

RESIDUAL PECTINESTERASE ACTIVITY IN DEHYDRATED ONION AND GARLIC PRODUCTS

ELISABETH GARCIA¹, MARISA ALVIAR-AGNEW² and
DIANE M. BARRETT^{1,3}

¹Department of Food Science and Technology
University of California
Davis, CA 95616-8598

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ABSTRACT

During the dehydration of onion and garlic products, use of high temperatures is undesirable due to the potential loss of aroma and flavor characteristics. As a consequence, residual pectinesterase (PE) activity may be found in these dehydrated spices. This study reports the presence of PE activity in raw onions and in dehydrated onion and garlic products. Pectinesterase activity is higher in the raw onion stem disks, and dehydrated products made from this tissue, than in the bulbs. Dehydrated onion products induced gelation of citrus pectin solutions and tomato purees. Although some inactivation of PE in dehydrated onion water suspensions and extracts was observed after 10 min at 50C, complete inactivation required 2 min at 82C. Commercial dehydration operations may require reevaluation to eliminate residual PE activity in dehydrated onion and garlic products.

INTRODUCTION

The two most important members of the *Allium* botanical genus, onion (*A. cepa*) and garlic (*A. sativum*) have been used for a long time; onions were cultivated in India in about 600 B.C., and in Egypt, garlic was used before 2000 B.C. (Rubatzki and Yamaguchi 1997). In addition to the consumption of fresh onions and garlic, both vegetables are often used as seasoning in dried forms. Different forms of dried onion and garlic are commercially available; onions can be sliced, chopped, minced, granulated and powdered, and although garlic can be found in the same categories, it is more often sold in powder or granulated

² Formerly with Gilroy Foods, Inc., Gilroy, CA.

³ Author to whom correspondence should be addressed. TEL: (530) 752-4800; FAX: (530) 752-4759; E-mail: dmbartlett@ucdavis.edu

forms. These spices are common ingredients in tomato products (ketchup, sauces, salsas), dried soup and sauce mixes, and salad dressings, among others (Brennan 1994).

California is responsible for three-fourths of the U.S. production of onion varieties for dehydration, which corresponds to about half of the California onion production. In recent years the production of dehydrated onion and garlic has been increasing. Dehydrated onions are the most important spice exported by the U.S.; it is estimated that in 1998 more than 30% of the dehydrated onion produced was exported (ADOGA 2000). Moreover, the domestic consumption of these spices has also shown an upward trend, attributed to the popularity of ethnic foods, such as salsa, pizza, among others, as well as to the use of more spices to compensate for less salt and fat in foods (Buzzanell *et al.* 1995).

In the processing of dehydrated onions it is desirable that the final product retains many of the flavor characteristics of the fresh onion; severe heat treatments are avoided to protect the sensory quality of the dehydrated product. Although dehydrated products can have substantially reduced enzymatic activity, due to the blanching of the raw commodity prior to dehydration, in most cases, onion and garlic are dried without prior blanching. Moreover, typical drying temperatures and treatment duration used in the drying process of onion and garlic spices do not lead to complete enzyme inactivation. It is well known that residual enzymatic activity can be responsible for quality defects such as lipoxygenase-catalyzed off-flavor in frozen peas and corn (Theerakulkait and Barrett 1995), enzymatic browning catalyzed by polyphenoloxidase, among others (Adams 1991). The presence of residual pectinesterase (pectin pectylhydrolase, E.C. 3.1.1.11) activity, which de-esterify pectins enhancing their gelling power (Matthew *et al.* 1990; El-Nawawi and Heikel 1997), may be a potential problem in the stability of some food products. Tomato products to which dehydrated onion and garlic ingredients are added have been reported to undergo a serious quality defect called gelation (Mersfelder and Ehrman 1985), in the same way described for concentrated orange juice (Wenzel *et al.* 1951), and gel formation in canned whole peeled tomatoes (Heil *et al.* 1989). This phenomenon is caused by pectinesterase, through hydrolysis of carboxylic acid esters of the native pectin up to a point when the de-esterified pectin coagulates with calcium ions forming a gel (Joslyn and Pilnik 1961; Blundstone *et al.* 1971; Chen *et al.* 1993). This is the same principle used for firming vegetables using low temperature blanching, which activates pectinesterase, before canning (Lee *et al.* 1979; Stanley *et al.* 1995).

The purpose of this study was to determine residual pectinesterase (PE) activity in a fresh white onion variety commonly used for dehydration, and in dehydrated onion and garlic products.

MATERIALS AND METHODS

Materials

Spice mixtures containing different proportions of onion and garlic powders were supplied by a food processor. A local grower kindly provided three batches of fresh white onion cv. Southport White Globe harvested on different days during the spring-summer of 1997, and ranging in weight from 18 to 175 g, as well as samples of onion powder and dehydrated minced and large chopped onion prepared from the same onion variety were kindly supplied by a local producer. In addition, crude onion flakes prepared from var. Southport White Globe processed for different times and temperatures were also analyzed for residual PE activity.

Pectinesterase (PE) Activity

Enzyme extracts were prepared by stirring samples of fresh onions (1 g) or dehydrated products (0.5 g) in 10 mL 0.2 M NaCl, for 30 min at 30C. After the extraction the pH of the suspension was adjusted to 7.0 with 0.05 N NaOH and the PE activity was determined according to Bartley *et al.* (1994) using citrus pectin (P-9135, Sigma Chemical Co., St. Louis, MO). PE activity was expressed in PE U/g, based on milliequivalents of ester hydrolyzed per minute per gram of sample. One PE U/g equals one *mEq* of ester hydrolyzed/min/g $\times 10^4$.

Gelation

Samples of onion powder, minced and chopped dehydrated onion were mixed with a water solution of citrus pectin to a final concentration of 0.6% pectin and 1% spice. All samples were kept at 25C for up to 24 h for visual assessment. In addition, one percent onion powder was added to a commercial sample of tomato puree and kept overnight at 25C to evaluate gelation.

Heat Treatment of Onion PE

Suspensions. Suspensions of 0.5 g of onion powder in 10 mL of 0.2 M NaCl were heated in a water bath for 10 min at different temperatures: 21, 30, 39, 49, 58, 68, and 73C. The extracting saline solution was preheated to the temperature being tested, and after 10 min of incubation with the onion powder, the suspensions were rapidly cooled in ice water for further assay of PE activity. All the PE assays were carried out at 30C for 15 min.

Extracts. Extracts of onion PE were prepared by stirring for 20 min onion powder in 0.2 M NaCl, at a ratio 0.5 g to 10 mL, and then centrifuged at

11,950 × g for 20 min, at 4C. The supernatant was used as a crude onion PE extract. Aliquots of 10 mL of PE extract were heated at different temperatures for 10 min. As described for the onion powder suspensions, PE activity was measured at 30C.

To evaluate the conditions required for PE heat inactivation, aliquots of 10 mL of onion powder suspensions were heated at 76C, 80C, and 82C for various time periods. After the heat treatment the extracts were rapidly cooled in ice water and assayed for residual PE activity at 30C. Another experiment was carried out with aliquots of 10 mL of onion PE extracts, which were heated at 59, 66, and 71C for various time periods, followed by cooling of the extracts and PE activity measurement.

Onion PE Temperature-Activity Profile

Onion extracts prepared as described above were also used to evaluate the optimum temperature for PE activity. Enzyme extracts were incubated with citrus pectin at temperatures from 20 to 69.5C for 15 min, and the enzyme activity was measured during this period.

RESULTS

Residual PE Activity of Spices

In a preliminary experiment commercial spice blends containing different proportions of onion and garlic powder were analyzed for residual PE activity (Fig. 1). All spice blends were prepared by mixing either onion or garlic powder with sugar in order to obtain the desired spice concentration. Enzyme activity was higher in all onion containing products than in garlic blends; no PE activity was detected in the sugar used to dilute the spices. Increase in the concentration of either onion or garlic powder in all the spice blends tested corresponded to a proportional increase in PE activity. Figure 2 presents a comparison of PE activity in dehydrated onion products. While minced and large chopped onions had approximately the same residual PE activity, the onion powder sample was the most active, with about a 4-fold greater PE activity.

Gelation

Pectin solutions to which were added either onion powder, dehydrated minced or chopped onions formed quite a consistent gel (Fig. 3A). Only after 15 min of mixing the ingredients the sample containing onion powder was already firm. Although adding minced onion also caused gelation, the resulting gel was not as firm as the gel obtained with onion powder. The sample

containing chopped onion also gelled, but this required standing overnight. In another test using onion powder or minced onion added to tomato puree, instead of a citrus pectin solution, a gel was also obtained (Fig. 3B and 3C).

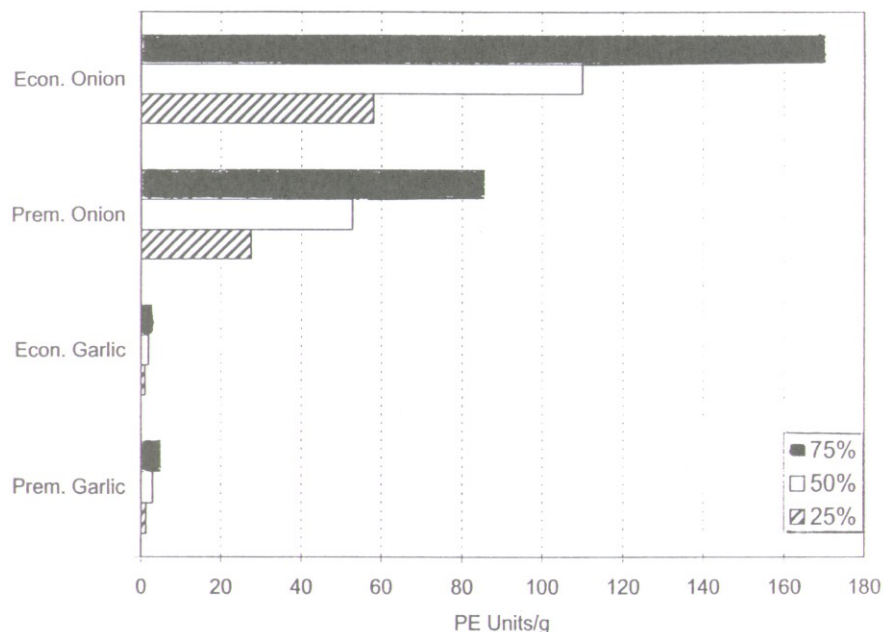


FIG. 1. PECTINESTERASE ACTIVITY IN PREMIUM AND ECONOMY SPICE BLENDS AT DIFFERENT CONCENTRATIONS OF ONION OR GARLIC POWDER

PE Activity in Fresh Onions

Fresh white onion cv. Southport White Globe, which is commonly cultivated in California for dehydration, was sampled for PE activity in two different regions of the bulb, the “stem disk”, and “upper bulb”, referred to herein only as “bulb” (Fig. 4). The PE activity ratio for stem disk/bulb shows an average of 3.8 times more activity in the stem disk, and the activity ranged from 1.4 to 17.6 fold more PE in the stem disk than in the bulb.

Heat Treatment of Onion PE

PE inactivation was accomplished at temperatures above 50C in samples heated for 10 min. When the assay temperature for PE activity measurement was varied, the classical curve for effect of temperature on enzyme activity was

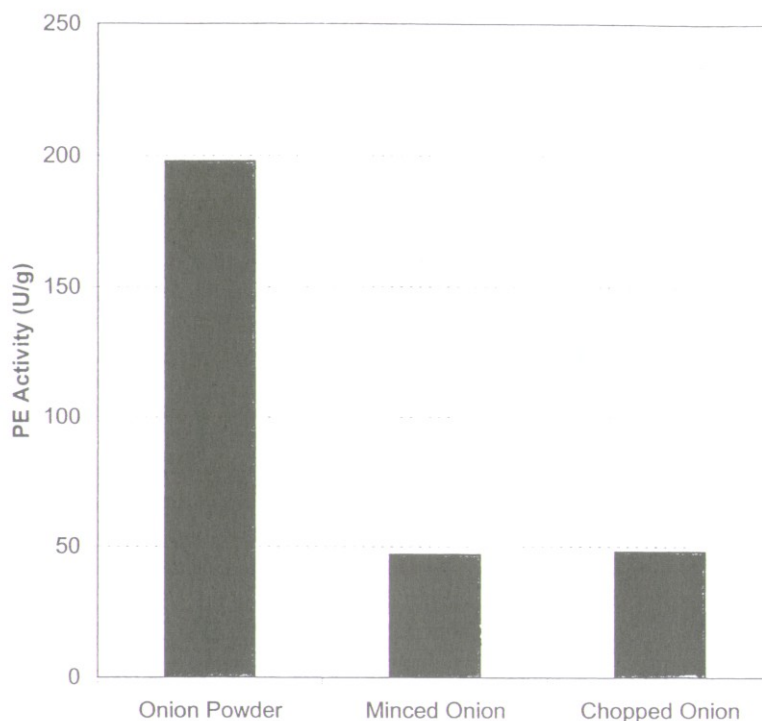
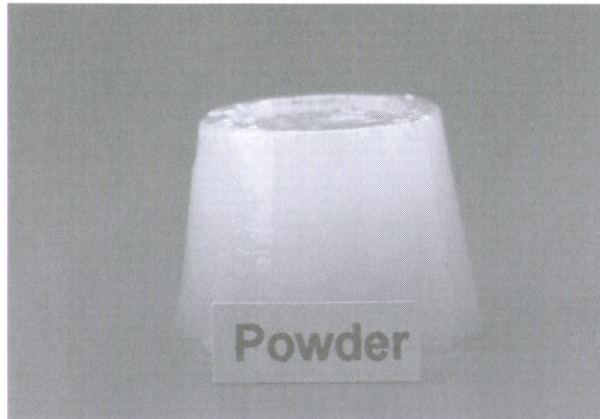


FIG. 2. PECTINESTERASE ACTIVITY IN DEHYDRATED ONION AND GARLIC PRODUCTS

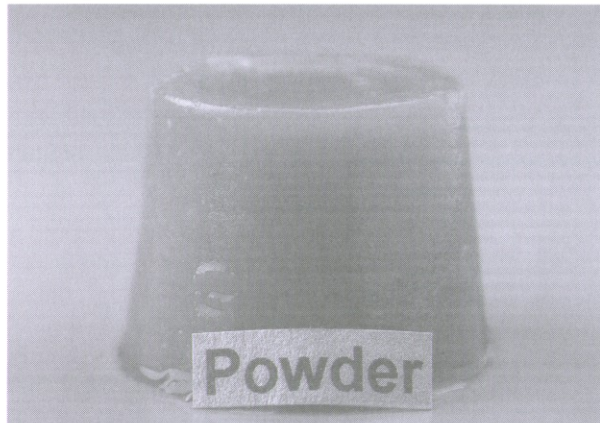
obtained (Fig. 5); maximum PE activity was detected at 55.5°C. Two sets of assays were carried out on heat inactivation of onion PE in suspensions and extracts, respectively. The results are presented in Fig. 6. Figure 6A shows the effect of heating on onion PE extracts, revealing that more than 10 min at 71°C was necessary for PE inactivation, while on Fig. 6B it is shown that higher temperatures are required for PE inactivation in onion powder suspensions. From these preliminary results the activation energy (E_a) was estimated as ~ 8,000 cal/mole.

Effects of Drying Conditions on PE Activity

In a preliminary study of dehydration conditions required for attaining PE inactivation, three sets of conditions were used, and will be referred to as mild, regular and high heat treatments. The average starting air drying temperature was 180°F (~82°C) for the mild treatment, 200°F (~93°C) for regular and 220°F (~104°C) for high; in all cases air temperature was brought down to 130°F as



3A



3B



3C

FIG. 3. VISUAL ASSESSMENT OF THE GELATION CAUSED BY THE ADDITION OF DEHYDRATED ONION TO A PECTIN SOLUTION (3A) AND TOMATO PUREE (3B AND 3C)

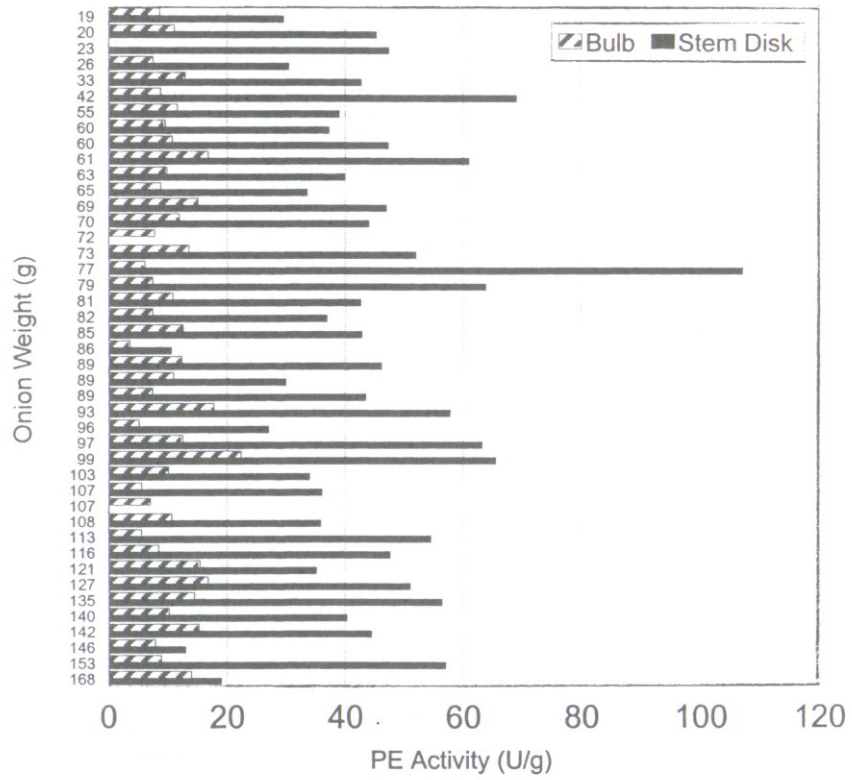
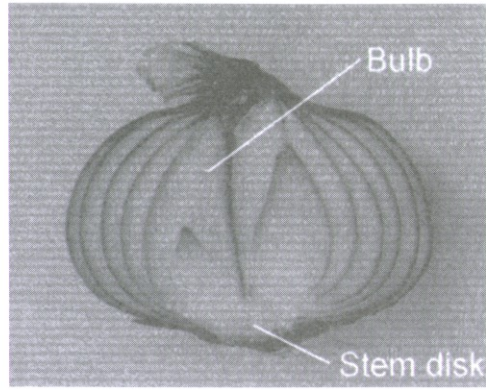


FIG. 4. VARIATION OF PECTINESTERASE ACTIVITY IN FRESH ONION CV. SOUTHPORT WHITE GLOBE

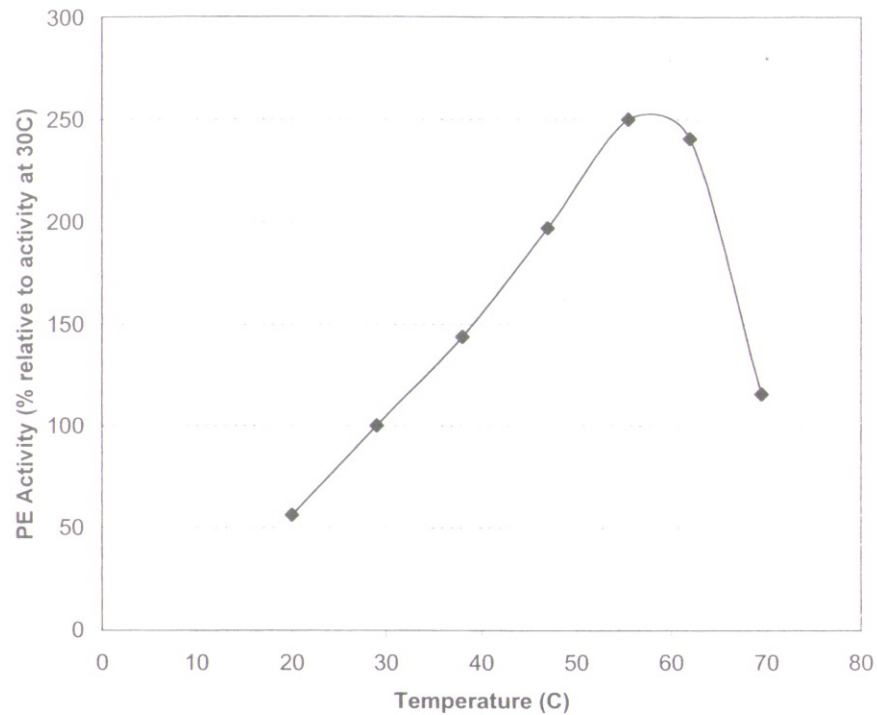


FIG. 5. EFFECT OF TEMPERATURE ON PECTINESTERASE ACTIVITY AND STABILITY

soon as the product dried, in order to avoid discoloration and quality degradation of the spices. The PE activities measured in the “stem disk” and “bulb” of onion flakes after these heat treatments are presented in Table 1. Considering the mild treatment as a standard, where 100% of PE activity is retained, an increase (regular treatment) in the temperature during drying led to inactivation of 20% of the PE activity in the bulb and 13% in the stem disk; further increase (high) in the drying conditions resulted in no further effect on the bulb PE over the inactivation achieved with the regular treatment, and in inactivation of 20% of the PE activity in the stem disk.

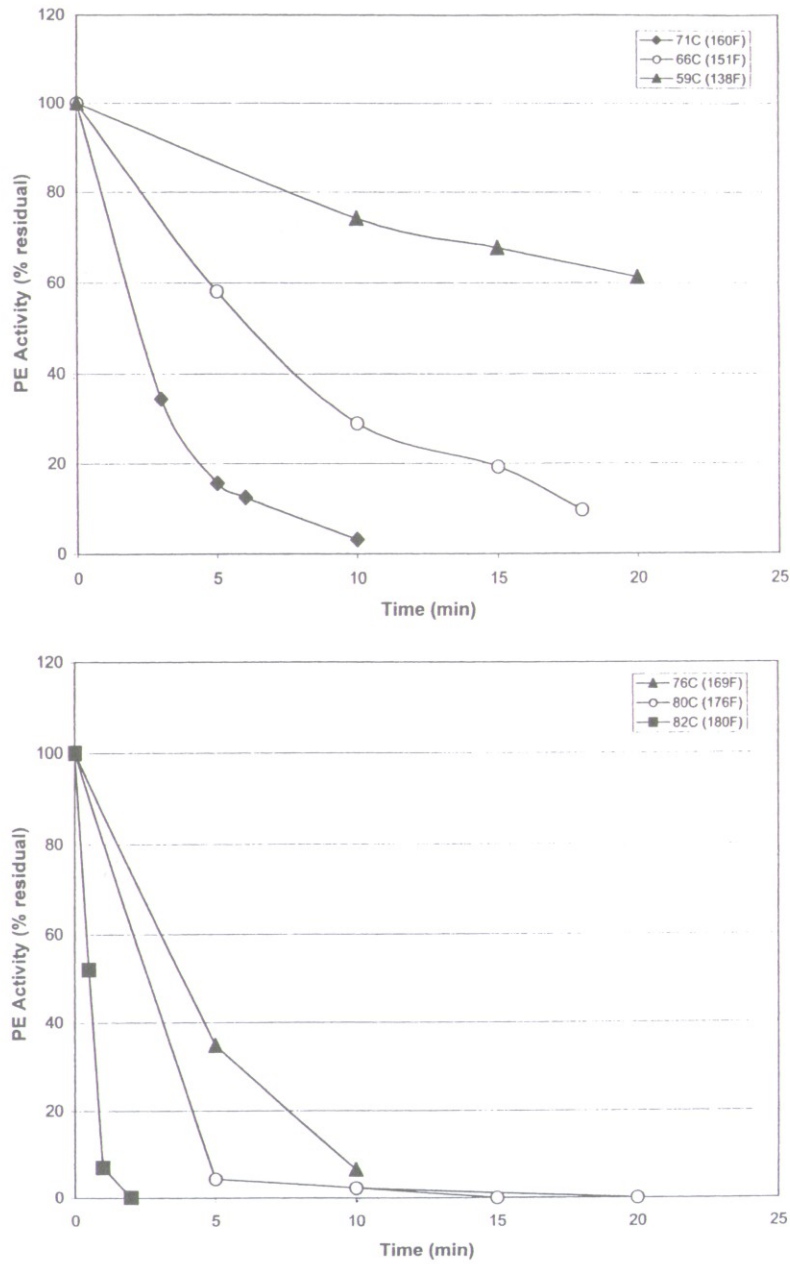


FIG. 6. HEAT TREATMENT OF (A) ONION POWDER EXTRACT AT 59C, 66C, 71C, AND (B) OF ONION POWDER SUSPENSION AT 76C, 80C AND 82C

TABLE 1.
RELATIVE VARIATION OF PE ACTIVITY (%) WITH PROCESSING

Temperature	Bulb	Stem Disk
Mild	100.0	100.0
Regular	79.1	87.1
High	79.9	79.7

DISCUSSION

Removal of water through dehydration processes is a common method of food preservation. Depending on whether blanching is used in the preparation of the vegetable prior to drying, and the severity of the heat treatment, enzymatic activity may be retained. Enzymes tend to be more stable to temperature when the vegetable tissue is intact, and enzyme stability is generally higher in dry environments. Although blanching is a common operation used in preparing vegetables for processing, often it is not one of the steps used in the dehydration of onion and garlic, because of the interest in retaining characteristic *Allium* flavors. The results of PE activity obtained from spice blends containing onion and garlic, as well as dehydrated onion products (Fig. 1 and 2) confirm that substantial residual PE activity is present in all products tested. Moreover, variability of PE activity in spices may be influenced by onion or garlic cultivar, possible presence of other spices containing residual PE activity, and variable moisture content. Nonetheless, depending on the use these spices, it is important to ensure that PE is inactivated prior to their addition to some final products, such as a tomato-based sauce or salsa. Alternatively, it is necessary to achieve thermal inactivation in the processing of the formulated product in order to avoid quality defects such as shown in Fig. 3. Similarly, gelation due to residual PE in oranges can also occur in concentrated orange juice, which has been very well studied (Blundstone *et al.* 1971).

Results on fresh onions showed that PE activity was consistently higher in the "stem disk" than in the "bulb" (Fig. 4). No correlation was found between onion bulb size and PE activity; a considerably high variation in PE levels was detected in the three batches of onions analyzed. The spice industry screens and air classifies the dehydrated products by size and shape; there are estimates that as much as one-third of the total dried onion results in onion powder (Van

Arsdel *et al.* 1973). It is expected that the more comminuted products (powdered and granulated), include proportionally larger amounts of "stem disks". This would explain the significantly higher (4-fold) PE activity reported in the onion powder, which originates from the stem disk, when compared to the other onion products (Fig. 2). The differences observed between "economy" and "premium" spice blends (Fig. 1) is also attributed to the different composition of these samples. The industry offers spices in a great variety of forms, and commonly in at least two grades, referred to as "economy" and "premium". "Economy" spices often include peels and root residues, while the "premium" grade contains only the bulb. In the case of onion blends (Fig. 1), results of the "economy" samples are higher (double) than the "premium" samples, probably due to the higher PE activity in the stem disk. In the case of garlic this was not observed; the difference has been attributed to the different anatomy of onion and garlic. When the garlic cloves are detached from the bulb the stem disk is separated, which do not happen in the case of onion bulbs. Possible explanations for the variation of PE activity in the two different regions of the bulb are that most of the PE is synthesized in the stem disk, and/or that different PE isozymes are expressed in these two regions of the onion bulb.

It is known that, in general, pectinesterases are activated in the presence of cations, and the concentration of NaCl in the extraction media may affect the enzymatic activity; some conditions were identified for tomato PE (Pressey and Avants 1972), but no information is available on onion PE. Moreover, different isozymes respond differently to variable concentrations of NaCl, and multiple forms of PE have been identified in several plant tissues. In the preparation of our samples 0.2 M NaCl was used, because higher PE activity was obtained with sodium chloride solution than with water. Nevertheless, all the PE measurements were carried out under identical conditions.

With an optimum temperature at 55C, PE activity declines rapidly at higher temperatures (Fig. 5). When extracts of onion PE were heated, it was clear that the enzyme requires temperatures above 70C for complete inactivation under feasible heating times. Using suspensions of onion powder, inactivation of PE was achieved at 82C after 2 min. An earlier work on gelation of tomato products caused by *Allium* spices recommended a heat treatment in water at 82C for about 20 min to ensure inactivation of PE (Mersfelder and Ehrman 1985). In the processing of orange juice, slightly increased heat treatment is also required for juices containing larger amounts of pulp (Rouse and Atkins 1952). The heat treatment required for inactivation of orange PE is 1 min at 90C (Versteeg *et al.* 1980); in fact, in the case of orange juice the pasteurization temperature required is more severe than that necessary for microbiological control of the product (Cameron and Grohmann 1996).

When preparing tomato-based products with spices, one possibility would be to inactivate onion PE concomitantly with the inactivation of tomato pectic

enzymes (polygalacturonase and pectinesterase). Tomato PE is inactivated during the "hot break" treatment frequently used in the preparation of tomato juice, where temperatures are in the range of 82 to 104C (Gould 1992). However, it is a common practice in the industry to store tomato paste prepared during the summer season for further use in the preparation of other tomato products, such as salsas, sauces, etc. In this case, the tomato pectic enzymes were previously inactivated, and except for the inactivation of the onion PE, the formulated product may not require much additional heating, due to the low pH of tomato products. Gelation of tomato-based products has occurred in industrially formulated products to which *Allium* spices (containing active PE) have been added and left in contact with the tomato base prior to heat processing of the formulated product. Nevertheless, it should be pointed out that although the GMP and HACCP requirements were adequately followed, the contact time between the tomato paste with the PE-active spices was sufficient to lead to development of gelation in the final canned product. It is suggested that to avoid excessive heating of the formulated products, in order to obtain a product of high quality flavor and consistency the heat treatment of the spices be carried out prior to their addition to the tomato base. Another possibility that can be envisioned is to blanch the onion or garlic before drying. This treatment could lead to complete inactivation of the enzymes prior to dehydration, however, it would certainly affect the sensory characteristics of the final product, which is undesirable. In addition, the problem could also be tentatively solved by the spice industry in the production of a high quality product free of active PE, if segregation of the stem disks could be carried out prior to drying. This could be achieved by removing the stem disk prior to slicing of the bulbs, and as the PE activity is lower in the bulb, probably less severe heat treatment would be required, thus helping to preserve the typical flavor of the dehydrated spices. It should be pointed out that the use of highertemperatures in the dehydration of spices can contribute to flavor losses, possible discoloration, and result in final product with a lower commercial value. Nevertheless, optimization of the process is necessary. Resulting products would have a higher cost, but this type of product would not be required for all spice uses.

In the process of drying fruits and vegetables the choice of temperature to be used is the result of a compromise between efficiency of the process, energy cost, and product quality. Although different driers are suitable for dehydration of onion and garlic, such as tunnel dryer, vertical bin dryer, and fluidized bed dryer, the most common system used is fluidized bed. Such dryers operate at initial temperatures around 110C, and gradually are reduced to about 85C, and later to around 65C, followed by finishing in stationary bins receiving dehumidified air at 45C. Often drying temperatures used in tunnel dryers are in the range of 75 to 55C (Lin 1997). Our results showed that the drying conditions (temperature and time) normally used are not sufficient to ensure complete PE

inactivation. In a preliminary test of dehydration conditions it was possible to achieve reduction of PE levels of 20% (Table 1); when a more severe heat treatment was used, however, there was no change in the residual PE activity in the bulb. For the three drying temperatures tested, the PE activity in dried onions was 2.3 to 5.8 times greater in the stem disk than the activity in the bulb. Evidently, further study on the optimization of the drying conditions is required, with measurement of the temperature not only of the dryer circulating air, but with probes to measure the temperature reached by the spices, and in parallel, an appropriate enzyme kinetics study should be conducted to follow the enzyme inactivation.

CONCLUSIONS

Under the common heating conditions used in the dehydration of onion and garlic, considerable pectinesterase activity is retained. This residual activity is sufficient to cause gelation of tomato-based products, if an appropriate heat treatment to inactivate PE in the spices is not applied during product formulation, particularly when formulating products using stored tomato paste previously heat treated. A minimum treatment of 0.5% spice suspensions at 82C for 2 min was required for PE inactivation in small scale lab conditions; for an industrial scale process the heating treatment may need to be adjusted.

PE activity levels in dehydrated onion can be reduced through the use of higher temperatures during the dehydration process; with a mild increase in the drying temperatures it was possible to achieve a 20% decrease in the enzymatic activity of the onion bulb, but higher temperature was required to obtain a comparable enzymatic reduction in the onion stem disk. If PE inactivation is targeted, further studies need to address dehydration conditions, involving changes in temperature and time of drying, and ideally evaluate the sensory qualities of the product.

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