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Extraction of Pectinesterase from Jalapeno Chili Pepper (*Capsicum annuum*) and Its Thermal Stability

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Abstract The effect of NaCl solution concentration (from 0 to 3 M) on the extraction of pectinesterase (PE) from jalapeno chili pepper (*Capsicum annuum*) was studied by determining its solubilization degree from the chili tissue. All concentrations of the salt favored the solubilization of PE in jalapeno chili pepper, compared to that in water. Maximum enzyme activity was obtained with NaCl 2.0 M. The effect of temperature on the PE activity of jalapeno chili pepper in the extracts was also studied. The PE residual activity of jalapeno pepper was 75% after 60 min of incubation at 55°C and 10% at 75°C. At 85-95 °C, PE residual activity was 5% after 5 min of incubation.

Keywords: chili, pectin methylesterase, solubilization, pectin

Introduction

Jalapeno chili pepper (*Capsicum annuum*) is a member of the genus *Capsicum* that includes 22 wild species and five domesticated species: *C. annuum*, *C. baccatum*, *C. chinese*, *C. frutescens* and *C. pubescens* (1). The word "chile" derives from the Mexican dialect Nahuatl "chili", and curiously this word has been adapted in countries like India, where the Spaniards introduced the chili in the 16th century. Chili or chile (in Spanish) was one of the most common tributary products of the pre-Hispanic period. Today, the chili is an important ingredient of the Mexicans diet at all social levels; it may therefore be considered as a common denominator between social classes. The chili is one of the attributes identifying the Mexican culture. Jalapeno chili peppers have been used as flavoring for the preparation of traditional Mexican foods (tacos, burritos, quesadillas, nachos or tortilla-chips, etc.) and their consumption has become very strong in the United States and the rest of the world. There are industrial products available in the market that take advantage of the special attributes of jalapeno chili pepper such as sauces, cheese dips, canned pepper rings, etc. In addition to the attractive green color of the jalapeno pepper, the most special attributes are pungency (2) and crunchy texture (3).

Softening or hardening during vegetable processing is largely due to the degradation or modification of pectic substances in the primary cell wall and middle lamella through enzymatic, chemical or physical treatments (4). Their change is related with the intensity of the treatment. For example, high-temperature, short-time (HT-ST) blanching decreases the texture and eradicates all biological activity, whereas in some cases low-temperature, long-time (LT-LT) blanching increases the texture and active biological activity (5, 6).

Pectinesterase (PE; EC 3.1.1.11), a demethoxylating enzyme of pectin that is commonly found in fruits and vegetables and it is also synthesized by various microorganisms, is responsible for texture vegetable modifications (7). Biochemically, the enzyme removes methoxyl groups from the homogalacturonan regions of the pectin molecule by a nucleophilic attack on the ester bound, resulting in the formation of an acyl-enzyme intermediary with the release of a methanol molecule, followed by a deacylation and hydrolysis of the acyl-enzymes to regenerate the enzyme and carboxylic acid, yielding low-methoxyl pectic substances (8).

LT-LT blanching has been used to activate PE in jalapeno peppers (3, 9). However, the thermostability of the enzyme has not been reported. Furthermore, extraction and quantification of endogenous PE is needed to measure the effect of temperature on the activation or inactivation process to design appropriate blanching conditions, either to inactivate or activate the enzyme (8). Fayyaz *et al.* (10) have recommended that an enzyme extraction study is advisable to develop and optimize an extraction procedure for a given plant material rather than to adopt any procedure published in the literature.

The aims of this work were, (1) to determine the effect of NaCl concentration on PE solubilization from jalapeno pepper, and (2) to determine the thermal effect of HT-ST and LT-LT treatments on PE extracts.

Materials and Methods

Materials Jalapeno chili pepper (*Capsicum annuum*) was obtained from a local supermarket (Saltillo, Coahuila, Mexico) in January 2001 and stored in a refrigerator at 5 °C until used. Pectin (galacturonic acid, 76%; methoxyl, 77%, P 9135) and galacturonic acid were purchased from Sigma-Aldrich (Mexico City, Mexico) and NaCl from Fermont (Mexico City, Mexico). Sodium hydroxide and

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acetic acid were from Productos Químicos Monterrey (Monterrey, Mexico). All other chemicals were of reagent grade.

Preparation of extract from jalapeno pepper Jalapeno pepper was washed carefully with water and dried with paper at room temperature. The fruits were cut into small pieces (0.5 × 0.5 cm) and the seeds were separated by hand. Material tissues were suspended in distilled water or NaCl solution (0.5-3.0 M) and quickly homogenized for 1 min in a blender (Osterizer®, Mexico city, Mexico). The ratio of tissue to extractant was 1:4 (w/v). The homogenates were filtrated through a filter paper and the retained material was discarded. Filtrates obtained were checked for conductivity with a hand-held conductivity meter (model WD-35 607-30, Oakton®, Vernon Hills, IL, USA). Subsequent filtrates were dialyzed in cellulose membranes against distilled water (1:100) overnight or during 12 hr under refrigeration and constant agitation. All extractions steps were carried out in duplicate, at 4°C. After dialysis filtrates were checked for volume and conductivity, and all samples were frozen at -20°C until used.

Assay for PE activity Frozen extracts were thawed by immersion in a water bath at 37°C under agitation. The filtrate was adjusted to pH 7.0 with NaOH 0.1 M or 0.01 M. PE activity was measured titrimetrically by the method of Kertesz (11), which estimates the free carboxyl groups formed in pectin as a result of enzyme demethylation. The amount of 0.010 M NaOH required to maintain a pH of 7.0 at 37°C was measured by the method described by Kertesz (11). Two milliliters of jalapeno chili pepper filtrate were added to 20 mL of citrus pectin solution (1.0 %, w/v). The reaction was continuously monitored at the correspondent pH for 30 min. One unit of PE was defined as the amount of enzyme able to release 1 μmole of carboxyl groups/min. PE activity was calculated using the following formula (12):

PE (U/mL) = [(mL NaOH)(Molarity of NaOH)(1000)]/ [(min)(mL sample)]

Thermal treatments Jalapeno pepper extract was prepared with 2 M NaCl, as described previously. The dialyzed extract (4 mL) was mixed with 1 mL of 0.5M Na-acetate buffer, pH 5.0 and placed in a 10 mL tube glass covered with screw-cup. The extracts were subjected to HT-ST or LT-LT treatments, after which the samples were quickly cooled in a water-ice bath. Controls were maintained in a water-ice bath for the corresponding times (0, 5, 10, 20, 40, and 60 min). All samples were dialyzed overnight at 4°C against water and residual activity was measured.

Comparison of PE activity from jalapeno chili pepper PE activity of jalapeno pepper extracts were compared with those of potato (*Solanum tuberosum* var. alpha), lime (*Citrus aurantifolia*) and orange (*C. sinensis*). PE extracts from whole potato were prepared by the method of Montañez-Saenz *et al.* (13). Lime and orange PE extracts were prepared from peels according to the methods of Contreras-Esquivel *et al.* (8) and Versteeg (14), respectively. All samples were prepared with the same ratio of tissue to extractant (1:4, w/v) and dialyzed overnight against water at 4°C. Extraction time for all samples was 30 min.

Experimental design and statistical analysis A monofactorial design was employed to evaluate the effect of NaCl concentration on PE extraction. Bifactorial design was used to evaluate the effect of thermal treatment on jalapeno pepper PE extraction. A monofactorial design was used to evaluate the PE activity of jalapeno pepper, lime, potato and orange extracts. The response variable was PE activity (U/mL of extract). Treatments were carried out in triplicate. The obtained results were analyzed using computer software for experimental designs (15).

Concentration of the crude extract A measured volume (500 mL) of the crude extract was taken in a dialysis membrane bag (cut off: 12.4 kDa) and concentrated against polyethylene glycol-6000 (PEG-6000) to about 20 times of the original volume. The concentrated sample was taken out and the dialysis bag was rinsed with distilled water to ensure maximum recovery.

Chemical analysis Glucose, total sugars, galacturonic acid and soluble protein contents of the extracts were measured before and after dialysis. The amount of glucose was determined using a glucose oxidase - peroxidase diagnostic kit (Winerlab, Rosario, Argentina). Total sugars and galacturonic acid were assayed as described by Fry (16). The protein concentration was determined according to Bradford microassay (BIO-RAD, Mexico City, Mexico) using bovine serum albumin as standard.

Results and Discussion

PE extraction from jalapeno chile pepper Before assaying the PE activity, all jalapeno extracts were dialyzed against distilled water for desalinization. The conductivity of the extracts at different NaCl concentrations, before and after the dialysis process, is shown in Fig. 1. Independently of the initial conductivity, the dialyzed extracts showed an efficient salt elimination. Extracts were evaluated qualita-tively for color and cloudiness. All extracts

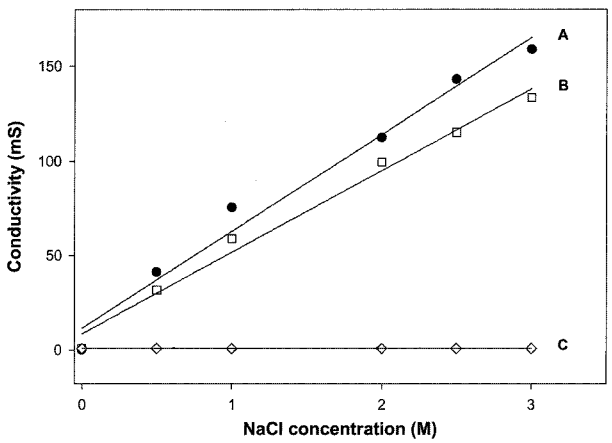

Fig. 1. Effect of dialysis on the conductivity of jalapeno pepper extracts. A) NaCl solutions (standards), B) Extracts filtrated before dialysis, C) Extracts filtrated after dialysis. See text for details.

showed a homo-genous green color before and after dialysis. Meanwhile, cloudiness was lost in all extracts indicating the presence of soluble pectin in the extracts that was demethoxylated by solublilized PE. More cloudiness was observed in treatments from 2.0 to 3.0 M NaCl. Citrus PE causes one of the most intensively studied problems in food techno-logy; namely, the cloud loss of citrus juices (7, 14). When jalapeno chili pepper is used for sauce preparation, cloud loss takes place when raw material is not blanched appropriately.

The effect of NaCl concentration on the extraction of PE from jalapeno pepper is shown in Fig. 2. The PE activity in the water extracts was very low. The activity increased substantially when NaCl solution was used as an extractant. A linear increase was observed from 0.5 to 2 M NaCl, while from 2 to 2.5 M there was no significant difference. Then a decrease was observed at 3.0 M NaCl. PE activity was seven times higher with 2.0 or 2.5 M NaCl than that observed for water. Differences between PE activity extracts can be explained by assuming that different PE isoenzymes are gradually released from the tissue as the ionic strength increases. When cell-wall tissues are incubated in NaCl solution, ionically bound proteins are released. Various NaCl concentrations for PE solubiliza-tion have been reported for optimal PE release from fruit or vegetal tissues. In citrus fruit, generally a low NaCl concentration (0.25-0.50 M) is necessary (8, 14), whereas for other vegetables like persimmon (*Diospyros kaki* L. f.) (17), papaya (*Carica papaya* L.) (10), soursop fruit (*Annona muricata* L.) (18), star-fruit (*Averrhoa carambola* L.) (19), jicama (20) and Peruvian carrot (21) higher ionic strength (1.0 to 3.0 M) is necessary to obtain an optimal PE desorption from the tissue. Galacturonic acid, glucose and total sugars were evaluated before and after dialysis of jalapeno chili extracts from 2 M NaCl (Table 1). Dialysis lowered the concentration of glucose and galacturonic acid in the extract. Total sugar showed a minor decrease, possibly because of the interaction with polymeric materials. Soluble protein was not detected in the extract. However, when extract was concentrated 20 times the protein content was 178 mg/L. A high yield (-80%) of PE was also obtained when polyethylene glycol 6000 was used.

Thermal treatments Jalapeno pepper PE extracts were subjected to LT-LT and HT-ST treatments at pH 5.0. The measured residual PE activities after LT-LT treatments are presented in Fig. 3. Data showed that jalapeno pepper PE is very stable at LT-LT incubation of 55°C for 60 min (-25 % loss of activity). Nevertheless, with HT-ST treatment, 10% residual activity was obtained at 75°C after 60 min (Fig. 3), and 5% PE residual activity was obtained at 85-95 °C after 5 min (Fig. 4). From Fig. 3 and 4, it can be seen that the unheated extract did not show any reduction in its PE activity when it was maintained on ice. The results show that HT-ST treatment required to inactivate the PE jalapeno agrees with that previously reported for acerola (12), papaya (22) and citrus (23). The stability of PE is affected by some factors, such as pH and ionic strength (23). In general, vegetal PE isoenzymes have a higher thermal stability (12). In accordance with preliminary studies of purification by ion-exchange (DEAE-Sepharose) chromatography of the jalapeno PE in our laboratory, there is more than one isoenzyme with PE activity.

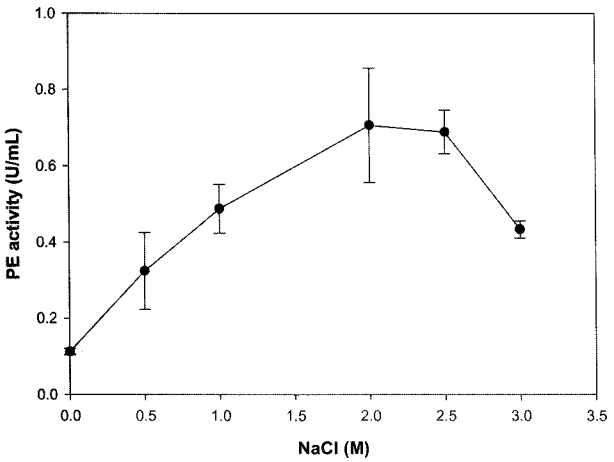

Fig. 2. Effect of NaCl concentration on PE solubilization (jalapeno chili pepper tissue).

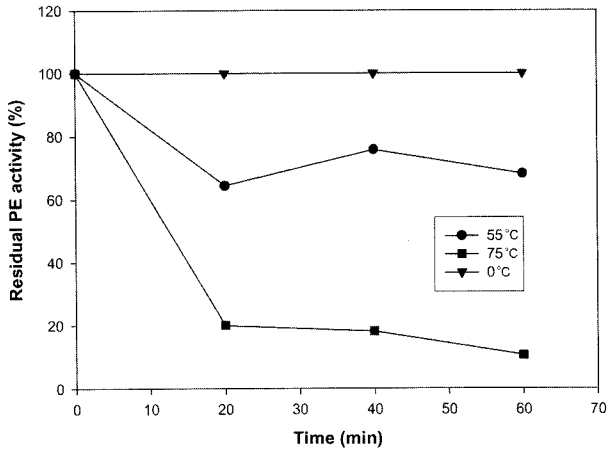

Fig. 3. Residual PE activity after low-temperature, long-time incubation of jalapeno chili extracts.

Table 1. Total sugars, galacturonic acid and glucose analysis for non-desalinated and desalinated extract from jalapeño chili pepper obtained with 2 M NaCl

Dialysis procedure	Total sugars (ppm)	Galacturonic acid (ppm)	Glucose (ppm)	Protein (ppm)
Before	3409.96±191.54	131.09±48.93	2407.70±48.81	N.D.
After	1551.33±205.01	20.94±7.33	310.52±71.84	N.D.

N.D.: not detected

Table 2. Comparison of PE activity titers from jalapeno pepper.

Source	NaCl (M)	Extraction time (min)	pH extraction	Activity (U/mL)	Reference
Jalapeno pepper	2.00	30	7.0	0.80±0.10	This work
Potato	1.00	30	7.0	2.30±0.50	(13)
Lime peel	0.50	30	7.0	70.2±7.32	(8)
Orange peel	0.25	30	7.0	40.3±0.39	(14)

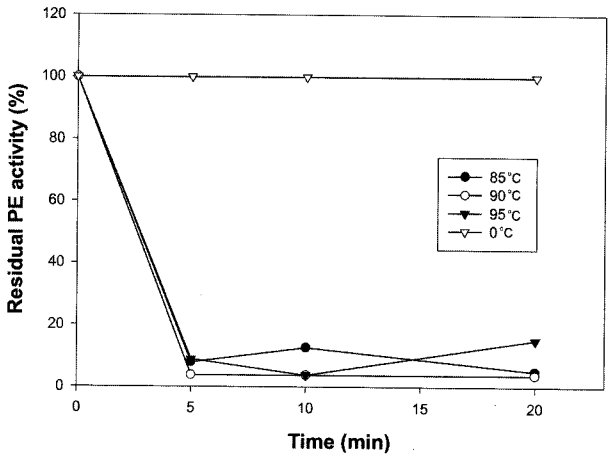

Fig. 4. Residual PE activity after high-temperature, short-time incubation of jalapeno chili extracts.

Comparison of PE activity from jalapeno pepper The data in Table 2 show that jalapeno chili pepper extract has a lower PE activity than lime or orange peel extracts. The PE activities for chili and potato extracts were similar in both cases. Citrus and tomatoes are fruits showing higher titers for PE activity (7). Knowledge of PE activity is very important to predict thermal activation, to increase texture or thermal inactivation, and to avoid cloud loss in sauces and juices. For example, Javeri *et al.* (24) and Banjongsinsiri *et al.* (25) have used vacuum infusion for exogenous citrus PE to improve the texture in peaches and strawberries with low endogenous PE activity. In accordance with the results of jalapeno PE activation carried out by Villarreal-Alba (3), it requires 40 min and 60°C to increase the texture of jalapeno chili. In order to diminish the time to enhance the texture in jalapeno pepper by PE treatment, the use of an exogenous enzyme either of vegetable or microbial origin is recommended.

Concluding remarks This study has suggested that a high percentage (-80%) of bulk PE is ionically adsorbed into vegetal tissue, which has been released using a NaCl solution. Furthermore, the results show that PE from jalapeno pepper is highly stable following LT-LT blanching (55°C). Further research is being conducted on the electrophoretic studies and PE purification from jalapeno pepper with details to be published in the near future.

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