Research Article

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EFFECTS OF DIFFERENT DRYING METHODS ON THE ACTIVITY OF PAPAIN FROM THE LATEX OF UNRIPE PAW-PAW (*CARICA PAPAYA*) FRUITS

Udobi Chinweizu Ejikeme[1*], Onazi Orie Blessing[2], Udobi, Ugochi Juliet[2], Ubulom Peace Me[1]
[1]Faculty of Pharmacy, University of Uyo, Uyo-Nigeria
[2]College and Science and Technology, Kaduna Polytechnic, Kaduna- Nigeria

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Abstract: The effect of different drying methods viz- sun, oven- cabinet, ambient temperature and vacuum drying on the activity of crude papain from *Carica papaya* latex was studied. Latex from an unripe (but matured) pawpaw which contained papain was dried using one of these methods and subsequently assayed for enzymatic activity. This was done relying on the ability of papain to hydrolyse protein using milk. Results obtained showed that the vacuum dried sample of latex had the highest activity while the cabinet dried sample completely lost activity. Results further confirmed that other factors which may be environmental, interplays with drying methods and time in the determination of the activity of papain from *Carica papaya* latex.

Keywords: Drying methods, Papain, *Carica papaya*, activity, Interplays, Latex

INTRODUCTION

Enzymes are proteins that catalyse the rate of chemical reactions. Almost all processes in a biological cell needs enzymes to occur at significant rate. Like all catalysts, enzymes work by lowering the activation energy for a reaction thus dramatically increasing the rate of the reaction. Most enzyme reactions are millions of times faster than those of comparable un-catalysed reaction. They differ from most other catalyst by being much more specific. In enzymatic reactions, molecules at the beginning of the process are called substrates and the enzyme converts them into different molecules called products (1)

Papain is a common enzyme which can be obtained from the latex of unripe *Carica papaya* fruits among other sources. It is an endolytic cysteine protease that has a strong biological activity and the decomposing power or ability of protein (2). Papain has been extensively studied and found to have wide industrial application and is therefore, of high research interest. Among the major known industrial application of papain are beer clarification, meat tenderization and as protein hydrolysate (3, 4)

The latex of *Carica papaya* is basically tapped by making an incision of 1-2mm deep on unripe fruits and allowing the latex to flow for about 4-6 minutes. The latex is then dried and then subjected to further treatment before final use. The activity of the papain obtained will to a great extent be dependent on the heat treatment the latex is subjected to at the time of drying due to the proteineous nature of the papain.

This paper presents the results of the study of the effect of different drying methods (which can be used for drying the latex of *Carica papaya* prior to the extraction of papain) on the activity of the papain therein. It also evaluates the roles drying time and other environmental factors play during the drying in determining the activity of papain from the latex of *Carica papaya*.

MATERIALS AND METHODS

Materials:

Unripe Paw-paw fruits were obtained from a garden inside the Finetex textile factory in Kaduna-Nigeria. The water used was obtained from the Nigerian bottling company Kaduna plant.

Latex tapping:

The latex tapping was done very early in the morning in the month of June, 2010 when the relative humidity was still high. This was done to enhance the latex flow since the latex will flow better at high relative humidity. An incision 2mm deep was made on a matured but unripe paw-paw fruit using a stainless steel blade and the latex was allowed to flow for about 6 minutes. The steel blade was used to carefully scrape the flowing latex into a petri dish containing buffered water (PH 7.0). It was subsequently transferred to a polyethylene lined box with a closed fitting and kept away from direct sunlight.

Latex drying:

The latex (with water) was strained using a muslin cloth to remove water so as to reduce drying time. 50g of the latex was then measured and dried using either of five different methods namely;

***Corresponding Author:**
Dr. Udobi Chinweizu Ejikeme,
Faculty of Pharmacy,
University of Uyo,
Uyo-Nigeria.

A. Sun drying
B. Cabinet drying
C. Oven drying at 55°C
D. Ambient temperature drying
E. Vacuum drying at 43°C

After, the dried latex (containing the required papain) was observed properly for appearance and colour, then packaged and subsequently used for the papain activity determination.

FLOW CHART SHOWING LATEX TREATMENT

Unripe paw-paw fruit
↓
Cutting/Incision (2mm depth)
↓
Tapping (6minutes)
↓
Collection into phosphate buffered water (PH 7.0)
↓
Straining (using muslin cloth)
↓
Drying and packaging

Papain activity assay:

A known quantity of the dry latex sample (containing papain) was dissolved in known volume of acetic acid and labelled A. 5g of dry powdered milk was also dissolved in 15mls of water, warmed to 30°c and labelled B. A and B were then thoroughly mixed and observed until the first sign of clotting (Lump formation) appeared. The experiment was repeated using different amounts of latex (containing papain) solution and the times taken for clotting to take place noted. Milk sample (B) that was not mixed with dry latex was used as a negative control.

RESULT

All results obtained are presented in tables and figures in the tables and figures section

Table.1: Showing Latex appearance after drying under different conditions

Sample	Appearance
A	Brownish crumbs
B	Lumpy and very brownish
C	Creamy rough powder
D	Brownish crumbs
E	Very fine creamy white powder

Table.2: Results showing time taken for hydrolysis of milk using several samples

Papain Concentration (gms/20mls)	Sample A (sec)	Sample B (sec)	Sample C (sec)	Sample D (sec)	Sample E (sec)
0	0	0	0	0	0
5	180	0	130	160	120
10	176	0	115	160	116
15	170	0	110	152	110
20	160	0	100	142	90
25	154	0	95	140	75

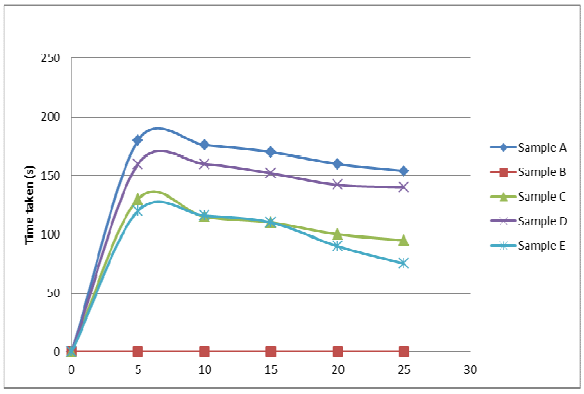

Fig.1: Graph showing the relationship between papain concentration and time of hydrolysis

DISCUSSION

Results obtained showed that sample E (vacuum dried latex), after drying, turned out to be the finest powder appearing closest to white when compared with all the other samples (Table1). Color is one of the known indices for papain quality and acceptability (5). It is generally accepted that the whiter a papain sample, the more its activity (6). Hence papain users generally prefer white fine powdered papain to brownish and crumble –like ones.

Results obtained also showed that the same sample E had the highest activity due to the latex contained therein. This may not be unconnected with the temperature and conditions of drying (43°C under vacuum) and the relatively short time of 3 hours it took to dry the latex. At the concentration of 25g/20mlw/v (Latex in milk), the first sign of hydrolysis was observed at about 75 seconds. The oven dried sample C was next in the rate of activity. The latex was dried at 55°C and the residence time was 18 hours. The time the sample took to dry may be responsible for the comparatively lower activity observed. When 5%w/v of the crude latex was used, it took 130 seconds for hydrolysis to occur and at 25 %w/v, it took 95 seconds for hydrolysis to occur (Table 2)

Sample D ranked third in terms of activity. This sample was left at ambient temperature to dry in a closed room and drying took 40 hours to be completed. There was no application of heat which would have affected its activity yet it had a lower activity as compared to vacuum and oven dried samples. The longer time of drying may be an explanation for the low activity obtained. This could also be as a result of the exposure of this sample while it was left to dry. At 5%w/v, it took 160 seconds for hydrolysis to occur and at 25%w/v, it took 140 seconds (Fig 1)

The Sun dried sample A was fourth in terms of activity. The sample was exposed to direct sun rays and drying was completed after 12 hours. It was exposed to

dust, insects, rodents and other environmental conditions which were uncontrolled. This may have contributed in lowering the activity of this sample. This result may be a suggestion that other environmental factors may be playing a role in the determination of the activity of the enzyme. At 5%w/v concentration, hydrolysis occurred at 180 seconds and at 25% w/v, it was at 154.

The least in terms of activity was sample B the cabinet dried sample. This sample was dried at a very high temperature and drying time was 4 hours. Despite the short residence time, the sample completely lost activity even at increased concentration of the enzyme. This is a further confirmation that enzyme activity can be adversely affected by high temperature.

CONCLUSION

From all the results obtained, it can be concluded that temperature of drying may not just be responsible for the inactivity of the enzyme. The time of exposure as well as some other yet to be identified environmental factors may be playing some very vital roles. Though papain will lose activity at very high temperature or even reasonably high temperatures, there may be an inter-play of time, temperature and the environment of drying in the determination of activity retained.

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