Mild Process for Dehydrated Food-grade Crude Papain Powder from Papaya Fresh Pulp: Lab-scale and Pilot Plant Experiments

Milena Lambri, Arianna Roda, Roberta Dordoni*, Maria Daria Fumi, Dante Marco De Faveri

Istituto di Enologia e Ingegneria Agro-Alimentare, Università Cattolica del Sacro Cuore
Via Emilia Parmense, 84, 29122 Piacenza, Italy
roberta.dordoni@unicatt.it

Proteases are protein digesting biocatalysts long time used in the food industry. Although many authors reported the crystallization of papain and chymopapain from papaya latex, the powder of crude papain had the largest application as food supplements due to its highly positive effect on the degradation of casein and whey proteins from cow's milk in the stomach of infants. As the industrial preparative procedures have not been extensively applied, this study aims at producing dehydrated crude papain from fresh papaya pulp, planning lab-scale trials, followed by process development toward the pilot industrial-scale.

In the lab-scale experiments, the enzyme activity (EA), expressed as protease unit (PU)/g, were evaluated on pulp and papain standard before and after a 2 h thermal treatment at 70 °C, 90 °C, and 120 °C, and the thermal behavior was monitored by means of differential scanning calorimeter (DSC). The process development toward the pilot-scaling optimized: the homogenization of the fresh pulp, followed by its filtration at high pressure (HP) in order to obtain the vegetation water and the pre-dehydrated pulp which was then oven dried varying the time-temperature conditions (4 h-80 °C; 2 h-120 °C; 30 min-150 °C). Proceeding at higher temperatures for a shorter time allowed obtaining commodity-related and technologically valid products.

In the final pilot-scale step, filtration was done with vertical HP filter press, and final dehydration was performed with 2-step turbo-drying: the first aimed to concentrate with 2 min air flow (500 m³/h) at 200 °C, the second aimed to dry with 10 min air flow (500 m³/h) at 120 °C. The resulting dehydrated pulp was grinded with ball-mill to obtain a stable powder. Starting from 90±2 % pulp moisture, the two turbo-drying steps lowered the water content from 75±4 % to 50±2 % and from 50±2 % to 8±1 %, respectively. The enzyme release from the final powder highlighted an EA of the food-grade crude papain powder extract of 28 PU/g. The thermal steps provided with turbo-driers permitted to maintain a fraction of sugars and pectin acting as a protective structure, so increasing the digestion effects provided by papain. Vegetation waters were ultra-filtered allowing at obtaining a concentrated pectin suspension and rich in nutrient waters which can be reused along the food chain.

Further efforts should be made to implement this procedure as potential alternative for the dehydrated crude papain production, deepening the impact of process variables on this matter.

1. Introduction

Carica papaya is a tropical and a herbaceous succulent plant growing in all tropical countries and many sub-tropical regions (Amri and Mamboya, 2012). Despite being highly appreciated worldwide for its flavor, nutritional qualities and digestive properties (Galindo-Estrella et al., 2009), papaya fruits do not sufficiently maintain desired fresh quality when shipped long distances due to an easily bruised soft skin and a short shelf life. This leads to both a large supply of pulp from unsightly fruit that is never distributed and low sales due to blemished fruit (De Freitas Borghi et al., 2009). Moreover, traditional thermal preservation methods may negatively alter papaya features. Thus to effectively utilize available papaya pulp,
processing requires a mild approach that enables retention of papaya natural properties. Analysis of the raw fruit provides the following results (per 100 g): 88.06 g water, 0.47 g protein, 0.26 g fat, 10.82 g total carbohydrate, 1.7 g fiber. Furthermore, papaya ranks highest per serving among fruit for carotenoids, potassium, fiber, and ascorbic acid content (USDA National Nutrient Database for Standard Reference). Nevertheless, the most important constituent is the digestive enzyme “papain.” Papain (EC 3.4.22.2) is an endolytic plant cysteine protease enzyme which may be extracted from the plant's latex, fruit, leaves and roots (Amri and Mamboya, 2012; Hitesh et al., 2012). It is a monomeric protein of 23.4 kDa, with a pI around 6.7 and a temperature of maximum activity of 37 °C; it is stable and active under a wide range of conditions. Papain enzyme can be fold in two different size of domains, each having hydrophobic core. The molecule is folded along three disulfide bridges creating a strong interaction among the side chains which contributes to the stability of the enzyme (Braia et al., 2013). Papain shows extensive proteolytic activity towards proteins, short chain peptides, amino acid esters and amide links (Uhlig, 1998). It preferentially cleaves peptide bonds involving basic amino acids, particularly arginine, lysine and residues following phenylalanine (Amri and Mamboya, 2012; Hitesh et al., 2012). According to its broad specificity papain has found a wide application, especially in food industry. It is being used to tenderize meat and meat products, in the manufacture of protein hydrolysates, in confectionery (to prepare chewing gum), brewing (to remove cloudiness in beer), and dairy industry (for cheese making). Similarly, it is further used in textile and tanning industries, for aroma and perfume production, for pharmaceutical applications, and also for effluent treatments (Chaiwuta et al., 2010). However, papain has achieved very large market share especially in the areas of food supplements being widely administered to aid the lyses of ingested casein in infants and children and more generally to promote the protein digestion in adults (Lambri et al., 2013).

Papain is a minor constituent among the papaya endopeptidases. As a matter of fact, papaya proteases are composed of 4 similar molecular weight cysteine proteases, which contribute 69–89% of total protein: less than 10 % of papain, 26–30 % of chymopapain, 23–28 % of glycol endopeptidase, and 14–26 % of carcain (Nitsawang et al., 2006). Papain is routinely purified from papaya latex obtained by cutting the skin of the unripe but almost mature fruit. Fresh latex is usually collected from locally grown Carica papaya. Initially, four to six longitudinal incisions are made on the fruit using a stain less steel knife. The resulting exuded latex is allowed to run down the fruit and drip into collecting devices attached around the trunk. Purification of papain from papaya latex has traditionally been achieved by salt precipitation and chromatography. However, the purified enzyme still remains contaminated with other proteases. A wide variety of synthetic and natural polyelectrolytes can interact with globular proteins to form stable protein–polyelectrolyte complexes that result in the formation of soluble or insoluble complexes and can be easily separated by simple decantation (Braia et al., 2013). An alternative purification strategy has involved the use of various chromatographic techniques including ion exchange, covalent, or affinity chromatography (Nitsawang et al., 2006). However, these methods led to low yields. They were also unscalable, time consuming, expensive and had multi-step protocol (Chaiwuta et al., 2010). Although many authors (Amri and Mamboya, 2012) reported the crystallization of papain and chymopapain from papaya latex, the powder of crude papain had the largest application as food supplements due to its highly positive effect on the degradation of casein and whey proteins from cow's milk in the stomach of infants. Despite being proven the enzymatic activity of freeze-dried papaya pulp used as a powerful digestive, the industrial preparative procedures of crude papain from fresh pulp have not been extensively applied.

In order to produce a shelf stable powder, containing active enzymes at low cost (for food and/or pharmaceutical applications) papaya pulp was mild processed through feasible and simple technologies, to minimize required processing and to maximize nutrient retention. To the aim at producing dehydrated crude papain from fresh papaya pulp, this study planned lab-scale trials, followed by process development toward the pilot industrial-scale.

2. Materials and methods

2.1 Analytical determinations

The papain enzyme activity (EA), expressed as protease unit (PU)/g, was determined following the Kunitz (1947) method, based on titration of tyrosine released, using casein as a substrate. The enzyme release from the papaya sample resulted only after an ultrasonic treatment (300 W for 30 min) (Zhang et al., 2013). All chemicals used in the analytical determinations were high-purity commercially available reagents. The results are expressed as mean ± standard deviation (n=3).
2.2 Lab-scale trials
The lab-scale experiments and the process development toward the pilot-scaling were arranged as detailed in Figure 1. EA was evaluated in papain standard (papain from papaya latex crude powder, 1.5-10 units/mg solid, Sigma Aldrich - St. Louis, MO, USA) and in fresh pulp from lab-scale trials before and after the thermal treatment at 70 °C, 90 °C, and 120 °C for 2 h. Thermal behavior was investigated on 20 mg samples by means of Setaram DSC-92 (Setaram, Caluire-France) differential scanning calorimeter (DSC): the working temperature ranged from 20 °C to 200 °C at 1 °C/min.

2.3 Pilot industrial-scale
Industrial-scale experiments were arranged according to the plan showed in Figure 2. In the final pilot-scale step, filtration was done with vertical HP filter press at 240 bars, and final dehydration was performed with 2-step turbo-technology (VOMM Impianti e Processi S.p.A., Milan-Italy): the first step was set with 2 minute air flow (500 m³/h) at 200 °C and drying chamber wall temperature at 180 °C; the second step was fixed with 10 minute air flow (500 m³/h) at 120 °C and drying chamber wall temperature at 100 °C. The EA values were measured both on the whole fruit and on the pulp after the main stages of the process. Enzyme release was determined on suitably homogenized and sonicated samples. Vegetation waters were characterized as density, net extract, ash, and pH, and analyzed in terms of sugars (glucose, fructose, sucrose), vitamins (A, C, B₁, B₂, and B₃), minerals (Ca, K, Na, Fe, P, Cu, Zn), according to AOAC (Official Methods of Analysis of AOAC International, 2005). Beta-carotene and lycopene were extracted and quantified according to the methods described by Nagata and Yamashita (1992) and Zuorro et al. (2013), respectively.

**Figure 1:** Lab-scale experiments and optimization trials toward pilot-scaling.

**Figure 2:** Industrial-scale pilot plant for dehydrated food-grade crude papain powder production from papaya fresh pulp.
3. Results and discussion

3.1 Lab-scale trials
In order to detect the temperature parameters corresponding to the complete enzyme denaturation, the EAs of fresh pulp and papain standard samples were evaluated before and after 2 h heating at 70 °C, 90 °C and 120 °C. Results were compared with the DSC thermal profiles and confirmed a high thermal resistance of the standard (Amri and Mamboya, 2012). The DSC profiles (shown in Figure 3) evidenced a denaturation heat requirement higher in the thermally pretreated than in the untreated enzyme standard. In the descending phase of the untreated enzyme denaturation peak, the DSC curve (Figure 3a) showed a further minor peak, missing in the DSC curve of the 120 °C pretreated enzyme standard (Figure 3b). This behavior was different to what observed by Zuorro and Lavecchia (2011) on coffee grounds extracted of variable amounts of phenolic compounds. The calorific value of their samples did not change on extraction of phenolic compounds. In our study, the change in DSC profile on enzyme thermal pretreatment was probably due to the presence of different enzyme molecular conformations or intramolecular rearrangements, especially with regard to the disulfide bonds, that increased both the thermal stability and the required enthalpy. To determine the enzyme heat stability in a complex matrix, such as the fresh fruit pulp, water and pectin content had to be decreased: high moisture reduces EA in ripe fruits and pectins (constituting the cell wall in pulp parenchyma) inhibit the enzyme release and the EA determination. For this reason, fresh pulp samples were thoroughly homogenized and sonicated prior to be filtered and oven pre-dehydrated. Under real conditions, pulp cell pectins rapidly react with the hydrochloric acid secreted by the stomach mucosa during digestion: the enzymes are released and their reaction begins on various substrates. In lab trials, it was not possible to acidify the enzyme solution before the test on casein substrate without altering the substrate itself. It was not even conceivable to add pectolytic enzymes because they cause a papain proteolytic activity decrease. Therefore papaya pulp homogenization and subsequent ultrasonic treatment were required for enzyme release measured as EA (Zhang et al. 2013), which was 0.5 PU/g in whole fruit. EA analysis evidenced high residual activity in all differently dried samples (30 PU/g dry matter). Proceeding at higher temperatures for a shorter time (30 min oven drying at 150 °C) allowed obtaining commodity-related and technologically valid products.

3.2 Pilot industrial-scale
The process layout reported in Figure 2 shows the mild technology required to obtain dehydrated food-grade crude papain powder from papaya fresh pulp by minimizing the raw material purification steps and by enhancing the by-products. After seeds and peel removing, fresh pulp homogenization was needed to increase filtration efficiency and facilitate subsequent drying operations, as well as to ensure uniform moisture level and improve EA in the final product. The HP filtration decreased the pulp moisture from 90±2 % to 75±4 %. Afterwards the pulp was extracted from the mats and sent into two steps turbo-drying equipment. Turbo-technology was based on the creation of a thin film of material in high turbulence by means of a turbine rotating inside a static horizontal chamber. The chamber was jacketed and a thermal fluid was circulating to keep the temperature constant inside the machine, according to the process need. These two turbo-drying steps lowered the pulp moisture from 75±4 % to 50±2% and from 50±2 % to 8±1%, respectively. The resulting dehydrated pulp was grinded with ball-mill to obtain a stable powder. The pulp powder was stored in inertized tanks and finally packaged under vacuum, protected from light and moisture.

Figure 3: DSC thermal profiles of papain standard before (a) and after (b) 2 h-120°C thermal treatment.
Pareto chart in Figure 4 reported the effect of each unit operation on the maximum EA obtained. As expected the increase of the EA was inversely proportional to the powder water content: after filtration and two turbo-drying steps protease activity of the papaya pulp increased from 3.0 PU/g to 4.5, and 28.0 PU/g respectively. The 2-step turbo technology accounted for the 90% of the EA increase; fresh pulp pressing and filtration were essential to remove part of the water before proceeding to the further dehydration steps. Vegetation waters contained several compounds with high added value, such as pectins, vitamins, minerals, sugars and other organic compounds that can be recovered and used in food and/or cosmetic industries. In particular, vegetation waters were characterized by 1.050±0.0002 mg/L density at 20 °C, 15.4±0.2 g/L net extract, 5.55±1.20 mg/L ash, and 5.5±0.1 pH, and a yellow-orange color. They also contained 60.0±3.0 g/L glucose, 5.1±2.8 g/L fructose, 3.4±0.4 g/L sucrose, 450±55 RE/100g vitamin A, 2000±100 mg/L vitamin C, 0.03±0.10 mg/100g vitamin B₁, 0.04±0.22 mg/100g vitamin B₂, 0.30±0.20 mg/100 g vitamin B₃, 0.54±0.05 mg/L lycopene, 32.16±3.85 mg/L beta-carotene, 123.11±8.12 mg/L Ca, 1190.5±13.0 mg/L K, 27.10±3.72 mg/L Na, 2.11±0.05 mg/L Fe, 77.94±5.07 mg/L P, 0.42±0.02 mg/L Cu, and 1.77±0.02 mg/L Zn.

Filter press separation was followed by tangential flow ultrafiltration in order to obtain concentrated pectin suspension (retentate) and more storable and rich in nutrients (sugars, vitamins, minerals) waters (permeate). Both permeate and retentate, suitably recovered, can be considered a good source of bioactive compounds with a high added value which can be reused along the food chain.

4. Conclusions

In this study dehydrated food-grade crude papain powder was obtained from papaya fresh pulp in order to maximize nutrient retention by minimizing processing requirements. Lab-scale trials evidenced high residual EA in all differently dried samples and allowed at proceeding at higher temperatures for a shorter time in order to arrange the subsequent steps of process development toward the pilot industrial-scale. In the final pilot-scale step, filtration was done with vertical HP filter press, and final dehydration was performed with 2-step turbo-drying: the first aimed to concentrate, and the second aimed to dry. The resulting dehydrated pulp was grinded with ball-mill to obtain a stable powder and the enzyme release highlighted an EA of 28 PU/g.

Although the yield of EA obtained with this mild-process was not very high if compared to the one obtained with the studies starting from papaya latex (Nitsawang et al., 2006), the thermal steps provided with turbo-driers allowed a high heat transfer with both low energy consumption and residence time, thus permitted to maintain a fraction of sugars and pectin acting as a physical barrier and protecting the papain enzyme like a “core”. This protective structure is then degraded by the hydrochloric acid in the stomach, rapidly releasing the enzyme and thus initiating the proteolysis reaction with various substrates, so increasing the digestion effects provided by papain. To better evaluate the impact of process variables on this matter, further investigations are needed. However, mild process and exploitation of by-products, as low-cost resources, could be anticipated for developing new products, reducing industrial waste and cost, and ultimately providing a positive economic and environmental impact.

Figure 4: Effect of the unit operations in pilot industrial-scale experiments on EA of dehydrated food-grade crude papain powder.
References


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