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Effect of temperature cycling on allinase activity in garlic

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ABSTRACT

Allinase, which catalyzes the conversion of alliin to allicin, the principal component of potential medicinal value in garlic, is a thermo-labile enzyme. The potential for allicin formation is determined by the quantity of allinase that remains active after the process of preserving garlic by drying. The kinetics of enzymatic activity loss during drying by temperature cycling or by constant temperature were evaluated and compared. Allicin-forming potential was 91% preserved by temperature cycling from 40 to 60 °C. It was found that sugars present in the garlic and the high molecular mass of the enzyme were responsible for protection against degradation at high drying temperatures. Preservation of the enzymatic activity under cyclical conditions occurred mainly with exposure to low temperatures for drying periods longer than those of constant drying conditions.

1. Introduction

The sulphur-containing compounds of garlic, primarily allicin and its by-products (ajoen E, vinyldithiins, etc.), have exhibited properties such as antimicrobial, antiviral and antifungal activities as well as a cancer-preventing effect (Block, 1992). When the enzyme allinase comes into contact with its substrate alliin in the presence of water, allicin is formed. Allicin is an unstable and thermo-labile compound that decomposes within hours or days to more stable sulphur-containing compounds (Jansen, Muller, & Knobloch, 1989; Rybak, Calvey, & Harnly, 2004). Alliin and allinase precursors must remain separated prior to consumption to preserve the allicin potential.

The allicin potential can be estimated by measuring enzymatic activity which determines the concentration of pyruvic acid or by measuring the allicin produced by the cell break down and the mixing of precursors. These two methods are based on the alliin–allinase reaction that produces two acid pyruvic molecules and one allicin molecule (Fig. 1). Nevertheless the pyruvic acid method has been preferred, because of the thermal instability of allicin and the complexity of the measurement method.

Commercial preparations of garlic contain variable sulphur compound profiles which reflect different processing methods. Only dehydrated garlic contains appreciable amounts of alliin (Lawson & Wang, 2001).

The principal processes of garlic preservation by dehydration are low-temperature convective drying (3 or 4 days at 50 °C), and freeze-drying (Staba, Lash, & Staba, 2001). These processes are very costly due to the long processing times which increase energy consumption and are suitable only for high-value-added products. Modifications of the hot air drying method have been proposed as alternatives for food product drying. Variable temperature drying has resulted in improved quality of dried products compared to the conventional convective drying method.

The goal of this study was to compare the effects of temperature cycling and constant temperature drying on the preservation of the allicin-forming potential in garlic.

2. Materials and methods

2.1. Dryer

A tunnel dryer was used (Rodríguez, Méndez, Martínez, & Diego, 2001). Air was heated by resistive elements under PID control (Love Controls, Dwyer Instruments, Michigan City, IN, USA). Air speed was controlled using a frequency variator (ABB Strömberg Drives Oy, Vaasa, Finland). Sample internal temperatures were measured with type T implantable thermocouples (Physitem Instrument Inc., Clifton, NJ, USA). Sample mass was measured throughout the drying period with a digital balance at 0.5 g resolution (Mettler Toledo, Barcelona, Spain). The thermocouples and the balance were connected to a data acquisition system. Mass data were recorded with Winwedge software (TAL-Technologies Inc., Philadelphia, PA, USA). An anemometer was used to measure air speed at 0.01 m/s resolution and relative humidity was measured

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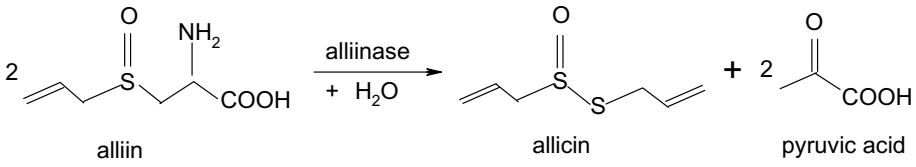

Fig. 1. Formation of allicin and pyruvic acid from alliin.

with a humidity sensor over an interval of 0–100% at 0.1% resolution. Temperature and humidity were recorded with MAC-14 software (Cole-Parmer, Vernon Hills, IL, USA).

2.2. Drying conditions

Garlic slices were dried at constant temperatures or at either cycled temperatures. Two types of cycle were studied: 40–60 °C (increasing cycle) and 60–40 °C (decreasing cycle). These sinusoidal cycles were each fitted to the time sequences shown in Table 1. The temperature profile is shown for the decreasing cycle in Fig. 2. Drying air speed was set at 2 m/s.

2.3. Experimental design

A 2×2 factorial experiment was done with two repetitions. Treatment effects were compared by analysis of variance (NCSS, Keysville, UT, USA).

2.4. Sample preparation

White garlic bulbs were purchased at a local supermarket and stored at ambient temperature. Ten to twelve bulbs were used for each experiment. Bulbs were separated into cloves which were peeled and cut into longitudinal slices averaging 2.5 mm thick. Slices were immediately placed on a tray in a single uniform layer.

Table 1
Cyclic drying conditions

Temperature cycle	Temperature interval (°C)	Cycle time sequence (min)
Increasing	40–60	20–10–20–10 20–20–20–20 20–40–20–40 20–60–20–60
Decreasing	60–40	20–10–20–10 20–20–20–20 20–40–20–40 20–60–20–60

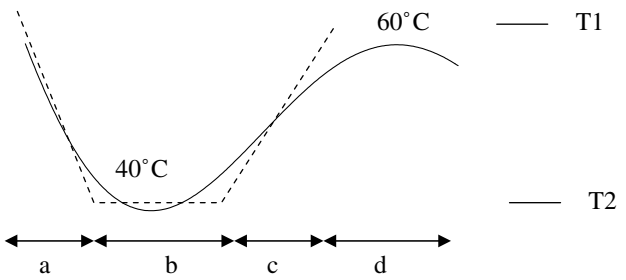

Fig. 2. Profile of oven temperature set point (dashed line) and measured air temperature in oven (continuous line) cycles (decreasing cycle shown) for drying garlic slices. (a) Phase of decreasing temperature, (b) constant temperature, (c) increasing temperature and (d) constant temperature. T1 – upper temperature limit, T2 – lower temperature limit.

The tray was placed in the pre-heated dryer at the desired temperature. Three slices were withdrawn from the dryer at various time intervals to determine the pyruvic acid content.

2.5. Final moisture content

Samples of about 2 g of product withdrawn from the dryer were weighed on an analytical balance (Mettler Toledo, Barcelona, Spain) and placed in a vacuum-drying oven at 50 °C. After 48 h, they were weighed again and the moisture content was determined from the difference. Phosphorus pentoxide was used as the drying agent for the air in the vacuum oven and in the desiccation chamber.

2.6. Pyruvic acid content

Allicin-forming potential was determined by measuring pyruvic acid content by the dinitrophenylhydrazine method (Schwimmer & Guadagni, 1962). Absorbance at 420 nm was measured using a spectrophotometer (Shimadzu, Kyoto, Japan). Pyruvic acid is the co-product of the enzymatic reaction that converts alliin to allicin (Fig. 1).

The pyruvic acid standard curve was prepared for concentrations of 0–0.5 μmol/mL.

Extracts of fresh garlic were prepared by grinding 5 g of slices for 2 min in 20 mL of water at 25 °C with a mortar and pestle. The suspensions were left to settle for 15 min and then filtered on Whatman no. 4 filter paper.

For dried garlic, extracts were prepared by grinding 2 g of slices for 1 min in a mini grinder mill (Krups, Medford, MA,USA) and mixing 1 g of powder with 20 mL of water with a magnetic stirrer (speed 50 rpm) at 25 °C for 10 min. The suspensions were left to stand for 15 min and then filtered on Whatman no. 4 filter paper.

3. Results and discussion

Figs. 3 and 4 show pyruvic acid content as a function of water content in the case of temperature cycling and constant temperature drying. The initial, average pyruvic acid content was 360.4 ± 23.5 μmol/g (dry weight basis) and decreased rapidly at water contents below 0.2 g/g of dry matter.

Analysis of variance showed that the effect of the temperature cycle was significant ($P < 0.05$). It was found that at a water content of 0.10 g/g of dry matter, the acceptable water content minimum to ensure chemistry and microbiology stability, the 40–60 °C temperature cycle preserved the allicin generating capability better than the 60–40 °C cycle did.

The 40–60 °C drying cyclic with the 20–20–20–20-min sequence preserved 91% of this activity, compared to 90% and 74% for constant temperatures of 40 and 60 °C, respectively.

Constant drying at 40 °C preserved the allicin-forming potential of the garlic at a level near that obtained by temperature cycling. A major energy saving was obtained as a result of reducing drying time by 50% in the case of cyclical drying. For the other cyclical drying conditions, pyruvic acid levels between 38% and 69% of the maximal values were obtained at a water content of 0.10 g^{-1} of dry matter.

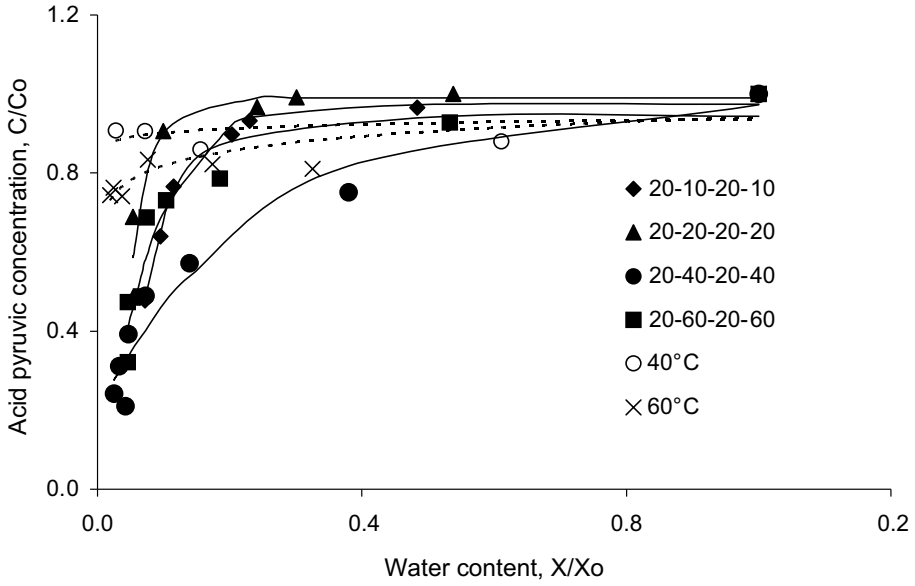

Fig. 3. Pyruvic acid concentration as a function of water content in garlic slices dried by 40–60 °C cycling and at constant temperatures.

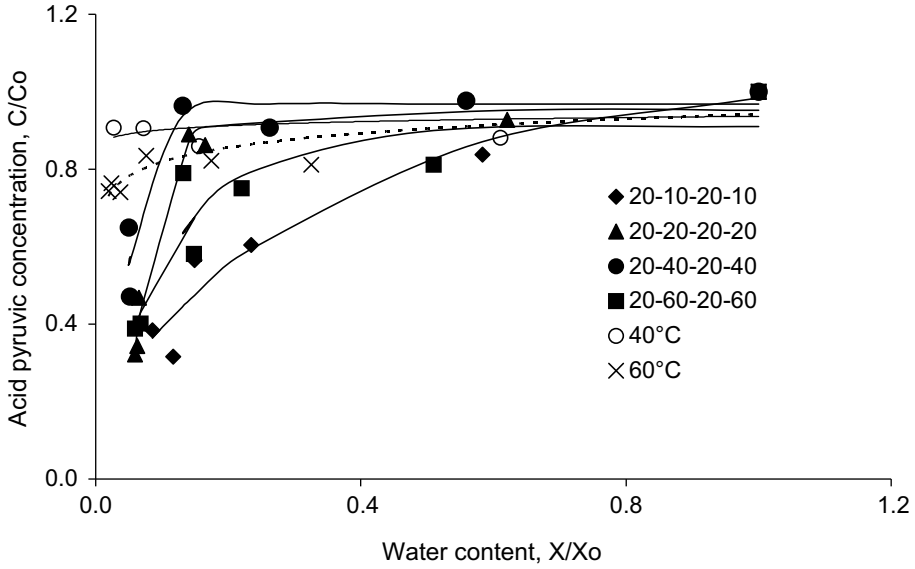

Fig. 4. Pyruvic acid concentration as a function of water content in garlic slices dried by 60–40 °C cycling and at constant temperatures.

3.1. Mechanisms of degradation

The loss of allicin-forming capacity in garlic may be caused by (a) partial or total destruction of allicin precursors, (b) partial or total inactivation of the enzyme allinase or (c) physical loss of compounds.

3.1.1. Destruction of precursors

Alliin, the precursor of allicin, breaks down at temperatures above 100 °C. The optimal temperature for the activity of allinase, which has a quaternary structure, is in the range of 35–37 °C. The reaction occurs when cells of fresh garlic are broken or when garlic powder is moistened with water, resulting in allicin formation within seconds thus the drying temperatures used in this study (40–60 °C), the enzyme may have been affected, while alliin would not have been.

3.1.2. Inactivation of the enzyme

The principal factor responsible for allinase inactivation is the drying temperature. The denaturing of this enzyme begins at 42 °C and temperatures above 60 °C inactivate it. The time required for the inside of the garlic slices to reach 40° was 135 min in drying air maintained constant at this temperature, while the critical temperature of 42 °C was reached within 10 min and quickly passed in drying air maintained at 60 °C. The preservation of the enzymatic activity during 40–60 °C temperature cycling may be explained by the longer time for which the inside of the product was at lower temperatures (150–200 min) for some time sequences (Fig. 5). In the case of the decreasing cycle (60–40 °C), the temperature inside the product reached 60 °C sooner and the time spent at the lower temperature was shorter (70–170 min, depending on time sequence), producing more extensive denaturing of the enzyme (Fig. 6).

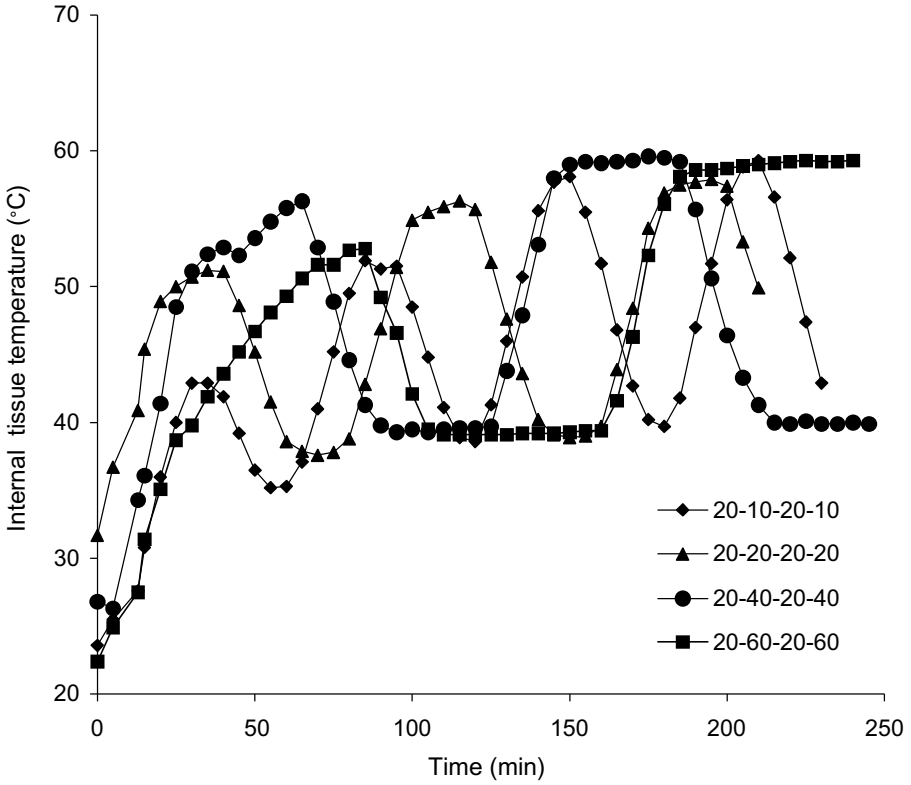

Fig. 5. Temperature measured inside garlic slices during drying by the different increasing temperature (40–60 °C) cycle time sequences.

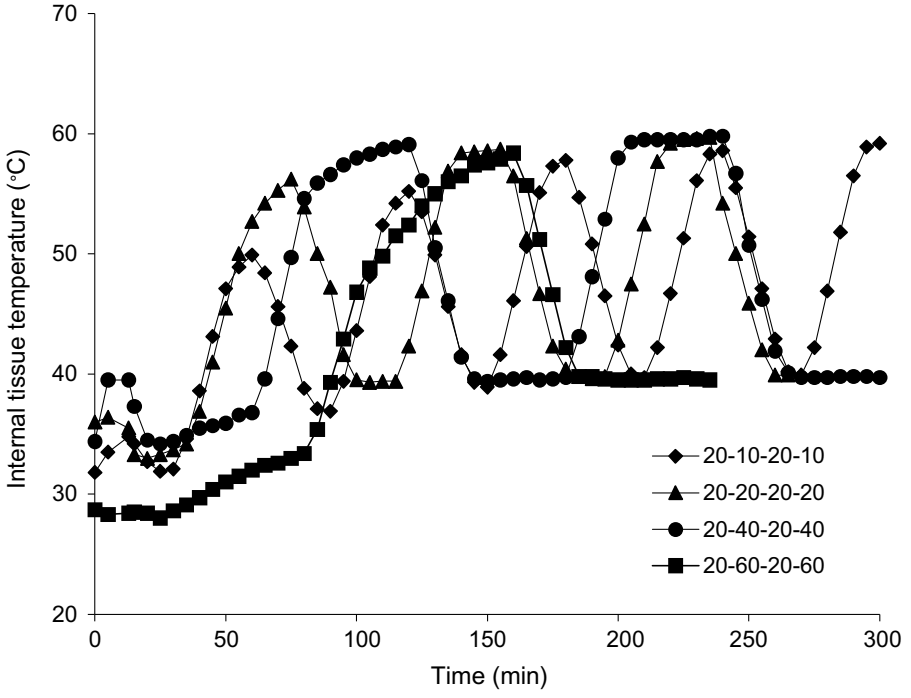

Fig. 6. Temperature measured inside garlic slices during drying by the different decreasing temperature (60–40 °C) cycle time sequences.

The initial drying temperature thus played a major role in the preservation of the enzyme activity, since it influenced the overall behaviour of the cycle and the internal temperature of the product. After a certain time, the internal temperature of the product during temperature cycling exceeded the enzyme denaturing temperature. Other factors are involved in the protection of the enzymatic activity.

In general, enzymes are more stable in the intracellular milieu of intact tissue, due to protective effects of other compounds present, such as proteins, carbohydrates, pectin and so on. In addition, enzymes of high molecular mass are more resistant to high temperatures. In garlic, the presence of fructose polymers (65% of the dry matter), pectin (11–12% in the skin, Alexander & Sulebele,

1973) and other saccharides of high molecular mass (Lawson, 1996) play a major protective role during drying. The molecular mass of allinase is about 103,000 Da (Kuettner, Hilgenfeld, & Weiss, 2002), which could also contribute to its stability. Furthermore, the loss of activity of the enzyme during a thermal treatment may be reversible or irreversible, depending on whether or not it undergoes changes to its secondary or tertiary structure, dissociates into its sub-units or dissociates from its co-factors. Kuettner et al. (2002) have determined that the enzyme allinase consists of two sub-units. Krest and Keusgen (1999) suggest that these sub-units dissociate during the drying of garlic.

3.1.3. Physical loss of compounds

Many researchers have shown that temperature and time are the principal factors involved in the breakdown of nutritional compounds. However, various types of physical breakdown that affect enzyme activity, such as the collapse of cell structure, become significant during the reduction in water content brought about by drying (Aguilera, 2003). Allicin-forming capacity is decreased both by mechanical destruction and shrinkage of cellular compartments. Contact and, hence, reaction between alliin and allinase (which are otherwise confined to separate cellular compartments in fresh intact garlic) become possible if the water content is still above a certain level when such destruction occurs.

Constant temperature produces sustained product shrinkage from the beginning of drying until the water content reaches 0.45 g/g dry matter (from an initial content of 1.29 g/g), while variable temperature drying produces less shrinkage until a content of 0.97 g/g is reached, at which point shrinkage remains almost constant until 0.50 g/g (data not shown). Allicin-forming capacity is decreased both by mechanical destruction and by shrinkage of cellular compartments.

4. Conclusions

We have studied the effects of different sinusoidal and constant drying conditions on allinase activity in garlic that produced water content of no more that 10%. Sinusoidal temperature cycle increasing from 40 to 60 °C in a 20–20–20–20–20 min sequence preserves allicin formation capacity at levels higher than drying at constant temperature or using a sinusoidal cycle decreasing from 60 to 40 °C. The preservation capacity is increased when using a 40–60 °C cycle as it keeps the product's internal temperature lower that other conditions tested as mentioned above. Moreover, less shrinkage is caused by sinusoidal cycle conditions than constant conditions, reducing cellular fissuring and cracking phenomena, thus diminishing the loss of allicin precursors. Furthermore, cyclical drying saved 50% of the drying time in comparison to constant drying.

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