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PREPARATION OF CRUDE AND REFINED PAPAIN FROM PAPAYA LATEX.

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ABSTRACT

Drying of fresh papaya latex with and without EDTA addition was studied with regard to the proteolytic activity of crude papain. The optimum concentration of EDTA added to fresh latex before drying was 0.2 % (w/w). Moisture content of the fresh latex treated with and without EDAT addition was decreased by 94.29 and 93.07%, receptively, after 7 hrs of drying at 55 ^{0}C. Drying of fresh latex reduced its proteolytic activity, gradually the presence of 0.2% (w/w) EDTA, added to the fresh latex, before drying preserved its proteolytic activity comparing with the control. Refined papain was isolated by a modified method, its proteolytic activity of the isolated papain was assayed, showing an enzyme unit of 7.08 /mg of sample and specific activity of 11.14 U/mg protein.

Keywords: *Papaya latex- crude and refined papain- proteolytic activity- EDTA*

INTRODUCTION

The importance of latex of papaya fruit (Carica papaya) as a source of enzymes was reported in 1873 in the Calecutta Medical Journal (Baines and Brocklehurst, 1979). They also reported that the main source of these enzymes present in papaya latex, is papain (EC 3.4.22.2), the most thoroughly characterized of the thiol proteinase. Crude papain is the name given to the dried latex obtained by tapping the green unripe papaya fruits and collecting the resulting exudate. Foyet (1972) indicated that tapping should be done early in the morning, preferably in the cloudy weather followed the rainy periods. Although some of the early studies on papain were carried out on enzyme prepared from the fresh latex, yet most of the workers used the enzyme isolated from dried latex, the state in which the material is exported from the producing countries, which are mainly the wet tropics (Jones and Mercier, 1974). Fresh latex is traditionally dried in forced- air driers or sun-dried. The latter product is of reduced activity possibly because of ultraviolet (u.v.) light sensitivity of the histidine residue in papain, that acting as one of the active centers of the enzyme which is essential for its activity (Arnon, 1970). The sun- dried product is darker in colour and smell strongly since microbial contamination is enhanced by the longer drying time. It is mportant to clarify that, the commercial evaluation of papain is often based on an assessment of colour and smell, but there is an increasing demand for the evaluation of the proteolytic criteria because of the unreliability of the usual sensory evaluation (Flynn, 1975). Boudart (1970) developed a drying procedure in which fresh latex is stirred to liquefy the thixotropic coagulum, then filtered, centrifuged under vacuum and spray dried. This process is applicable on a large-scale, but is inappropriate to

many countries (e.g. Costa Rica) where papain would be considered as a by-product of the fruit on an intermediate scale. Hoon et al., (1997 a) used the response surface methodology (RSM) to optimize the extraction of crude papain from papaya latex. The resuits showed that the optimum conditions were at a concentration of 4% NaHSO3, 120 min extraction time and pH 7.6; under these conditions, 793.16 mg papain were extracted from 1g papaya latex.

Robinson (1975) reported that dried latex contains several enzyme activities namely, papain, chymopapain "A" and "B" as well as papaya peptidase "A". The role of papain in stabilizing the thiolate-imidazolium ion pair was examined and the potential energy pathway for the subsequent attack of the cysteine anion and proton transfer from the imidazolium cation was determined by Harrison *et al.*, (1997). The demand for crude papain that used in food industry is increasing by up to 95% (Flynn, 1975). The papain applications in food industries include:

- Brewing for beer stabilization, barley and malt treatment.
- Dairy industry for treatment of vegetable proteins
- Meat industry for tenderization, fat extraction and meat hydrolyzed protein concentrates.
- Fish industry for hydrloysates- fish protein concentrates and fish meals.
- Juices industry for fruit juices and wines.
- Dietetic industry for baby food.
- Baking industry for biscuits and baked products.

Other non-food industries in which papain is used are the pharmaceutical extracts, aromas and perfumes.

The present work aims to find a simple method for producing crude papain by applying slight modifications based on the method of Baines and Brocklehurst (1979) to optimize the yield of papain from highly soluble papaya latex, while avoiding contamination by other enzymes.

MATERIALS AND METHODS

Materials:

Latex collection:

Latex was collected from green papaya fruits in the early morning, between 5.30-7.00 a.m., by means of three longitudinal incisions (2-2.5 mm depth) in each fruit from the same plant at Sabahia farm, Alexandria. A stainless steel blade inserted in a curved wooden handle was used to cut the fruit epidermis after which cuts were distributed equally on the fruit surface. The fluid latex was collected in Petri dishes and coagulated latex on the cut surface (about 20% by weight of the yield) was collected 30 min after the incision and mixed with the latex previously collected in the petri dishes to ensure uniformity (Madrigal *et al.*, 1980). The yielded latex was transported in an ice- box to the Laboratory of Food Technology Sabahia Hort. Research. Station, Alexandria.

Methods:

1- **Pre-drying treatment:** Fresh collected latex (100g) was divided into two portions, one of which was used as a control, and the other was subjected to ethylene diamine tetraacetic acid, sodium salt (EDTA). EDTA was added to the fresh latex at concentrations of 0.1, 0.2, 0.3 and 0.4% (w/w) prior to drying according to the method of Ortiz *et al.*, (1980).

2- **Latex drying:** Latex was dried in heated air-drying oven at 55 ºC (±2 ºC) for 7 hrs, to reach moisture content about 6%. The samples were dried on Petri dishes, the walls of which had been reduced to approximately 1.5 mm in hight to minimize air flow disturbances. The ratio between sample weight and surface area (0.16 g per cm2) was maintained by stirring the latex to liquefy it prior careful addition to the dishes. Dried samples were packed in polyethylene bags and stored in other black one at 4 ºC prior to subsequent analysis (within 2 weeks). This method was described by Ortiz *et al.*, (1980).

3- **Preparation of isolated papain:** The method of Baines and Brocklehurst (1979) was applied with some modifications. The obtained papain was free from contamination by chymopapains A and B (E.C 3.4.22.6). A solution of the fresh latex containing 65 mg of protein / ml (Plummer,1978) was prepared by dissolving the appropriate amount of latex in 20 mM of mercapto ethanol of pH 5.7 instead of 20 mM of cysteine , in the original method , containing 1m M EDTA. The pH of the latex solution (250 ml containing 65 mg protein/ml) was adjusted to pH 9 by adding 1 M-Na oH with stirring, during 10-15 min at room temperature. Small amount of grey-white precipitate formed was removed by centrifugation (20.000 g, 4ºC, 30 min) and discarded. The produced supernatant was adjusted to 0.45 saturation by adding solid ammonium sulfate with stirring during 20 min. The white precipitate was isolated by centrifugation as mentioned before and dissolved in 250 ml of 1mM EDTA. The solution was adjusted to 0.40 saturation by the addition of ammonium sulfate salt, with stirring during 20 min. Again, the white precipitate was isolated by centrifugation as mentioned before and then dissolved in 250 ml of 0.1 M phosphate buffer, pH 7.5 containing 20 mM mercapto ethanol and 1 mM EDTA at room temperature. Solid Nacl (25 g) was added with stirring during 15 min, and the precipitate was isolated by centrifugation. The white precipitate was suspended in 100 ml of 0.1 M phosphate buffer pH 6.8, containing 20 m M mercapto ethanol and I m M EDTA at room temperature. The suspension was left at this temperature for 30 min and then at 4 ºC for 18 hrs. The crystals were isolated by centrifugation as above, assayed for papain activity and then stored at 4 ºC as a suspension in 0.1 M sodium acetate buffer, at pH 5.0, containing 24.3% (w/v) ammonium sulfate.

4-Proteolytic activity: The following samples were subjected for assaying their proteolytic activity:

The fresh latex containing 0.0, 0.1, 0.2, 0.3 and 0.4% EDTA (w/w)
The fresh latex with and without 0.2% EDTA (w/w) during the drying period
The isolated papain.

Proteolytic activity was assayed by the action of the enzyme on 4% casein solution at pH 7 by titration acidimetrically, after the addition of neutral formaldehyde according to Barman (1974). The enzyme was activated prior to addition to the substrate solution by sodium bisulphite 4% and the incubation time was 20 min at 60 ؛C.

Enzyme unit (U) was expressed as the amount that librates /um amino nitrogen per minute per mg sample from 4% casein at pH 7 and at 60 ؛C; whereas specific activity was expressed as U/mg protein.

5. **Moisture content:** Moisture content of the fresh papaya latex was determined according to AOAC (1995) using a vacuum oven at 80 ؛C. Also, latex samples with and without EDTA (0.2%) (w/w) were subjected to moisture content determination hourly during drying period.
6. **protein content:** Protein content of the fresh and dried papaya latex, as well as that of the latex containing different concentrations of EDTA salt, and the isolated papain were subjected for protein determination according to Plummer (1978).

RESULTS AND DISCUSSIONS

1- Effect of pre-drying treatment on the proteolytic activity of latex:-

Fig (1) shows the effect of EDTA addition to fresh latex on the resultant proteolytic activity of crude papain. The activity of crude papain increased up to 20% at 0.2% concentration of added EDTA and then a slight decrease was demonstrated at 0.3 and 0.4% of EDTA contentrations. The obtained results are inagreement with Ortiz *et al.*, (1980) who reported that the addition of EDTA (0.2% w/w of fresh latex) and sodium bisulphite (1% w/w of fresh latex) increased the proteolytic activity yields by 25% and 21%, respectively, relative to the control crude papain.

2-Effect of drying treatment on the proteolytic activity of crude papain:

The proteolytic activity of crude papain is found to be the same if it was dened either from exudated latex or from that portion (about 20% of latex yield) which coagulates on the fruit surface on tapping (Ortiz *et al.*, 1980). Therefore, the fresh collected latex- obtained within the investigated work- was stirred to liquefy the thixotropic coagulum and then dried at 55 ؛C in an air driven electrically heated oven. The effect of drying period on the moisture content of the crude papain and its resultant activities with and without EDTA addition (0.2% w/w of fresh latex) are shown in Fig (2) and Fig (3), respectively. An initial moisture content was 84 and 85.15% at zero time and decreased to be around 5% for latex samples with and without EDTA addition, respectively after 7 hrs of drying period. Whereas, its proteolytic activity decreased by 18.60 and 20% for the same samples, respectively after the same period at 55 ؛C.

In conclusion, the drying papaya latex is recommended to carry out at 55 ؛C in the presence of 0.2% EDTA (w/w of fresh latex) because EDTA had an effect on the drying rates of latex as well as activated and preserved its proteolytic activity.

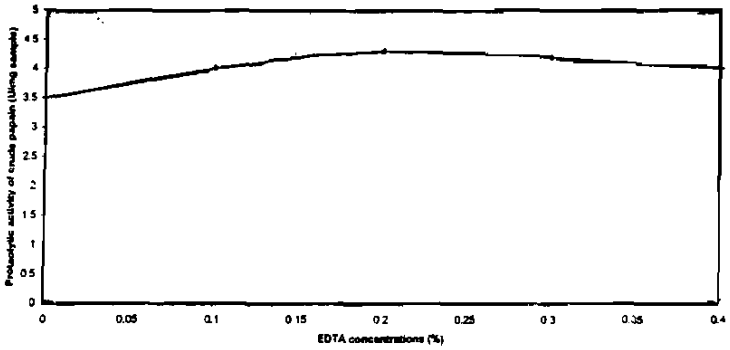

Fig (1): Effect of EDTA addition of fresh latex (%w/w) on the resultant proteolytic activity of crude papain.

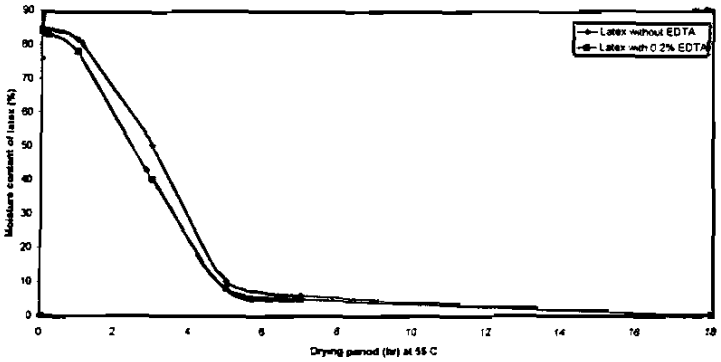

Fig (2): Effec of drying period on moiture content of crude papain with and without 0.2% EDTA

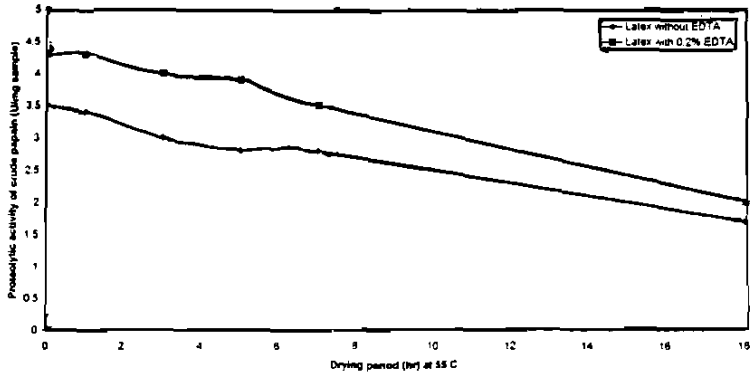

Fig (3): Effec of drying period on proteolytic activity of crude papain with and without 0.2% EDTA

Hinkel (1951) reported that the residual proteolytic activity after oven drying at 55 ºC and after sun-drying had the same effect, but drying at 70 ºC reduced the activity. An initial decrease in papain activity of about 20% was also noticed by Ortiz *et al.*, (1980), after the first 6 hrs of storage period under tropical ambient condition, prior to drying, and the optimum drying temperature is between 50 to 55 ºC.

Table (1) shows that there is no difference between the yield percent of crude papain produced by EDTA addition (0.2% w/w of fresh latex) and the control. The colour and smell of these two crude papain preparation were similar being cream-white and plane, respectively. Jones and Mercier (1974) reported that the dried latex is known as crude papain showed a colour varying from cream-white to red-brown. These variations are due to the oxidation of the latex, or to the burning, caused by excess heat applied during drying.

The protein content, proteolytic activity and specific activity of crude papain containing, EDTA salt were slightly higher than these of the control.

Table (1): Physical properties, protein content, proteolytic activity and specific activity of crude papain*.

Properties / Treatments	Crude papain with EDTA (0.2% w/w)	Crude papain without EDTA addition
Yield (%)	20.80	20.75
Colour	Cream-white	Cream-white
Smell	Plane	Plane
Protein content (mg/ mg sample)	0.28	0.25
Proteolytic activity (U/ mg sample)	3.50	2.80
Specific activity (U/mg proteim)	12.50	11.20

*** Crude papain is the papaya latex dried at 55 ºC for 7 hrs in an air oven.**

3-Isolation of refined papain from papaya latex:

Refined papain was prepared from fresh latex using the method of Baines and Brocklehurst (1979) with some modifications. The modified method optimizes the yield of papain, while avoiding contamination by other enzymes. The fresh latex is refined, immediately after its collection. The concentration of protein in fresh latex and refined papain are shown in Table (2). According to this modified method, the latex solution must contain 65 mg of protein/ml, therefore, it is convenient to work with 50 g fresh latex in 300 ml of 20 mM mercapto ethanol, at a pH of 5.7. The insoluble material was removed at pH 9. The papaya latex extract consists of ammonium sulfate fractionation, followed by sodium chloride fractionation and crystallization. This procedure yielded about 1.13 g of papain crystals from 50 g fresh latex with a yield of 2.26%. The crystals were suspended in 0.1 M sodium acetate buffer, pH5 containing 24.3 % (w/v) (NH4)2 SO4. In such a case; 1 ml of this buffer contained 32.85 mg of resulting crystals. When the protein content and proteolytic activity of the refined papain were determined, and were found to be 0.7 mg/mg sample and 7.80 U/mg sample, respectively. Baines and Brocklehurst (1979) found that the product of resulting crystals was approx.

1.5 g, when prepared form 25g spray dried latex. Whereas Hoon *et al.*, (1997b) reported that the extraction and purification of crude papain were optimized by response surface methodology (RSM), with the function being expressed in terms of a quadratic polynomial equation. Adequacy of the model equation for optimum response values was tested and optimum conditions of protein recovery were 38.2 mg/ml of protein, 40% ethanol concn. and (–8 ◦C) precipitation temperature.

Table (2): Comparison between fresh latex and refined papain with respect to their protein content and proteolytic activity.

Component (mg)	Protein content (mg)	Proteolytic activity (U) **
Fresh latex*	0.5	3.50
Refind papain	0.7	7.80

***Fresh latex without EDTA addition.**
**** U: Unit of activity / mg sample.**

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تحضير انزيم البابين الخام والنقى من السائل اللبنى (لاتكس) المسال مـــن ثمـــار الباباظ.

سلوى داتيال روفائيل

قسم تصنيع الحاصلات البستانية- معهد بحوث تكنولوجيا الاغذية- مركز البحوث الزراعية

تم دراسة اضافة مادة ثنائى الامين رابع حمض خليك (EDTA) وايضا عملية تجفيف لمادة الاتكـس مـن ثمار الباباظ. ووجد ان انسب اضافة لمادة (EDTA) كانت عند تركيز ٠,٢% بالوزن من الاتكس الطـــازج وذلك قبل عملية التجفيف. انخفض المحتوى الرطوبى للاتكس في وجود مادة (EDTA) وأيضا فـــي حالـــة عدم وجودها الى حوالى ٥% بعد سبعه ساعات تجفيف على درجة ٥٥؛ م في فرن هـــواء ســـاخن خفضـــت عملية التجفيف النشاط البروتيوليتى (المحلل للبروتين) للاتكس الطازج ولكن باضافة مادة EDTA بنسـبة ٠,٢% (بالوزن من اللاتكس الطازج) قبل عملية التجفيف حافظ علـــى نشـــاطها الـــبروتيوليتى مقارنـــه بالكنترول بمقدار ١,٤%٠ استحدثت طريقة معدلة لتنقية انزيم البابين من لاتكـــس البابـــاظ وقـــدر النشـــاط البروتيوليني لهذا الانزيم النقى. وقد ادى التعديل في الطريقة الى زيادة احتفاظ الانزيم بنشاطة وزيادة العـــائد مقدرا بالوحدات الانزيمية و النشاط النوعى لمستحضر البابين النقى .