EFFECTS OF IONIC AND NON-IONIC SURFACTANTS ON PAPAIN ACTIVITY

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ABSTRACT. *Papain was extracted from the latex of green Carica papaya fruit. It is stable and easy to obtain in relatively large quantities in tropical countries. Since papain is useful for incorporating into surfactants of toiletry and personal care, the objective of this study was to investigate the activity in the presence of common surfactants such as ionic surfactant sodium laureth sulfate (SLES) and non-ionic surfactant cocomide di-ethanol amide (CDE). Besides that, an attempt was made to determine the stability and the effect of various surfactants on papain enzyme. It was found that papain exhibited an optimum activity at pH 7 and it was concluded that the variation of the concentration of surfactants did not impair the activity of papain, since the results showed a positive effect on the enzyme.*

KEYWORDS. *Papain, sodium laureth sulfate, cocomide diethanol amide, shower gel.*

INTRODUCTION

Carica papaya L., more commonly known as the papaya, belongs to the family of *Caricaceae*. Its classification is as follows: Division: Magnoliophyta, Class: Magnoliopside, Subclass: Dilleniidae, Order: Violales. The most important and unique property of the Carica papaya, however, is the proteolytic activity of the enzyme papain, which is present in the milky juice or latex of the green fruits. Papain, an activated and refined proteolytic enzyme is an endoprotease belonging to the cysteine endopeptidase family (Samson, 1986).

Papain has a molecular weight of 23,000 daltons (Dreuth *et al.*, 1968), an optimum pH and temperature in range of 6.0 - 7.0 and 65 to 80 °C respectively (Harton *et al.*, 2002). Papain has wide specificity. It degrades most protein substrates more extensively than pancreatic proteases. It is also an esterase (Glazier *et al.*, 1971). The action of papain on leucine methyl ester produces an insoluble polyleucine peptide. Papain breaks down the intercellular matrix of cartilage and it is activated by cysteine, sulfide, and sulfite. Activity is enhanced when heavy metal chelating agents such as EDTA are also present. Papain concentrate is a food grade proteolytic enzyme preparation isolated from the latex of the Carica papaya fruit. It is characterized by its ability to hydrolyze large proteins into smaller peptides and amino acids (Arnon, 1970).

Papain belongs to the family of eukaryotic thiol proteases, a group of proteolytic enzymes, which contains the active site cystein. Probably the aspect of cysteine proteases of primary importance for their catalytic activity is the high nucleophilicity of the active site thiol group. It is now generally accepted that the active form of papain and of cysteine proteases consists of a thiolate-imidazolium ion pair as shown in Figure 1 (Beveridge, 1996).

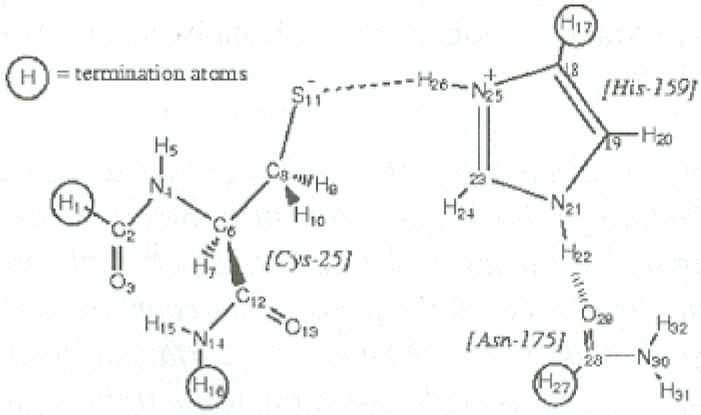

Figure 1. The active form of papain and of cysteine proteases consists of a thiolate-imidazolium ion pair (Beveridge, 1996).

Papain has a wide range of applications in different industries, especially in personal care products such as soap, shower gel and food industry (Uhlig, 1998). It is also being used to tenderize meat and meat products, in the manufacture of protein hydrolysis, in confectionery industry (to prepare chewing gum), in brewing industry to remove cloudiness in beer and in dairy industry (for cheese) (Chaplin, 2002). Similarly, it is used in pharmaceutical industry, textile industry and tanning industry (Uhlig, 1998).

The starting point of this work was the tapping of papaya latex from green papaya fruit proceeded by the extraction of papain from papaya latex. To gain more understanding of the physical and chemical properties of papain, experiments were carried out with various pH's and various surfactants.

EXPERIMENTAL METHOD

Extraction of Papain From Papaya Latex

Naturally, papaya is richer in latex than the leaves and the trunk of the tree. The greener the fruit the more active is the papain. In the present investigation extraction of papain was carried out on papaya green fruits which were about 90-100 days old. The latex was obtained by making incisions (scratches) on the surface of the green fruits early in the morning. The scratches are made 0.2 cm deep at 1.25 cm apart and are best done on the same tree two weeks later. Tappers hold a plastic cup, clay cup or glass beneath the fruit to catch the latex. The collected latex was dried by using vacuum oven to get the best quality latex powder. Drying times vary but an approximate guide is 4-5 hours at a temperature of about 35-40°C (Moore, 1980).

The procedure for extracting papain was a 4% latex solution thoroughly mixed by using a homogenizer. Then the latex solution was filtered with a vacuum pump using the filtration paper size of 0.45 mm (Baines and Broklehurst, 1979). After filtering, the solution was added with an amount of ethanol until there were significant signs of sedimentation in the solution. This mixture would then be ready to be centrifuged. It was centrifuged for about 30 minutes at 4000 rpm. When the solution was being centrifuged, the sediment would stick at the bottom of the tube. The sediment was taken out after decantation and heated in an oven at 50°C to evaporate the ethanol. After the sediment was fully dried, the papain was used for pH and surfactant tests on papain activity.

Effect of pH

The Milk Clot Assay is a very accurate and yet simple test procedure which measures the amount of time required to form clotted milk in the presence of the proteolytic enzyme under specified and controlled conditions, e.g. temperature, concentration (Arnon, 1970).

In this method, 10 g of papain sample from a solution at a concentration of 1 g of papain in 100 g of 0.01% acetic acid was added to 20 g of milk in a solution at a concentration of 2.5 g of milk powder in 100 g of water, which was heated to 50 °C in a water bath. The contents were thoroughly mixed and then observed until the first sign of coagulation (formation of lumps) was detected. The time taken to reach this stage, from when the papain was added to the milk, was recorded.

The experiments were carried out at different pH's (pH 4.0 – 10.0) of papain solutions by adding 1 % NaOH or 1 % HCl to get an acidic or alkaline solution. The pH of the solution was adjusted by using a pH meter. The activity of the papain sample was then shown by plotting the time taken to clot milk against different pH's.

Effect of Surfactants

In this study, two different surfactants Sodium Laureth Sulfate (SLES) and Cocomide Di-Ethanol Amide (CDE) were used to determine the effect on activity papain. The papain solution was prepared by dissolving 2 g of crude papain sample in 100 ml of distilled water. The concentrations of SLES and CDE that was added to the papain solution ranged from 1% to 6% (wt/wt). All six mixtures were kept in closed systems at room temperature for one day to let the reaction between papain and SLES run its course. After one day, the mixtures were analyzed by using the spectrophotometric method (Houle-Grant, 1994). Hence the effect of surfactant on papain activity was shown by plotting the graph of absorbance against various concentrations of surfactants.

RESULTS AND DISCUSSION

Result of Extraction

To analyze the effect of pH, the temperature and concentration of papain were kept constant at 50°C and 1 g papain in 100 g of 0.01% acetic acid (1 wt%). The pH values tested in this study covered both acidic and alkaline regions. The graph in Figure 2 illustrates that the fastest time for the milk clotting is in neutral region, which is pH 7. Meanwhile, in the highest alkaline region, which is at pH 10, it takes over 160 sec to clot the milk.

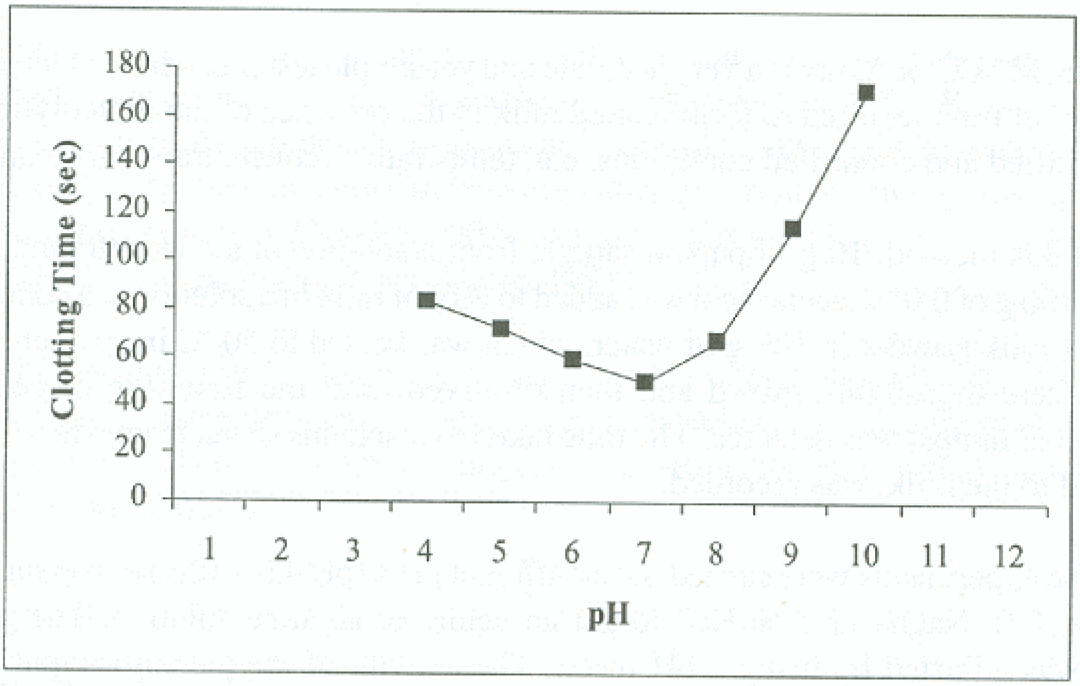

Figure 2. Effect of pH on papain enzyme activity (Temp,50°C and Conc, 1 wt%)

An attempt to explain the mechanism of the formation of colloidal milk is given below: reaction through hydrolysis of a specific peptide linkage takes place between phenylalanine and methionine residue (-Phe_{105}-Met_{106}-) in the k-casein protein present in the milk Figure 3.

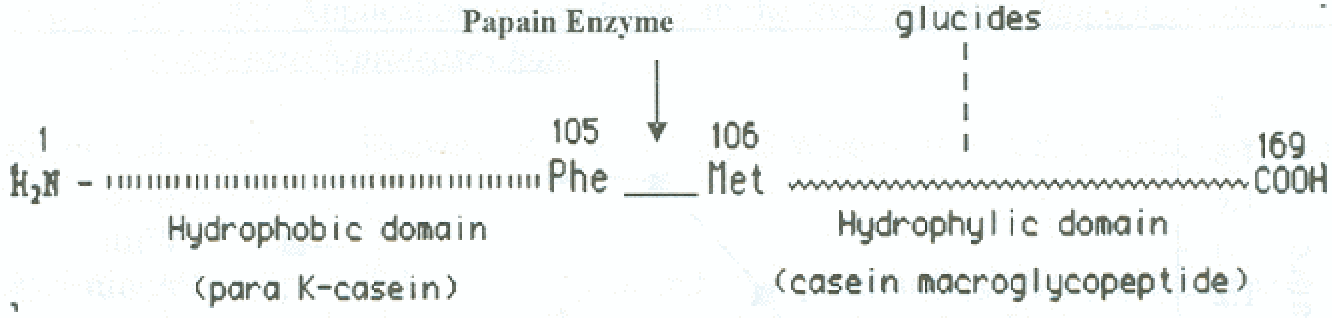

Figure 3. Hydrolysis of a specific peptide linkage in the k-casein protein present in the milk.

The k-casein acts by stabilizing the colloidal nature of the milk, its hydrophobic N-terminal region associating with the lipophilic regions of the otherwise insoluble a and b-casein molecules, whilst its negatively charged C-terminal region associates with the water and prevents the casein micelles from growing too large. Hydrolysis of the labile peptide linkage between these two domains results in the release of a hydrophilic glycosylated and phosphorylated oligopeptide and the hydrophobic para k-casein, which removes this protective effect, thus allowing the coagulation of milk to form curds.

Influence of surfactants on papain activity

The absorbances of different concentrations of SLES and CDE are shown in the Figure 4 and Figure 5. It is evident that the values of absorbance for the test of the papain sample present in the solution were higher than the solution without papain sample. Hence, it can be concluded that the presence of both surfactants, SLES and CDE provided a positive effect on papain enzyme activity.

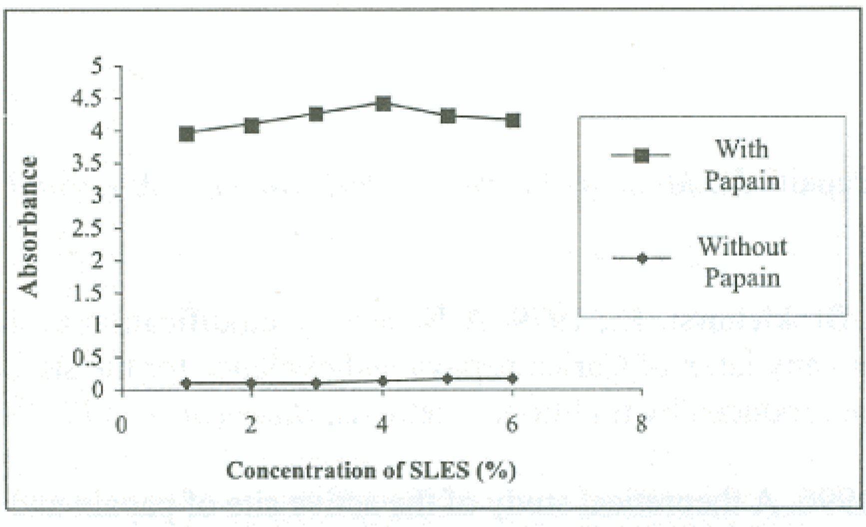

Figure 4. Effect of SLES on papain enzyme activity.

Nevertheless, papain enzyme still continued to exhibit activity without being influenced by the presence of surfactants.

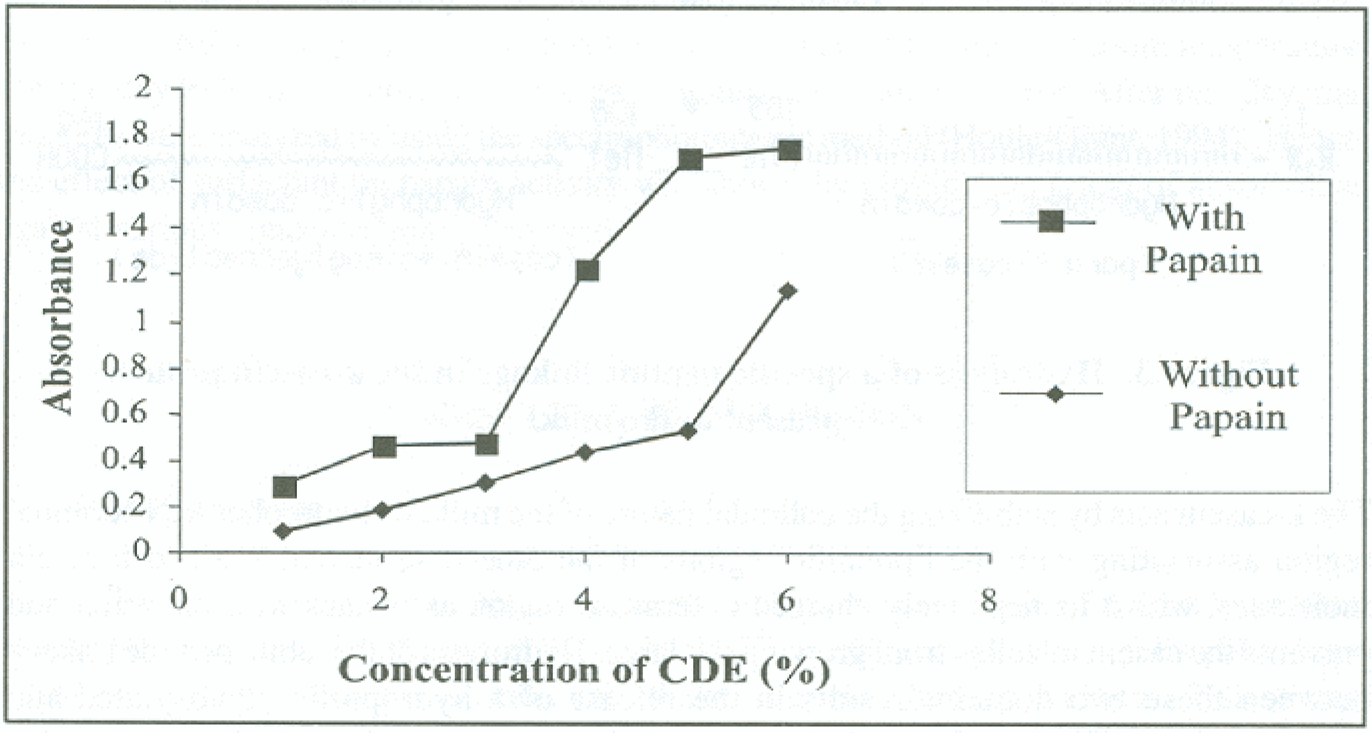

Figure 5. Effect of CDE on papain enzyme activity.

CONCLUSIONS

In this study, papain activity was shown to be more active at the neutral pH region. The activity turned lower when in either acidic or alkaline region. Meanwhile, the addition of surfactant in papain solution proved to have a positive effect on papain activity. Papain enzyme is hence a proper choice for use with surfactants for toiletry and personal care preparations.

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