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Enzyme inactivation kinetics and colour changes in Garlic (*Allium sativum* L.) blanched under different conditions

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ABSTRACT

The inactivation kinetics of the enzymes peroxidase, polyphenoloxidase and inulinase and changes in the color parameters L^*, a^* and b^* of garlic cloves cut in slices, were studied during steam blanching at 100 °C and water at 80 and 90 °C. The garlic cloves were peeled, cut into slices and distributed uniformly in metal baskets and one batch placed in an autoclave generating steam at a temperature of 100 °C; and the others in water baths at 80 and 90 °C. The best blanching conditions were in steam for 4 min, where no changes in texture were observed, and the enzymatic activities were reduced by 93.53%, 92.15% and 81.96% for peroxidase, polyphenoloxidase and inulinase, respectively. Under these conditions the inulin concentration reduced by 3.72%. The color parameter L^* increased with increase in blanching time, the samples becoming lighter in color, and the parameters a^* and b^* decreased, obtaining slices that were greener and bluer.

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1. Introduction

Garlic (*Allium sativum* L.) is classified as an energetic food, the principal chemical constituents being allicin, carbohydrates, phosphoric and sulfuric acids, proteins and mineral salts (Rahman and Lowe, 2006). Allicin is responsible for the characteristic odor and the antimicrobial, anti-inflammatory, anti-thrombosis, anti-cancer and anti-atherosclerosis activities and also for the antioxidant effect (Rabinkov et al., 1998). One of the carbohydrates present is inulin, which belongs to the group of polysaccharides called fructans.

Inulin is found as a reserve carbohydrate in various vegetables, fruits and cereals, including garlic, chicory, artichokes and yacon. It is classified as a prebiotic substance and used in a wide variety of food products since it presents excellent technological attributes, for example as a fat substitute and as dietary fiber (Roberfroid, 1993).

When partially hydrolyzed, inulin produces an extensive group of different compounds with an F_n structure such as: inulobiose (F_2), inulotriose (F_3) and inulotetraose (F_4), and GF_n compounds such as: sucrose (GF), kestose (GF_2), nistose (GF_3) and fructofuranosyl nystose (GF_4), which maintain their prebiotic properties (Ronkarta et al., 2007).

These oligosaccharides are not hydrolyzed by the digestive enzymes of the small intestine, and, as a consequence, do not increase glycemic index or the blood inulin levels, being ideal for diabetics (Leonel et al., 2006). The loss of activity of inulinase has been observed at temperatures above 70 °C (Sharma et al., 2006) where the catalytic activity is destroyed, thus maintaining the integrity of the inulin (Böhm et al., 2005).

Heat inactivation of the enzymes is fundamental due to the importance of preserving the color of the raw material before any transformation (Ndiaye et al., 2009). In the case of garlic, after peeling, the cloves are exposed to the environment and suffer undesirable changes in quality, such as rapid browning (Mayer, 2006). Polyphenoloxidase is responsible for the appearance of brown substances due to oxidative polymerization of the quinones, which can be avoided using methods such as heat treatment and pH changes (Schweiggert et al., 2005).

Prolonged hot water blanching leads to a series of undesirable alterations in the food including losses of color, flavor and texture. According to Schweiggert et al. (2005), most of nutrients are soluble in water, resulting in a greater loss during hot water blanching than during steam blanching.

The objective of the present work is to study the inactivation kinetics of the enzymes peroxidase (POD), polyphenoloxidase (PPO) and inulinase, and the variation in color of garlic slices during water blanching at 80 and 90 °C and steam blanching at 100 °C, for different times. The concentrations of inulin, glucose and fructose in the garlic with and without blanching were also investigated.

2. Material and methods

2.1. Material

The garlic (*Allium sativum* L.), cultivated in the city of Nova Pádua in the state of Rio Grande do Sul, Brazil, was acquired

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directly from the producer. The heads were cleaned and selected considering the absence of visual injury and infections and also uniformity of size and color. They were then stored at room temperature (22 ± 2 °C) until used.

2.2. Experimental procedure

The garlic cloves were peeled and sliced using a food processor, presenting diameters and thicknesses of 15 ± 2.40 and 1 ± 0.35 mm, respectively. The samples were then submitted to a blanching process in which the slices were placed in metal baskets in water baths containing 2 L of water previously heated to 80 or 90 °C. With respect to steam blanching, the slices were uniformly distributed in metal baskets and placed in an autoclave generating steam at 100 °C and atmospheric pressure. Times of 1, 2, 4, 6, 8 and 10 min were used in both cases. After blanching, the samples were cooled in an ice bath for 3 min (Agüero et al., 2008). The effects of blanching time and temperature on the activities of POD, PPO and inulinase were evaluated at this stage and the color parameters of L^*, a^* and b^* measured. The concentrations of inulin, glucose and fructose were determined in the samples prepared under the blanching conditions in water at 80 or 90 °C and in steam at 100 °C.

2.3. Preparation of the enzyme extracts

Following the methodology of Serradell et al. (2000) the samples were ground with 0.05 M phosphate buffer (pH 7.0) at a maximum of 4 °C in a proportion of 1:5 in the case of POD and PPO and 1:1 in the case of inulinase. The suspensions were then vacuum filtered through Whatman number 01 filter paper to remove larger particles and the filtrates centrifuged at 1680g for 10 min at 4 °C. The supernatants were again vacuum filtered through Whatman number 01 filter paper to obtain the enzyme extracts.

2.4. Peroxidase activity

POD activity was determined using the method described by Hultin et al. (1966), which consisted of mixing 3 mL enzyme extract with 5 mL (0.1 M, pH 5.0) phosphate buffer, 0.5 mL (3%, v/v) hydrogen peroxide and 0.5 mL guaiacol. The mixture was incubated at 30 °C for 5 min and 1 mL (30%, w/v) sodium bisulfate then added to interrupt the enzymatic reaction and the reading carried out in a UV–visible spectrophotometer (Ultrospec 3100pro) at 470 nm.

2.5. Polyphenoloxidase activity

PPO activity was determined by the method described by Teisson (1979) using a UV–visible spectrophotometer (Ultrospec 3100pro). An aliquot of 0.5 mL of enzyme extract was mixed with 1.8 mL (0.05 M, pH 7.0) phosphate buffer and 0.05 mL 10 mM catechol and incubated in a water bath at 30 °C for 30 min. An aliquot of 0.8 mL 2 N perchloric acid was then added to interrupt the enzymatic reaction and the absorbance read at 395 nm.

2.6. Inulinase activity

Inulinase activity was determined using the methodology of Sharma and Gill (2007) with some modifications. The trial consisted of using 0.4 mL enzyme extract, 0.45 mL (2%, w/v) inulin dissolved in 0.1 M citrate–phosphate buffer (pH 6.0) and 0.15 mL (0.1 M, pH 6.0) citrate–phosphate buffer. The mixture was incubated at 30 °C for 1 h and the reaction then interrupted by placing in a boiling water bath (100 °C) for 10 min. One milliliter dinitrosalicylic acid was then added and the absorbance read in a UV–visible spectrophotometer (Ultrospec 3100pro) at 570 nm.

One unit of enzyme activity was defined as an increase of 0.001 absorbance units per min of reaction at 470, 395 and 570 nm for peroxidase, polyphenoloxidase and inulinase, respectively, under the conditions of each assay (Sun and Song, 2003).

2.7. Color measurement

The garlic samples were ground and placed in Petri dishes (diameter = 5 cm and height = 1 cm) and filled to the top (Ancos et al., 1999). The color was measured by direct reading in a Minolta colorimeter (Chroma Meter CR 410, tristimulus type, 2° observer, beam diameter of 50 mm, 0° viewing angle and illuminant C, D_{65} with wide-area illumination) using the tridimensional CIE$L^*a^*b^*$ system, where L^* indicates the luminosity (varying from 0 = black to 100 = white), a^* is a measurement varying from green (−60) to red (+60) and b^* varies from blue (−60) to yellow (+60). The instrument was calibrated using a white ceramic plate (L^* = 97.47; a^* = 0.08; b^* = 1.76). The parameters $L^*a^*b^*$ were used to describe the *Chroma* (Eq. (1)), *Hue* (Eq. (2)), total color difference (ΔE) (Eq. (3)) and the browning index (*BI*) (Eq. (4)) during blanching (Palou et al., 1999)

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}, \tag{1}$$

$$Hue = \tan^{-1}\left(\frac{b^*}{a^*}\right), \tag{2}$$

$$\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2}, \tag{3}$$

$$\text{IE} = \frac{[100(x - 0.31)]}{0.17} \tag{4}$$

where,

$$x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \tag{5}$$

The subscript 'o' refers to the reading of the color of the fresh garlic, used as the reference. ΔE indicates the change in color of the samples when compared with fresh garlic.

2.8. Determination of sugars by HPLC

The samples were prepared according to the methodology cited by Scher et al. (2009) and adapted according to the method proposed by Toneli et al. (2007). Two grams of garlic were ground with 60 mL water at 90 °C. The product was heated in a water bath at 80 °C for 1 h with constant stirring and the suspension then cooled to room temperature and centrifuged (Centrifuge 5415R) at 1680g for 15 min at 25 °C. The supernatant was filtered through Whatman n°1 filter paper and the through a 22 μm membrane filter and stored at −18 °C. For the analysis, the samples were preheated in a water bath at 80 °C and then placed in an ultrasonic bath for 4 min before injection into the HPLC.

The inulin, glucose and fructose contents were determined using an adaptation of the method described by Zuleta and Sambucetti (2001) by direct determination using high performance liquid chromatography (HPLC-RI) with a Perkin Elmer series 200 chromatograph, refractive index detector, water (Milli-Q) as the mobile phase at 0.6 mL/min, temperature of 80 °C, a Phenomenex Rezex RHM Monosaccharide column (300 × 7.8 mm) and a total run time of 13 min.

2.9. Kinetic analysis of enzyme inactivation and browning index

The first order biphasic model proposed by Ling and Lund (1978) was used to describe the kinetics of the heat inactivation of the enzymes POD, PPO and inulinase and the browning index (*BI*). This model consists of the separation into two different groups