Process Biochemistry 44 (2009) 77–82

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Proteolytic activity in enzymatic extracts from *Carica papaya* L. cv. Maradol harvest by-products

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ARTICLE INFO

Article history:
Received 14 July 2008
Received in revised form 12 September 2008
Accepted 16 September 2008

Keywords:
Carica papaya
Papaya by-products
Enzymatic extracts
Latex
Cysteine proteases
Zymography profiles

ABSTRACT

Proteolytic activity and the cysteine protease profile were determined for enzymatic extracts (EE) from *Carica papaya* L. cv. Maradol harvest by-products (stems, unripe fruit, petioles and leaves). The proportion of each by-product type in the sampled plantation was calculated. Polypeptide bands were identified by SDS–PAGE for each EE and molecular weight calculated for the cysteine proteases. Leaf and fruit tissue had the highest protein contents of the by-products. Leaf tissue also produced the highest total EE yield. All the SDS–PAGE gels for the EE's exhibited an approximately 23 kDa band probably corresponding to papain. The zymography profiles of the EE's were similar, with bands at approximately >202.8, 76.8, 55.4 and 46.5 kDa. The fruit EE had the highest specific proteolytic activity and the leaf EE the lowest. Fruit and stem by-products are the most promising for proteolytic enzyme extraction.

1. Introduction

Papaya belongs to the *Caricaceae* family, which encompasses four genera. The most important of these is *Carica*, with a wide diversity of species including *Carica papaya* L. cv. Maradol, a variety well known in Mexico [1]. The Maradol variety was introduced into Mexico in 1978 and its commercial cultivation has been slowly replacing that of criollo varieties since 1998 [2]. In 2006, Mexico produced 798,589 tons of papaya and was the principal papaya exporter to the United States, accounting for 65% of imports [3]. This same year the state of Yucatan, Mexico produced 54,497 tons of papaya, making it the fourth largest producer nationwide [4].

Papaya fruit is highly appreciated worldwide for its flavor, nutritional qualities and digestive properties [5]. When unripe, it contains the enzyme papain (EC 3.4.22.2), a cysteine protease with action similar to that of the pepsin in gastric juice. Papain has multiple applications in the food industry (e.g. a clarifier in beers [6], a meat tenderizer and in preparation of protein hydrolysates [7]) and the pharmaceutical industry (e.g. in treatments for osteoporosis, arthritis, vascular diseases and cancer) [8,9]. The latex which contains papain is harvested from unripe fruit by making incisions in the fruit surface during a 4–5 day period and collecting the latex until is stops flowing (a few minutes to a number of hours) [10]. Three other cysteine proteases have been isolated from papaya latex: chymopapain (EC 3.4.22.6); caricain (EC 3.4.22.30); and papaya proteinase IV (EC 3.4.22.25) [11]. These have been purified and biochemically characterized [12]. In 1937, Balls et al. [13] developed a process for isolating and purifying papain from fresh papaya latex, which was later modified by Kimmel and Smith [14] in what has become the classic method for obtaining commercial papain from dry latex.

Papaya is harvested almost year round and during fruit harvest by-products are produced from removal of plants at the end of their productive cycle (approximately 2 years continuous production) or diseased plants, as well as in the form of unripe fruit or fruit that does not meet quality specifications (2–5% of total production) [15]. Under normal conditions, planting density in Yucatan can be as high as 2700 plants per hectare [1], meaning a large quantity of by-products are generated. These by-products are generally disposed of in open areas, although the fruit is occasionally used as animal feed, particularly in the dry season when forage is scarce. High transport costs seriously limit any secondary uses and in most cases this waste is left to rot, producing phytopathogens that cuase ecological problems and pose a risk to human health.

One potential alternative use for these wastes is extraction of proteolytic enzymes such as papain and other cysteine proteases, which are known to be present in different plant tissues (roots, stem, petiole and leaves), and not just in the fruit [12,13,16]. The

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doi:10.1016/j.procbio.2008.09.013

study objective was to produce enzymatic extracts from Maradol papaya harvest by-products using the salting out method, and determine the proteolytic activity and cysteine protease profile of these extracts.

2. Materials and methods

2.1. Biological material

Raw material was collected at Rancho San Pedro (Carretera Mérida-Tizimín Km. 139.5), Yucatan state, Mexico. Plant material included in the sample was harvest by-products consisting of culled plants that had completed the production cycle, damaged, unripe fruit and fruit that did not meet quality specifications (i.e. size and shape). By-products were classified by plant tissue type: (a) green fruit, (b) leaves, (c) stems, and (d) petioles (Fig. 1). The nature of the raw material and analysis time did not allow for simultaneous processing of all four by-products, therefore separate samplings were done for each by-product type, for a total of four samplings. All samples were stored in polyethylene bags at 4 °C until analysis.

The different proportions of by-products in the plantation were determined by random sampling of ten plants. These were disarticulated into the four tissue types, the material for each type weighed, and the resulting data used to calculate the proportions:

$$\%\,\text{By} - \text{product} = \left(\frac{\text{By} - \text{product weight}}{\text{Total plant weight}}\right) \times 100$$

Sample analysis was done in duplicate, and calculations done for the mean, standard deviation and variation coefficient of the data. Data analysis was done with a Duncan's comparison of means using the Statgraphics Plus ver. 4.1 software.

2.2. Proximate analysis

Proximate composition of the by-products was determined following standard methods [17]. Nitrogen (AOAC method 954.01), fat (920.39), ash (923.03), crude fibre (962.09) and moisture (925.09) contents were determined. Protein content was calculated as nitrogen × 6.25 and carbohydrate content was estimated as nitrogen-free extract (NFE).

2.3. Enzyme extraction

The enzymatic extracts (EE) were obtained according to Balls et al. [13] and Glibota et al. [16], with some modifications. By-product samples (20 g dry base) were ground separately by type in a blender (Osterizer). Each was mixed with 0.05 N phosphates buffer (Na_2HPO_4—citric acid) at a 1:6 ratio, and the extraction run at pH 7.0 for 1 h in an ice bath under constant agitation. The resulting liquid was centrifuged at 2500 × *g* and 10 °C for 30 min and the supernatant removed. Enzyme recovery from the supernatant was done by salting out. Ammonium sulfate was added slowly to the supernatant until reaching 40% saturation and the mixture centrifuged. The precipitate was dyalized against water at 4 °C for 24 h with 6000–8000 Da pore size Spectrapor membranes (Spectrum Labs Cat # 265132665) under constant agitation to eliminate excess salt in the extract. The product was lyophilized at −47 °C and 13 × 10^{-3} Mbars.

For comparitive purposes, fresh latex was collected from unripe fruit by making incisions in the fruit and collecting the latex until it stopped flowing. The latex was stored at −20 °C until analysis.

Protein content was determined for the EEs and the fresh latex following Bradford [18]. Approximately 10 mg of EE were used, and the latex was diluted with 50 mM phosphates buffer at a 1:10 ratio.

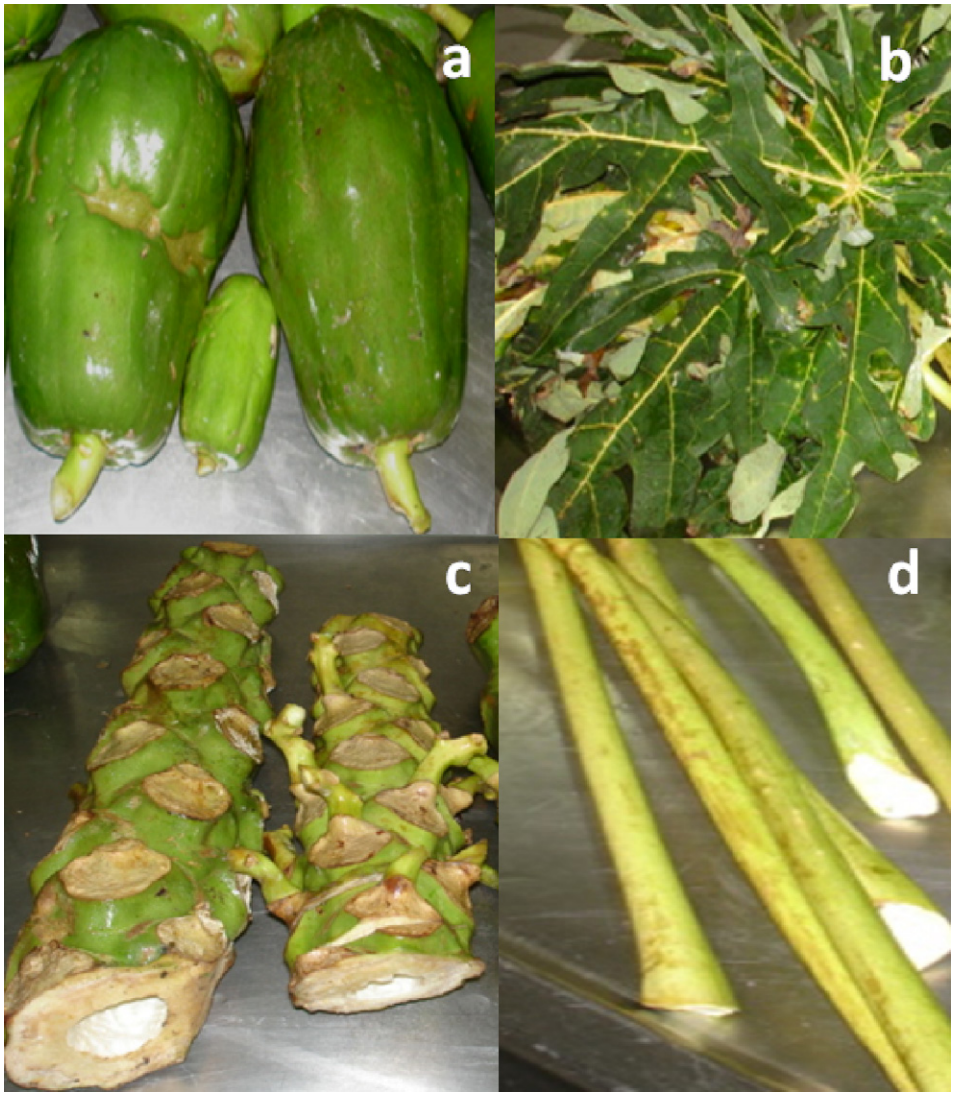

Fig. 1. *Carica papaya* L. cv. Maradol harvest byproducts: (a) fruit, (b) leaves, (c) stems, (d) petioles.