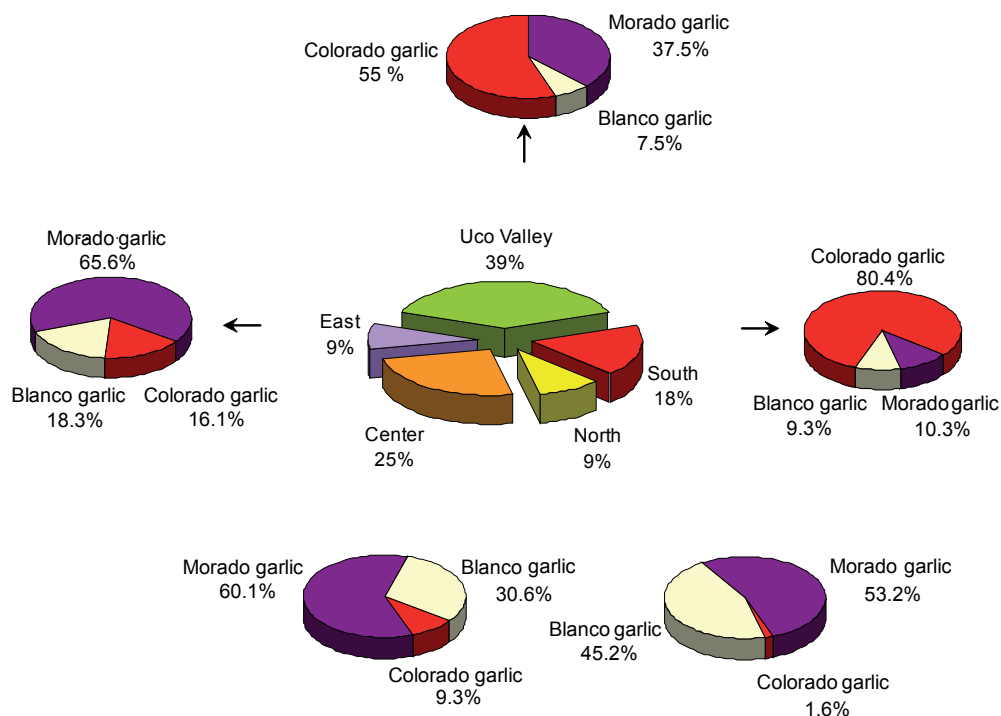


Figure 5. Distribution of surface with garlic in Mendoza province. Season 2009/2010.
Figura 5. Distribución superficial de ajos en la provincial de Mendoza. Estación 2009/2010.

CONCLUSIONS

The increasing health consciousness has been one of the most important stimulating factors for rapid global growth of the functional foods field. The increase of production in some countries and the emergence of new producer countries, forced to rethinking the production and marketing. Argentine is usually a supplier of garlic "bulk", without any added value.

Product differentiation is an interesting strategy to address the market more competitive. Notably, Se has been evidenced to play a vital role in human health and nutrition due to their numerous health benefit effects. Unquestionably, a distinct product could be fortified garlics with Se.

Thus, a bulk production could be transformed into the creation of a functional food with high added value.

REFERENCES

1. Arthur, J. R.; Nicol, F.; Beckett, G. J. 1993. Selenium deficiency, thyroid hormone metabolism, and thyroid hormone deiodinases. *American Journal of Clinical Nutrition*, 57(2): 236-239.
2. Bañuelos, G. S. 2002. Bioremediation and biodegradation: Irrigation of broccoli and canola with boron- and selenium-laden effluent. *Journal of Environmental Quality*, 31(6): 1802-1808.
3. Beilstein, M. A.; Whanger, P. D. 1987. Metabolism of selenomethionine and effects of interacting compounds by mammalian cells in culture. *Journal of Inorganic Biochemistry*, 29(2): 137-152.
4. Broadley, M. R.; White, P. J.; Bryson, R. J.; Meacham, M. C.; Bowen, H. C.; Johnson, S. E.; Hawkesford, M. J.; McGrath, S. P.; Zhao, F. J.; Beward, N.; Harriman, M.; Tucker, M. 2006. Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society*, 65(2): 169-181.
5. Burba, J. L. 2003. Ajos argentinos. Diferenciarse para ser más competitivos. *IDIA XXI*, 4(4): 45-49.
6. Burba, J. L. 2009. Mejoramiento genético y producción de "semilla" de ajo (*Allium sativum* L.). Posibilidades de adaptación a diferentes ambientes. *Revista Colombiana de ciencias hortícolas*, 3(1): 28-44.
7. Burba, J.L., Cavagnaro, P.F. 2010. Guía elemental para el buen uso y aprovechamiento del ajo Ediciones Instituto Nacional de Tecnología Agropecuaria (INTA), Documento Proyecto Ajo/INTA 096.
8. Cai, X. J.; Uden, P. C.; Block, E.; Zhang, X.; Quimby, B. D.; Sullivan, J. J. 1994. *Allium chemistry*: Identification of natural abundance organoselenium volatiles from garlic, elephant garlic, onion, and Chinese chive using headspace gas chromatography with atomic emission detection. *Journal of Agricultural and Food Chemistry*, 42(10): 2081-2084.
9. Cai, X. J.; Block, E.; Uden, P. C.; Quimby, B. D.; Sullivan, J. J. 1995. *Allium chemistry*: Identification of natural abundance organoselenium compounds in human breath after ingestion of garlic using gas chromatography with atomic emission detection. *Journal of Agricultural and Food Chemistry*, 43(7): 1751-1753.
10. Chen, X.; Yang, G.; Chen, J. 1980. Studies on the relations of selenium and Keshan disease. *Biological Trace Element Research*, 2(2): 91-107.
11. Chen, L.; Yang, F.; Xu, J.; Hu, Y.; Hu, Q.; Zhang, Y.; Pan, G. 2002. Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry*, 50(18): 5128-5130.
12. Chen, C.; Zhao, J.; Gao, Y.; Chai, Z. 2005. Tissue contents and intracellular distribution of selenium and iodine in human tissues studied by neutron activation analysis. *Chemia Analytyczna*, 50(1): 169-178.
13. Combs Jr, G. F.; Gray, W. P. 1998. Chemopreventive agents: Selenium. *Pharmacology and Therapeutics*, 79(3): 179-192.
14. Combs Jr, G. F. 2005. Current evidence and research needs to support a health claim for selenium and cancer prevention. *Journal of Nutrition*, 135(2): 343-347.
15. Cornelis, R.; Caruso, J.; Crews, H.; Heumann, K. 2003. In: *Handbook of Elemental Speciation II: Species in The Environment, Food, Medicine and Occupational Health*, ed Wiley, Chichester, vol 2, 69-93.
16. Cuderman, P.; Kreft, I.; Germ, M.; Kovačević, M.; Stibilj, V. 2008. Selenium species in selenium-enriched and drought-exposed potatoes. *Journal of Agricultural and Food Chemistry*, 56(19): 9114-9120.
17. De Martino, A.; Filomeni, G.; Aquilano, K.; Ciriolo, M. R.; Rotilio, G. 2006. Effects of water garlic extracts on cell cycle and viability of HepG2 hepatoma cells. *Journal of Nutritional Biochemistry*, 17(11): 742-749.
18. Dong, Y.; Lisk, D.; Block, E.; Ip, C. 2001. Characterization of the biological activity of γ -Glutamyl-Se-methylselenocysteine: A novel, naturally occurring anticancer agent from garlic. *Cancer Research*, 61(7): 2923-2928.
19. Dumont, E.; Ogra, Y.; Vanhaecke, F.; Suzuki, K. T.; Cornelis, R. 2006. Liquid chromatography-mass spectrometry (LC-MS): A powerful combination for selenium speciation in garlic (*Allium sativum*). *Analytical and Bioanalytical Chemistry*, 384(5): 1196-1206.
20. Dumont, E.; Vanhaecke, F.; Cornelis, R. 2006. Selenium speciation from food source to metabolites: A critical review. *Analytical and Bioanalytical Chemistry*, 385(7): 1304-1323.
21. El-Bayoumy, K.; Sinha, R.; Pinto, J. T.; Rivlin, R. S. 2006. Cancer chemoprevention by garlic and garlic-containing sulfur and selenium compounds. *Journal of Nutrition*, 136(3): 864-869.
22. Finley, J. W. 2005. Selenium accumulation in plant foods. *Nutrition Reviews*, 63(6 1): 196-202.
23. Germ, M.; Stibilj, V.; Osvald, J.; Kreft, I. 2007. Effect of selenium foliar application on chicory (*Cichorium intybus* L.). *Journal of Agricultural and Food Chemistry*, 55(3): 795-798.
24. Gmshinsky, I. V.; Mazo, V. K. 2006. Mineral substance in human nutrition. Selenium: Absorption and bioavailability. *Voprosy Pitaniia*, 75(5): 15-21.

25. Golubev, F. V.; Golubkina, N. A.; Gorbunov, Y. N. 2003. Mineral Composition of Wild Onions and Their Nutritional Value. *Applied Biochemistry and Microbiology*, 39(5): 532-535.
26. Gómez, L. J. G.; Sánchez-Muniz, F. J. 2000. Review: Cardiovascular effect of garlic (*Allium sativum*). Revisión: Efectos cardiovasculares del ajo (*Allium sativum*), 50(3): 219-229.
27. Gómez-Ariza, J. L.; Garcla-Barrera, T.; Lorenzo, F.; Bernal, V.; Villegas, M. J.; Oliveira, V. 2004. Use of mass spectrometry techniques for the characterization of metal bound to proteins (metalloomics) in biological systems. *Analytica Chimica Acta*, 524(1-2): 15-22.
28. Hudson, T. S.; Carlson, B. A.; Hoeneroff, M. J.; Young, H. A.; Sordillo, L.; Muller, W. J.; Hatfield, D. L.; Green, J. E. 2012. Selenoproteins reduce susceptibility to DMBA-induced mammary carcinogenesis. *Carcinogenesis*, 33(6): 1225-1230.
29. Instituto de Desarrollo Rural (IDR). 2010. Relevamiento Hortícola Provincial (Mendoza) del Instituto de Desarrollo Rural.
30. International Programme on Chemical Safety (IPCS - INCHEM). Environmental Health Criteria: Selenium, 1987.
31. Ip, C.; Lisk, D. J.; Stoewsand, G. S. 1992. Mammary cancer prevention by regular garlic and selenium-enriched garlic. *Nutrition and Cancer*, 17(3): 279-286.
32. Ip, C.; Lisk, D. J. 1994. Enrichment of selenium in allium vegetables for cancer prevention. *Carcinogenesis*, 15(9): 1881-1885.
33. Ip, C.; Lisk, D. J.; Thompson, H. J. 1996. Selenium-enriched garlic inhibits the early stage but not the late stage of mammary carcinogenesis. *Carcinogenesis*, 17(9): 1979-1982.
34. Izgi, B.; Gucer, S.; Jaćimović, R. 2006. Determination of selenium in garlic (*Allium sativum*) and onion (*Allium cepa*) by electro thermal atomic absorption spectrometry. *Food Chemistry*, 99(3): 630-637.
35. Jaffe, W. G. 1973. Selenium in food-plants and feeds. *Toxicology and nutrition. Qualitas Plantarum Plant Foods for Human Nutrition*, 23(1-3): 191-204.
36. Kápolna, E.; Hillestrøm, P. R.; Laursen, K. H.; Husted, S.; Larsen, E. H. 2009. Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chemistry*, 115(4): 1357-1363.
37. Kotrebai, M.; Birringer, M.; Tyson, J. F.; Block, E.; Uden, P. C. 2000. Selenium speciation in enriched and natural samples by HPLC-ICP-MS and HPLC-ESI-MS with perfluorinated carboxylic acid ion-pairing agents. *Analyst*, 125(1): 71-78.
38. Lauchli, A. 1993. Selenium in plants: Uptake, functions, and environmental toxicity. *Botanica Acta*, 106(6): 455-468.
39. LeBoeuf, R.A., Laishes, B.A., Hoekstra, W.G. 1985. Effects of selenium on cell proliferation in rat liver and mammalian cells as indicated by cytokinetic and biochemical analysis. *Cancer Research*, 45(11): 5496-5504.
40. LeBoeuf, R. A., Hoekstra, W. G. 1985. Changes in cellular glutathione levels: Possible relation to selenium-mediated anticarcinogenesis. *Federation Proceedings*, 44(9): 2563-2567.
41. Meija, J.; Montes-Bayón, M.; Le Duc, D. L.; Terry, N.; Caruso, J. A. 2002. Simultaneous monitoring of volatile selenium and sulfur species from se accumulating plants (wild type and genetically modified) by GC/MS and GC/ICPMS using solid-phase microextraction for sample introduction. *Analytical Chemistry*, 74(22): 5837-5844.
42. Menrad, K. 2003. Market and marketing of functional food in Europe. *Journal of Food Engineering*, 56(2-3): 181-188.
43. Mihajlović, M. 1992. Selenium toxicity in domestic animals. *Toksicnost selena kod domaćih životinja* (42): 131-144.
44. Montes-Bayón, M.; Grant, T. D.; Meija, J.; Caruso, J. A. 2002. Selenium in plants by mass spectrometric techniques: Developments in bio-analytical methods: Plenary lecture. *Journal of Analytical Atomic Spectrometry*, 17(9): 1015-1023.
45. Mounicou, S.; Dernovics, M.; Bierla, K.; Szpunar, J. 2009. A sequential extraction procedure for an insight into selenium speciation in garlic. *Talanta*, 77(5): 1877-1882.
46. Neuhierl, B.; Thanbichler, M.; Lottspeich, F.; Böck, A. 1999. A family of S-methylmethionine-dependent thiol/selenol methyltransferases. Role in selenium tolerance and evolutionary relation. *Journal of Biological Chemistry*, 274(9): 5407-5414.
47. Nordberg, G. F.; Fowler, B. A.; Nordberg, M.; Friberg, L. 2007. Handbook on the toxicology of metals, AP, Atlanta, GA, USA.
48. Olson, O. E.; Novacek, E. J.; Whitehead, E. I.; Palmer, I. S. 1970. Investigations on selenium in wheat. *Phytochemistry*, 9(6): 1181-1188.

49. Pedrero, Z.; Madrid, Y. 2009. Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. *Analytica Chimica Acta*, 634(2): 135-152.
50. Peng, A.; Yang, C.; Rui, H.; Li, H. 1992. Study on the pathogenic factors of Kashin-Beck disease. *Journal of Toxicology and Environmental Health*, 35(2): 79-90.
51. Pereyra, M.; Pappalardo, L. 2007. Contexto internacional en la temporada de comercialización de ajo 2006/2007. X Curso taller sobre producción, comercialización e industrialización de Ajo. p. 11-12.
52. Poggi, V.; Arcioni, A.; Filippini, P.; Pifferi, P. G. 2000. Foliar application of selenite and selenate to potato (*Solanum tuberosum*): Effect of a ligand agent on selenium content of tubers. *Journal of Agricultural and Food Chemistry*, 48(10): 4749-4751.
53. Portela, J. A. 2003. La calidad como meta. *IDIA XXI*, 4(4): 50-54.
54. Ramachandran, K. N.; Kaveeshwar, R.; Gupta, V. K. 1993. Spectrophotometric determination of selenium with 6-amino-1-naphthol-3-sulphonic acid (J-acid) and its application in trace analysis. *Talanta*, 40(6): 781-784.
55. Recommended Dietary Allowances (RDA). 1989. Recommended Dietary Allowances, 10th ed. National Academy of Sciences National Academy Press, Washington D. C., U. S. A.
56. Ríos, J. J.; Rosales, M. A.; Blasco, B.; Cervilla, L. M.; Romero, L.; Ruiz, J. M. 2008. Biofortification of Se and induction of the antioxidant capacity in lettuce plants. *Scientia Horticulturae*, 116(3): 248-255.
57. Romero, M. E. 2010. Utilización de promotores naturales sel-plex en el engorde de pollos parrilleros. Escuela de Ingeniería Zootécnica, Facultad de Ciencias Pecuarias, Ecuador.
58. Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science*, 179(4073): 588-590.
59. Schroeder, H. A.; Frost, D.V.; Balassa, J. J. 1970. Essential trace metals in man: Selenium. *Journal of Chronic Diseases*, 23(4): 227-243.
60. Schwarz, K.; Foltz, C. M. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *Journal of American Chemical Society*, 79: 3292 - 3293.
61. Selenium. T. P. F. 2003. Agency for Toxic Substances and Disease Registry (ATSDR).
62. Seo, T. C.; Spallholz, J. E.; Yun, H. K.; Kim, S. W. 2008. Selenium-enriched garlic and cabbage as a dietary selenium source for broilers. *Journal of Medicinal Food*, 11(4): 687-692.
63. Shi, W.; Chance, M. R. 2008. Metallomics and metalloproteomics. *Cellular and Molecular Life Sciences*, 65(19): 3040-3048.
64. Shi, W.; Chance, M. R. 2010. Metalloproteomics: forward and reverse approaches in metalloprotein structural and functional characterization. *Current Opinion in Chemical Biology*, In Press, Corrected Proof.
65. Silvestri, V.; Rigoni, C.; López Frasca, A.; Togno, L. 2007. Asociaciones entre clones y anomalías morfológicas en ajo "tipo colorado" (*Allium sativum* L.). *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza, Argentina*, 39(2): 43-49.
66. Slekovec, M.; Goessler, W. 2005. Accumulation of selenium in natural plants and selenium supplement vegetable and selenium speciation by HPLC-ICPMS. *Chemical speciation and bioavailability*, 17(2): 63-73.
67. Soto Vargas, V. C.; González, R. E.; Sance, M. M.; Burba, J. L.; Camargo, A. B. 2010. Efecto de la interacción genotipo-ambiente sobre la expresión del contenido de alicina y ácido pirúvico en ajo (*Allium sativum* L.). *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza, Argentina*, 42(2): 15-22.
68. Terry, N.; Zayed, A. M.; De Souza, M. P.; Tarun, A. S. 2000. Selenium in higher plants. *Annual Review of Plant Biology*, 51: 401-432.
69. Tsuneyoshi, T.; Yoshida, J.; Sasaoka, T. 2006. Hydroponic cultivation offers a practical means of producing selenium-enriched garlic. *Journal of Nutrition*, 136(3): 870-872.
70. Urala, N.; Lähteenmäki, L. 2007. Consumers' changing attitudes towards functional foods. *Food Quality and Preference*, 18(1): 1-12.
71. Ursini, F.; Bindoli, A. 1987. The role of selenium peroxidases in the protection against oxidative damage of membranes. *Chemistry and Physics of Lipids*, 44(2-4): 255-276.
72. Ventura, M. G.; Stibilj, V.; Freitas, M. d. C.; Pacheco, A. M. G. 2009. Determination of ultratrace levels of selenium in fruit and vegetable samples grown and consumed in Portugal. *Food Chemistry*, 115(1): 200-206.
73. Verbeke, W. 2005. Consumer acceptance of functional foods: socio-demographic, cognitive and attitudinal determinants. *Food Quality and Preference*, 16(1): 45-57.
74. Verbeke, W. 2006. Functional foods: Consumer willingness to compromise on taste for health? *Food Quality and Preference*, 17(1-2): 126-131.

75. Whanger, P. D. 2002. Selenocompounds in plants and animals and their biological significance. *Journal of the American College of Nutrition*, 21(3): 223-232.
76. Yan, L.; Demars, L. C. 2012. Dietary supplementation with methylseleninic acid, but not selenomethionine, reduces spontaneous metastasis of Lewis lung carcinoma in mice. *International Journal of Cancer*, 131(6): 1260-1266.
77. Zayed, A.; Lytle, C. M.; Terry, N. 1998. Accumulation and volatilization of different chemical species of selenium by plants. *Planta*, 206(2): 284-292.
78. Zhu, Y. G.; Pilon-Smits, E. A. H.; Zhao, F. J.; Williams, P. N.; Meharg, A. A. 2009. Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. *Trends in Plant Science*, 14(8): 436-442.
79. Zhu, Y. G.; Pilon-Smits, E. A. H.; Zhao, F. J.; Williams, P. N.; Meharg, A. A. 2009. Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation, *Trends in Plant Science*, p. 436 - 442.

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