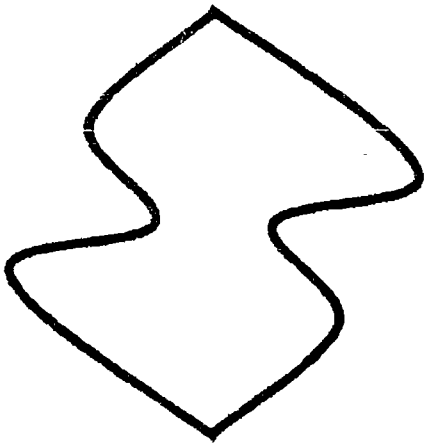
BOOK OF ABSTRACTS

3rd International Conference on High Pressure Bioscience and Biotechnology



hpbb 2004

September 26 – 30, 2004
Rio de Janeiro, Brazil
Hotel Pestana

PEPPER PECTINMETHYLESTERASE CATALYSED CONVERSION REACTION. THE EFFECT OF THERMAL AND HIGH-PRESSURE TREATMENTS

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ABSTRACT

INTRODUCTION

The popularity of peppers (Capsicum annuum) suddenly increased in the past few years due to their application in a wide range of food products. One of several examples most appreciated in the young consumers communities is the dippings for French fries and tacos, where peppers can be found as one of the ingredients. During industrial processing of food products, modifications within the pectin structure can often occur, either caused by chemical conversions, either by the action of endogenous pectin degrading enzymes, namely pectin methylesterase (PME) and polygalacturonase (PG). The reactions catalysed by these enzymes can influence the functional properties of foodstuffs in a positive or negative way. It is well known that the synergistic action of these two enzymes in tomato-based products can lead to undesirable changes during the industrial processing (e.g., decrease in viscosity). However, by controlling PME activity, at the same time selectively inactivating PG, some quality parameters, namely texture, of most vegetables can be preserved or even improved. Previous enzyme inactivation studies have shown that, under suitable high-pressure/temperature conditions, PG can be inactivated, whereas PME can remain active (Crelier et al., 2001; Fachin et al., 2003; Verlent et al., 2004). The firming effect that most vegetables and fruits exhibit after precooking at moderate temperature has been described in the literature and can be attributed to the activity of PME. Due to PME action on the cell wall materials, in particular pectic substances, low methoxyl pectin is formed, which can be cross-linked in presence of divalent cations. The aim of this work is the study of the effect of thermal and combined thermal/high-pressure treatments on purified pepper PME activity in the presence of pectin in a model system (pH 5.6, the pH of the green bell peppers under study).

MATERIALS AND METHODS

Extraction and purification of green bell pepper PME

Green bell peppers (*Capsicum annuum*), purchased from a local auction (Mechelen, Belgium), were cut into small pieces, frozen in liquid nitrogen and stored at –80°C until use. PME was extracted from peppers with 0.2 M Tris(hydroxymethyl)-aminomethan buffer (i.e., Tris buffer) (pH 8.0) with 1 M NaCl, followed by purification using affinity chromatography on a NHS-Sepharose-PME-inhibitor column, according to Ly-Nguyen

et al. (2002). The PME fractions were pooled together and concentrated with Centricon Plus 10 (Millipore) for further analysis.

Activity studies

Isothermal experiments were performed in a water bath with temperature control, whereas a laboratory pilot scale, multivessel high-pressure equipment (Resato, Roden, The Netherlands) was used to perform the isobaric/isothermal experiments. The enzymatic reaction catalysed by purified pepper PME was initiated at atmospheric pressure by adding purified pepper PME to 30 mL to 0.4% (w/v) pectin solution. The pH during the enzymatic reaction was controlled by dissolving the pectin in citrate buffer at pH 5.6, containing 0.4 M of NaCl. To perform the isothermal experiments, individual pyrex tubes were filled with the substrate-enzyme solution and incubated in a thermostated water bath for preset time interval. An equilibration period was considered to ensure isothermal conditions. The temperature range applied for the determination of purified pepper PME activity during thermal treatments was from 18°C to 65°C. For isothermal/isobaric experiments, flexible microtubes were filled with the substrate-enzyme solution and enclosed in the pressure vessels, already equilibrated at a preset temperature. Pressure was build up slowly (100 MPa/min) to minimize adiabatic heating. As for isothermal treatments, after reaching the desired pressure, an equilibration period was taken into account to allow temperature to evolve to its desired value. After this moment, the other vessels were decompressed after preset time intervals. The pressure range studied varied from 200 MPa to 600 MPa, while temperature range was from 35°C to 60°C. After both isothermal and isothermal/isobaric treatments, the reaction was quenched (heat-shock treatment) and the release of methanol, produced during the reaction of PME on pectin as a function of time, was determined colorimetrically according to Klavons and Bennett (1986). The amount of methanol formed in a pectin solution, without the enzyme addition, was also determined due to the possibility of chemical hydrolysis.

DATA ANALYSIS

The reaction catalysed by purified pepper PME was followed by measuring the release of methanol during thermal or combined pressure/temperature treatment. The PME activity (μg MeOH/mL pectin solution, min) was estimated from the initial linear part of the curve obtained by plotting the amount of methanol formed as a function of time and denoted as V_0. Due to the variations of the diluted solution of purified pepper PME daily prepared, it was necessary to divide this activity by the amount of units formed as determined under the standard assay conditions (measured titrimetrically at pH 7.0, 22°C). The normalized PME activity is denoted as V'_0. Once the initial rates of pepper-PME-catalysed methanol formation (V'_0) at different temperatures are known, the temperature dependence of V'_0 at given pressure, expressed by the activation energy (E_a, kJ/mol), can be estimated in the temperature area in which the reaction accelerates, using the Arrhenius model [Eq. (1)]:

$$V'_0 = V'_{0,ref} \exp\left[\frac{E_a}{R_{ref}}\left(\frac{1}{T_0} - \frac{1}{T}\right)\right] \qquad (1)$$

As a measure of the pressure dependence of V'_0 at a given temperature, the activation volume (V_a, cm^3/mol) can be estimated using the Eyring equation [Eq. (2)]:

$$V_0' = V_{0,ref}' \exp\left[\frac{-V_a}{RT}(P - P_{ref})\right] \qquad (2)$$

The activation energy and the activation volume can be estimated by linear-regression analysis of the natural logarithm of V'_0 versus the reciprocal of absolute temperature or versus pressure, respectively.

RESULTS AND DISCUSSION

Different concentrations of enzyme and substrate were assayed so that it was possible to identify an appropriate experimental set-up. The enzyme concentration was adjusted so that the amount of methanol formed by PME increased linearly with time, during at least 7 minutes. Therefore, the action of PME could be accurately estimated from the slope of the methanol formation versus treatment time. The pectin concentration was increased gradually until substrate saturation was attained. For all experiments described, an enzyme concentration of 8-16 units/mL and a substrate concentration of 0.4% (w/v) of pectin were used. Under these conditions, PME saturation by the substrate ([S]>Km) was maintained and hence maximal activity could be determined. Methanol formation in a pectin solution without PME addition was also investigated under the studied conditions, and no chemical hydrolysis of pectin at pH 5.6 was observed. Spontaneous demethylation of pectin occurs under mild alkaline conditions and room temperature (Renard and Thibault, 1996) and the rate of chemical saponification is accelerated at higher temperatures (Van Buren, 1979).

The pressure range studied varied from atmospheric pressure to 600 MPa, while temperature range was from 35°C to 60°C. The results are depicted in Figure 1. At pH 5.6 and atmospheric pressure, the optimal temperature for activity of purified pepper PME was found within the range of 50-55°C. For commercial tomato PME, an optimum temperature of 55°C within the pH range of 7.0 to 7.5 was also observed, while purified tomato PME presented 45°C and 35°C as an optimum temperature at pH 8.0 and 4.4, respectively (Verlent *et al.*, 2004; Van Broeck *et al.*, 2000).

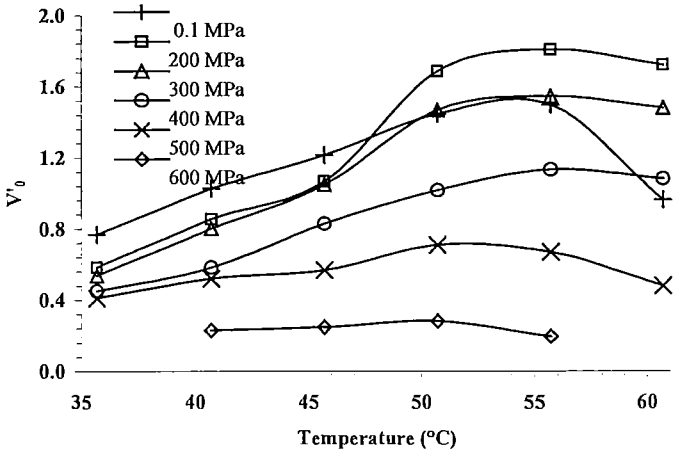

Figure 1 Purified pepper PME activity (V'_0) as a function of temperature.

The optimal temperature range for purified pepper PME activity at elevated pressures was broader (50-60°C) when compared to atmospheric pressure (Figure 2). A shift in the optimal temperature at elevated pressures has already been observed for commercial and purified tomato PME (Van den Broeck et al., 2000; Verlent et al., 2003). The optimal temperature at elevated pressures shifted to higher values as compared to atmospheric pressure. Optimal temperature shift has also been observed for other enzymatic reactions. PG-catalyzed hydrolysis of polygalacturonic acid at pH 4.4 shifted to lower values as compared to atmospheric pressure (Verlent et al., 2004).

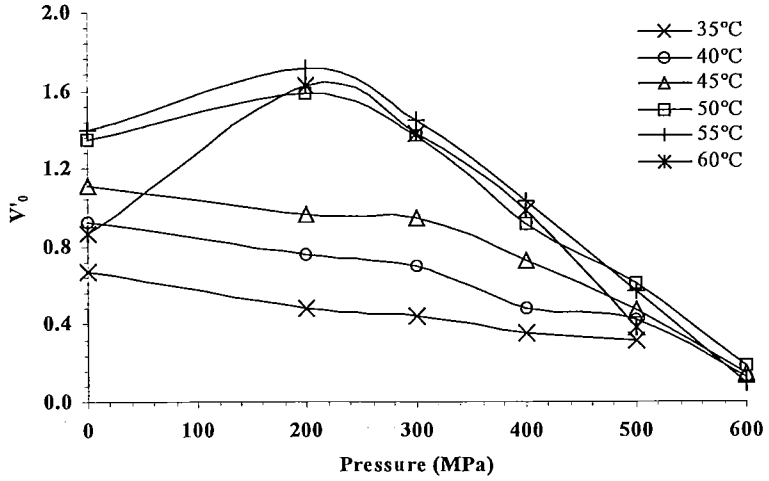

Figure 2 Purified pepper PME activity (V'_0) as a function of pressure.

At 200 MPa, a higher enzymatic activity of purified pepper PME was observed in the temperature domain of 50-60°C, as compared to atmospheric pressure, whereas at 300 MPa, a higher activity was only noted at temperatures above 55°C. As a consequence of this patterns (e.g., an increase in temperature and acceleration of the reaction under pressure), the catalytic activity of purified pepper PME at temperatures above 55°C, is significantly greater at pressures up to 300 MPa as compared to atmospheric pressure. At temperatures lower than the optimal temperature (i.e., T<50°C), pepper PME activity decreased with increasing pressure. But for temperatures higher than 50°C, there was an enhancement of the pressure effect up to 200-300 MPa, followed by a decrease for higher pressures. For tomato PME there was a clear enhancing effect of pressure on the PME activity under the thermal/high-pressure conditions studied (35-65°C and 150 MPa-600MPa) when compared to atmospheric pressure (Verlent et al., 2004).

The temperature dependence of V'_0 at a given pressure (E_a) in the temperature range in which the reaction is accelerated, and the pressure dependence of V'_0 at the different temperatures (V_a), could be described by Arrhenius and Eyring equations, respectively (Table 1). From the analysis of Table 1, the temperature sensitivity of V'_0 seemed to be almost unaffected by pressure up 400 MPa. Since the activation volumes were positive, pressure retards this enzymatic conversion. The activation volumes slightly increased with increasing temperature up to 55°C; therefore, the pressure sensitivity of V'_0 slightly increased with increasing temperature.

Table 1 Pressure and temperature dependence of purified pepper PME-catalysed pectin demethylation (V'_0) at pH 5.6.

T (°C)	V_a (cm^3/mol)	R^2
35	3.93±0.19[a]	0.99
40	4.23±0.72	0.92
45	6.45±1.76	0.87
50	8.77±1.20	0.96
55	9.75±1.69	0.93
60	17.86±4.86	0.93

P (MPa)	E_a (kJ/mol)	R^2
0.1	31.16±4.51[a]	0.94
200	54.91±5.77	0.97
300	51.55±6.33	0.96
400	47.08±4.81	0.97
500	26.51±4.82	0.94
600	27.72±4.98	0.97

[a] Standard error regression.

Treatments that increase PME activity, such as temperature and pressure, may be interesting for improving the texture of fruits and vegetables. As the activation of PME at elevated pressure/temperature is more pronounced than at atmospheric pressure, care should be taken in order to avoid a too enhanced deesterification of pectin and consequently, the production of uncookable products.

ACKNOWLEDGEMENTS

S. Castro gratefully acknowledges financial support from the Fundação para a Ciência e Tecnologia (Grant BD/6642/2001), the Fund for Scientific research – Flanders and the research council of the Katholieke Universiteit Leuven.

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