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COMMUNICATION

Effect of Blanching Treatments on Antioxidant Activity and Thiosulfinate Degradation of Garlic (*Allium sativum L.*)

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Abstract The degradation kinetics of thiosulfinates and the reduction of antioxidant activity of garlic bulbs cut in slices were studied during steam at 100 °C and in water at 80 and 90 °C. Blanching process led to significant reduction of the antioxidant activity and thiosulfinate contents. The reaction rate constants for loss of thiosulfinate species and antioxidant activity increased with blanching temperature, with activation energies of 7.67 and 89.75 kJ/mol, respectively. The antioxidant activity showed a significant correlation with thiosulfinates (R=0.7604), and both the antioxidant activity and thiosulfinate contents decreased with increasing blanching time. The antioxidant activity did not show significant differences after 6 min of steam blanching and 8 min of water blanching at 90 and 80 °C. Regarding the thiosulfinate concentration, there was no significant difference after 8 min of blanching for all temperatures.

Keywords Antioxidant activity · Thiosulfinates · Degradation kinetics · Garlic

Introduction

Garlic bulbs contain organosulfur components, particularly thiosulfinates, including γ-glutamylcysteines and cysteine sulfoxides (particularly alliin, which represents approximately 80 % of cysteine sulfoxide in garlic). The cysteine sulfoxides and the γ-glutamylcysteines are approximately 95 % of total sulfur in fresh garlic (Holub et al. 2002). During planting, germination, and storage, only part of the γ-glutamylcysteines are gradually hydrolyzed and oxidized to cysteine sulfoxides (alliin). In processing the garlic, the amino acid alliin [(+)-*S*-allyl-L-cysteine sulfoxide] rapidly interacts with the enzyme alliinase to produce allicin (diallyl thiosulfinate), which represents about 70 % of the sulfur components. These compounds are responsible for the pungent odor, whose unstable molecule is highly reactive and quickly decays into other thiosulfinates such as diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS), dithiins, and ajoene (Amagase et al. 2001). At the same time, the γ-glutamylcysteines that have not been converted to cysteine sulfoxides are transformed into *S*-allyl cysteine (SAC) by a different metabolic pathway. *S*-allyl cysteine is a stable and odorless compound that has the ability to lower cholesterol and to inhibit carcinogenic processes (Amagase and Milner 1993). Several studies have shown that allicin, DAS, DADS, and DATS, which are volatile compounds, possess antioxidant activity (Borek 2001).

According to Banerjee et al. (2003), thiosulfinates not only have antioxidant activity but also can stimulate the synthesis of glutathione, an important intracellular antioxidant. However, the antioxidant activity of thiosulfinates is affected by heat treatment of garlic due to the inactivation of the enzyme alliinase, responsible for the formation of these compounds interacting with alliin (Staba et al. 2001). The antioxidant activity may also be associated with the presence of phenolic compounds, including flavonoids (quercetin, kaempferol, and mericitin) (Willett 1994). According to Bozin et al. (2008), there are few data characterizing the potential antioxidant properties related to flavonoids and phenolic compounds in garlic.

Blanching is a heat treatment to which the vegetables are subjected prior to further processing. It consists of heating at high temperature in water or steam or less frequently using microwaves, radio frequency, or infrared radiation (Szymanek 2011). It is a fundamental operation due to the vital importance of maintaining the quality attributes such as color and texture, besides its benefits in terms of microbial destruction

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and enzyme inactivation (Jaiswal et al. 2012), such as polyphenol oxidase. When garlic is peeled, the bulbs are exposed to the environment and suffer undesirable changes in quality including rapid browning. Therefore, this pretreatment is necessary before processing to reduce changes in the phytochemicals and obtain a stable product (Heras-Ramírez et al. 2012).

Several studies on the blanching of garlic prior to drying, freezing, cooking, and fermentation have been carried out (Fante and Noreña 2013; James et al. 2009; Chung and Kim 2009; Beato et al. 2012). However, none of them studied the effect of different blanching conditions on the total deactivation of the enzymes alliinase and γ-glutamyl transpeptidase, the degradation of thiosulfinates, or the loss of antioxidant activity. However, in order to predict the effect of the temperature and time of blanching on the quality of the food, the kinetic parameters of thermal destruction must be estimated.

The aim of this study was to evaluate the degradation kinetics of thiosulfinates and the reduction of antioxidant activity in garlic slices during water blanching at 80 and 90 °C and steam blanching at 100 °C for different times.

Material and Methods

Material

Garlic (*Allium sativum* L.) was acquired directly from the producer in the city of Flores da Cunha, Rio Grande do Sul State, Brazil. The bulbs were cleaned and selected by considering the absence of visual damage and infections, as well as the uniformity of size and color, and stored at room temperature (22±2 °C) until the time of use.

Experimental Procedure

The garlic bulbs were peeled and cut into slices using a food processor, presenting diameter and thickness of 15±2.40 and 1 ±0.35 mm, respectively. The samples were submitted to a blanching process, which consisted of placing the slices in a basket in a bath with 2 L of preheated water at 80 and 90 °C. For the steam blanching, the slices were uniformly distributed in baskets and placed in an autoclave generating steam at 100 °C at atmospheric pressure. The times of 1, 2, 4, 6, 8, and 10 min were used in both assays (Fante and Noreña 2012). After blanching, the samples were quickly cooled in an ice bath for 3 min. In this step, the effect of blanching time and temperature on the antioxidant activity and thiosulfinate content was evaluated.

Antioxidant Activity

The antioxidant activity was determined by the ability of the compounds in garlic to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to the method described by Brand-Williams et al. (1995), with minor modifications. For the extraction process, 5-g sample was added to 20 mL methanol, the mixture was homogenized and centrifuged in a refrigerated centrifuge (Sigma 4 K15) at 3,000×g for 15 min at 4 °C, and the garlic extract was stored at 4 °C until analysis. A stock solution of DPPH at 0.024 % (*w*/*v*) in methanol was prepared and maintained under refrigeration. For the working solution, the stock solution was diluted to 0.044 % in methanol, and the absorbance was adjusted to 1.1 ±0.02. Subsequently, 100 μL of the garlic extract was added to 3.9 mL of DPPH working solution. The absorbance was measured after 1 h using a wavelength of 517 nm in a UV-visible spectrophotometer (Shimadzu-1240). The standard curve was prepared using DPPH concentrations from 0 to 60 μmol/L. Similarly, equal proportions of 100 μL methanol and 3.9-mL DPPH working solution were used as a blank. The results were expressed as percentage of antioxidant activity, according to the equation:

$$(\%) = [1-(A/A_0)] \times 100 \quad (1)$$

where A is the absorbance of the sample and A_0 is the absorbance of the blank.

Thiosulfinates

One gram of garlic was homogenized in 5 mL of HEPES buffer (50 mM, pH 7.5) according to the methodology proposed by Li et al. (2007). The homogenate was kept at room temperature for 10 min to ensure complete enzymatic conversion to thiosulfinates, and subsequently, it was filtered through no. 1 filter paper to obtain the garlic extract. At the time of the analysis, a 20-mM cysteine solution was prepared in HEPES buffer (50 mM, pH 7.5), by adding 5-mL cysteine solution to 1-mL garlic extract, and allowed to stand for 15 min. Then, 1 mL of this solution was diluted to 100-mL distilled water, and 4.5 mL of the diluted solution was added to 0.5 mL of DTNB solution (1.5 mM). After 15 min, the absorbance was measured at 412 nm in UV-Vis spectrophotometer (Shimadzu-1240). For the blank, 5-mL cysteine solution was added to 1-mL distilled water, and 1-mL homogenate was diluted to 100 mL. Then, 4.5-mL diluted solution was added to 0.5-mL DTNB solution (1.5 mM). The results were expressed in micromole per gram of dry basis (d.b.) of thiosulfinates, according to the equation:

$$\mu mol/g(d.b.) = (\Delta_{412} \times 100)/(2 \times 14,150) \quad (2)$$

where $\Delta_{412}=A_0-A$, 14,150 is the molar extinction coefficient of 2-nitro-5-thiobenzoate, and 2 is the half of the amount of reduced cysteine, indicating the thiosulfinate content (Li et al. 2007).

Kinetics of Antioxidant Activity

The combined kinetic model was used to describe the degradation kinetics of the antioxidant activity. This model is shown in Eq. (3):

$$y = k_c - (k_c - C_o)\exp^{(-kt)} \quad (3)$$

where y refers to the antioxidant activity or the color parameters in a given time, k_c is the residual activity of the antioxidant activity after a certain treatment time, C_o is the antioxidant activity or color parameter at the initial time (t=0), k is the reaction rate constant (min^{-1}), and t is the blanching time. The rate constant (k) was estimated from the regression analysis of the values of y versus blanching time.

The temperature dependence on degradation of the antioxidant activity was determined by the Arrhenius equation:

$$k = k_0 \ exp^{-Ea/RT} \quad (4)$$

where E_a is the activation energy (kJ/mol), k_0 is the pre-exponential factor (min^{-1}), R is the universal gas constant, and T is the absolute temperature (K).

Kinetics of Thiosulfinates

The behavior of the degradation kinetics of thiosulfinates was described using the modified logistic model (Eq. (5)), where y refers to the concentration of thiosulfinates in a given time, a and b are the adjustment parameters, k is the reaction constant (min^{-1}), and t is the time.

$$\boldsymbol{y} = \boldsymbol{a} - \frac{a}{1 + exp^{(b-kt)}} \quad (5)$$

For the Kinetic model adopted, the rate constant (k, min^{-1}) was estimated from regression analysis of values of y versus blanching time. The dependence of the rate constant with temperature was represented by the Arrhenius equation (Eq. (4)).

Statistical Analysis

ANOVA was used for statistical analysis, and the treatments were compared by Tukey's mean multiple comparison test, using the software SAS 9.3. SigmaPlot 8.0 was used to estimate the parameters of the kinetic models by regression analysis. The quality statistical indicators were the coefficient of determination (R^2) and the mean square error (MSE, defined as the sum of squared residuals divided by the corresponding degrees of freedom). The Pearson correlation coefficient was also calculated in order to measure the association or relationship between the variables.

Results and Discussion

Antioxidant Activity

The fresh garlic of the present study presented initial antioxidant activity of 17.06 %. During blanching, a significant loss (p<0.05) of antioxidant activity was observed (Fig. 1) over time, when samples were subjected to blanching in water at 80 to 90 °C and in steam. There was no significant difference (p>0.05) in the antioxidant activity after 6 min of blanching in steam and after 8 min of blanching in water at 90 and 80 °C. In contrast, after 10 min, the antioxidant activity decreased to 6.16±0.36, 6.13±0.02, and 8.34±0.02 % for steam blanching and water blanching at 90 and 80 °C, respectively, corresponding to activity losses of 63.89, 64.07, and 51.11 %.

This fact is due to the damage to the plant tissue by heating, with consequent exposure of antioxidant compounds (Lin and Chang 2005). Leaching with water may also bring about losses of antioxidant compounds (Nicoli et al. 1999). Yin and Cheng (1998) studied the antioxidant activity of *Allium* family and reported losses when garlic was subjected to heat treatment, suggesting that the loss of activity may be related to the thiosulfinate degradation and inactivation of the enzyme alliinase by heat, blocking up the conversion of alliin to allicin. Willett (1994) reported that phenolic compounds such as flavonoids also contribute to the antioxidant activity but the heat treatment may promote degradation of these compounds. Prasad et al. (1996) studied the effect of heating on the antioxidant activity of garlic and reported that its activity was reduced approximately 10 % when garlic was heated to 100 °C.

Thiosulfinates

The concentration of thiosulfinates in fresh garlic was 21.18± 0.11 μmol/g (d.b). Block et al. (1992) studied several species

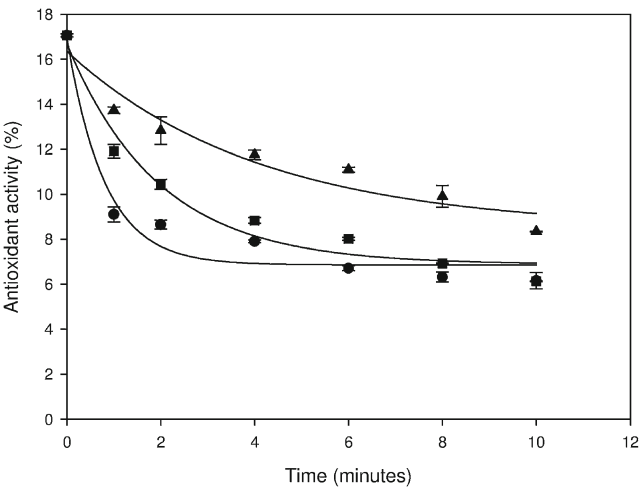

Fig. 1 Loss of antioxidant activity in garlic at different times and blanching conditions. (*circle*) Steam at 100 °C, (*square*) water at 90 °C, (*triangle*) water at 80 °C, (*line*) combined kinetic model

of garlic and found thiosulfinate contents ranging from 0.15 to 21 μmol/g for leeks and wild garlic (*Allium ursinum*), respectively. This variation in thiosulfinate contents in *Allium* species is dependent on the stages of germination and storage. According to Holub et al. (2002), there is an increase in the formation of γ-glutamylcysteine, which is a precursor of thiosulfinates, as decreasing temperature. Block et al. (1992) reported concentrations of 15 μmol/g in garlic grown at an average temperature of 21 °C, while Miron et al. (1998) found thiosulfinate concentration of 15.43 μmol/g in commercial garlic.

During water blanching at 80 and 90 °C and steam blanching at 100 °C, the thiosulfinate concentration decreased over time (Fig. 2), once there was no significant difference ($p>0.05$) in the first 2 min, but the concentration decreased significantly ($p<0.05$) after this period; however, after 8 min, no significant difference ($p>0.05$) was observed for all temperatures.

Likewise, for constant blanching periods, there was no significant difference ($p>0.05$) between thiosulfinate concentration and temperature, except for the 6-min period, in which a significant difference ($p<0.05$) in water blanching at 80 °C was observed. The thiosulfinate concentration decreased to 6.11±0.20, 6.92±0.09, and 7.58±0.92 μmol/g (d.b) after 10 min of steam blanching and water blanching at 90 and 80 °C, respectively, corresponding to losses of 71.15, 67.33, and 64.21 %. Yin and Cheng (1991) reported that the strong activity of organosulfur compounds is reduced under high temperatures, because the enzyme alliinase, which is responsible for the formation of thiosulfinates, is inactivated by heat (Miron et al. 1998), confirming the high loss of these compounds in the present study.

The Pearson correlation showed a positive correlation between the antioxidant activity and the thiosulfinates (R=0.7604). Yin and Cheng (1998) studied the antioxidant activity of garlic and observed that the decrease in antioxidant activity was related to a decrease in thiosulfinate content. These authors suggested that the antioxidant activity was due not only to thiosulfinates but also to other compounds that provide antioxidant activity in garlic, such as the phenolics, which were not evaluated in this study. Lawson et al. (1991) also observed a reduction in the antioxidant activity of garlic when subjected to blanching, due to the loss of phenolic compounds.

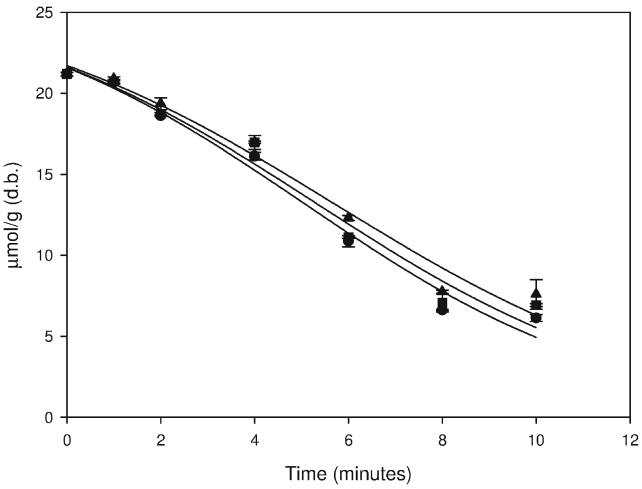

Fig. 2 Loss of thiosulfinates contents in garlic at different times and blanching conditions. (*circle*) Steam at 100 °C, (*square*) water at 90 °C, (*triangle*) water at 80 °C, (*line*) modified logistic model

Degradation Kinetics of Antioxidant Activity

Estimation of kinetic parameters for the antioxidant activity followed the combined model (Eq. (7)), which provided a good fit of the data, with R^2 values greater than 0.94 and MSE values less than 0.88 (Table 1). It is observed that the rate constant (k) increased significantly ($p<0.05$) with temperature, from 0.243 to 1.255 min^{-1}. The adjustment curves are shown in Fig. 1. The temperature dependence on the rate constant was defined by the Arrhenius equation (Eq. (4)), resulting in an activation energy value of 89.75 kJ/mol, with a determination coefficient of 0.99. High activation energy value indicates a strong temperature dependence, which means that the reaction takes place very slowly at lower temperatures but relatively fast at higher temperatures.

Jaiswal et al. (2012) studied the degradation kinetics of the antioxidant activity of cabbage at 80 to 100 °C and observed that increasing blanching temperature k values increased from 0.269 to 0.414 min^{-1}, with activation energy of 22.37 kJ/mol. Igual et al. (2013) studied the thermal inactivation kinetics of pectin methylesterase and peroxidase on the antioxidant capacity of grape jelly processed by heat treatment (45–75 °C) and high pressure (550–700 MPa) and applied the fractional conversion kinetic model. The authors reported that the

Table 1 Estimation of kinetic parameters, corresponding regression coefficients (R^2), and mean square error (MSE) for the degradation of the antioxidant activity and thiosulfinates for different blanching conditions

Antioxidant activity					
	C_o	k_c	k (min^{-1})	R^2	MSE
80 °C	16.334±0.78	8.439±1.48	0.243±0.060[a]	0.94	0.874
90 °C	16.667±0.76	6.884±0.56	0.507±0.033[b]	0.97	0.801
100 °C	16.962±0.87	6.861±0.44	1.255±0.081[c]	0.96	0.882
Degradation of thiosulfinates					
	a	b	k (min^{-1})	R^2	MSE
80 °C	22.281±1.777	1.188±0.373	0.229±0.015[a]	0.98	0.048
90 °C	22.140±2.025	1.192±0.428	0.243±0.017[b]	0.98	0.056
100 °C	21.767±1.315	1.275±0.307	0.263±0.018[c]	0.99	0.043

Values expressed as mean ± standard deviation. Different letters in the same column indicate a significant difference ($p<0.05$)

MSE is defined as the sum of squared residuals divided by the corresponding degrees of freedom

degradation rate constant (k) decreased with increasing temperature, but there was no significant difference in antioxidant activity ($p>0.05$).

Degradation Kinetics of Thiosulfinates

The concentration of thiosulfinates is considered an indicator of garlic flavor intensity (Amagase et al. 2001). The characteristic flavor (pungency) or aroma of garlic is due to volatile matter, which mainly consists of sulfur compounds. When garlic cloves are crushed, the cells rupture and release the enzyme alliinase from vacuoles, which meets alliin present in the vegetable tissue, converting it into allicin that decomposes rapidly in many sulfurous substances (Koch and Lawson 1996).

When zero-order and first-order models were used to study the losses of thiosulfinates, the experimental data did not fit well to these models, resulting in correlation coefficients below 0.90. Kaymak-Ertekin and Gedik (2005) mentioned that to describe the degradation kinetics of thiosulfinates, different mathematical models were needed, due to the complexity of the reactions involved in the deterioration and variability inherent in the product.

The results from the equations using the modified logistic (Eq. (5)) in relation to the kinetic parameters and the determination coefficients of the thiosulfinate degradation under different blanching conditions are shown in Table 1. This model showed fit to the data with R^2 values greater than 0.98 and MSE values ranging from 0.04 to 0.06, indicating that the model describe the data satisfactorily. The rate constant (k, min^{-1}) increased significantly ($p<0.05$) with the temperature. The adjustment curve is shown in Fig. 2. The temperature dependence and the degradation rate constant of thiosulfinates were related to the Arrhenius equation (Eq. (4)), and the result of the activation energy was 7.67 kJ/mol, with a determination coefficient of 0.99. Low values of activation energy indicate that thiosulfinates are sensitive to high temperatures (Jaiswal et al. 2012). Ilić et al. (2011) reported that the activation energy of the decomposition of allicin was 14.7 kJ/mol.

Kaymak-Ertekin and Gedik (2005) studied the degradation of thiosulfinates in onion slices during drying at various temperatures from 50 to 75 °C and different air velocities (0.6 to 1.5 m/s) and found losses of thiosulfinates with increasing drying temperature; however, thiosulfinate concentrations were not influenced by the rate of the drying air. The kinetic model that best fit the data was the second order, with R^2 values ranging from 0.90 to 0.99, with activation energy of 48.05 kJ/mol.

Conclusions

The results of this study provided information regarding the changes in kinetic stability of the thiosulfinates and the antioxidant activity during the blanching of garlic. The experimental results indicated that the modified logistic and modified first-order kinetic models provided an adequate description of the behavior of the degradation of thiosulfinates and the loss of antioxidant activity, respectively. The rate constants for the loss of thiosulfinates and antioxidant activity increased with blanching temperature, with activation energies of 7.67 and 89.75 kJ/mol, respectively. Although the results revealed a very good positive correlation between the antioxidant activity and thiosulfinates, other compounds could be involved in the antioxidant activity.

In order to optimize the blanching process for garlic, it is recommended that these results be used in combination with a heat transfer model in future studies.

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