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THE EXTRACTION OF PAPAIN ENZYME FROM *SEKAKI* AND EXOTICA PAPAYA AND ITS EFFECT ON THE AGED EGG LAYER CHICKEN MEAT TEXTURE

UMA DEVI NADARAJAH

THIS THESIS IS PRESENTED TO FULFILL TERMS AND CONDITIONS FOR BACHELOR DEGREE OF FOOD SCIENCE WITH HONOURS (FOOD SCIENCE AND NUTRITION)

SCHOOL OF FOOD SCIENCE AND NUTRITION

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2010

DECLARATION

Hereby, that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

The Extraction of Papain Enzyme From *Sekaki* and Exotica Papaya and its Effect on the Aged Egg Layer Chicken Meat Texture

Aged egg layer chicken meat is the chicken meat which has a very hard texture. Therefore this aged egg layer chicken meat was tested with papain enzyme extract in this study to produce softer texture. The objective of this study is to determine the effectiveness of the papain enzyme to tenderize the aged chicken meat. Secondly, to select the most effective variety of papaya that acts as the best to tenderize the aged chicken meat. Lastly to compare which temperature and marinating time is the most suitable for papain enzyme activation towards tenderizing the aged chicken meat. The papain enzyme extract was extracted from the young papaya. The papain enzyme extract underwent 1:200 dilution. Then, the papain enzyme was used to marinate the chicken meat at three different temperatures and three different time. The temperature was 10°C (T1), 37°C (T2) and 55°C (T3). The marinating time was half an hour, one hour and one and an half hour. Sensory test was then conducted to determine the best combination treatment which had produce the softest chicken meat. This study had showed that papain enzyme was effective to tenderize the aged chicken meat. Secondly, Exotica papain enzyme was the most effective variety compared to *Sekaki* papain enzyme. Lastly, the combination treatment of exotica papain enzyme with one hour marinating time and marinating temperature of 10 °C (EH2T1), was the most suitable for papain enzyme activation towards tenderizing the aged chicken meat. In conclusion, papain enzyme extract from Exotica papaya was proven to be effective in tenderizing aged chicken meat, where a combination treatment of EH2T1 was chosen as the best.

ABSTRAK

Pengekstrakan Enzim Betik daripada Betik Sekaki dan Betik Eksotika dan Kesannya ke atas Tekstur Daging Ayam Tua Bertelur

Daging ayam penelur tua merupakan daging ayam yang mempunyai tekstur yang keras. Maka, dalam kajian ini, daging ayam penelur tua ini diuji dengan ekstrak enzim papain untuk menghasilkan tektur daging ayam penelur tua yang lembut. Objektif kajian ini adalah untuk menentukan keberkesanan enzim papain dalam melembutkan daging ayam penelur tua. Objektif kedua, adalah untuk memilih enzim papain daripada varieti betik yang paling berkesan dalam melembutkan daging ayam penelur tua. Objektif ketiga, adalah untuk membandingkan kombinasi suhu pemerapan dan masa pemerapan yang paling sesuai untuk bertindak bersama enzim papain dalam menghasilkan daging ayam penelur tua yang paling lembut. Ekstrak enzim papain ini diekstrak dari buah betik muda yang masih melekat di pokok. Ekstrak papain enzim ini digunakan untuk memerap daging ayam tua dengan tiga jenis suhu perapan dan tiga jenis masa perapan. Suhu perapan tersebut adalah 10°C (T1), 37°C (T2) and 55°C (T3). Tiga jenis masa perapan adalah 0.5 jam (H1), 1 jam (H2) dan 1.5 jam (H3). Penilaian sesori dijalankan bagi menentukan kombinasi perlakuan yang terbaik yang menghasilkan daging ayam tua yang paling lembut. Dalam kajian ini, ekstrak enzim papain dibuktikan efektif dalam melembutkan daging ayam penelur tua. Papain enzim ekstrak daripada varieti Eksotika lebih berkesan berbanding dengan varieti *Sekaki* papain ekstrak. Kombinasi ujian EH1T2, ekstrak enzim papain Eksotika dengan suhu pemerapan 10°C dan masa pemerapan 1 jam didapati paling berkesan dan bersesuaian dalam melembutkan daging ayam tua berbanding kombinasi ujian yang lain. Secara kesimpulannya, ekstrak enzim papain daripada variety betik Eksotika telah dibuktikan keberkesanan dalam melembutkan daging ayam penelu, di mana kombinasi perlakuan EH2T1 telah dipilih sebagai terbaik.

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CHAPTER 1

INTRODUCTION

1.1 Papaya

1.1.1 Papaya tree

The papaya, *Carica papaya* L., is a member of the small family Caricaceae allied to the Passifloraceae (Lembaga Pemasaran Pertanian Persekutuan, 2008). Papaya tree usually grows up to 1.8-3 m for the first year. It is reaching 6-9 m in height, with a hollow green or deep-purple stem becoming 30-40 cm or more thick at the base and roughened by leaf scars. The leaves emerge directly from the upper part of the stem in a spiral on nearly horizontal petioles 1 to 30-105 cm long, hollow, succulent, green or more or less dark purple. The blade, deeply divided into 5 to 9 main segments, each irregularly subdivided, varies from 30-60 cm in width and has prominent yellowish ribs and veins. The life of a leaf is 4 to 6 months. Both the stem and leaves contain copious white milky latex (Galindo, 2009).

The 5-petalled flowers are fleshy, waxy and slightly fragrant. Some plants bear only short-stalked pistillate (female) flowers, waxy and ivory-white; or hermaprodite (perfect) flowers (having female and male organs), ivory-white with bright-yellow anthers and borne on short stalks; while others may bear only staminate (male) flowers, clustered on panicles to 1.5-1.8 m long. There may even be monoecious plants having both male and female flowers. Some plants at certain seasons produce short-stalked male flowers, at other times perfect flowers. This change of sex may occur temporarily during high temperatures in midsummer. Some "all-male" plants occasionally bear, at the tip of the spray, small flowers with perfect pistils and these produce abnormally slender fruits. Male or hermaphrodite plants may change completely to female plants after being beheaded (Department of Agriculture, 1898).

Papaya plant is a giant herbaceous and dicotyledonous with a soft-wooled hollow stem. It usually has a simple trunk but branching is easily induced by injury at the top. It may also develop several lateral branches as the plant becomes older. Trees may grow to a height of 2 – 6 m depending on the ecology and the care provided. Under excellent conditions some plants can produced for twenty years. It bears large, deeply-lobed leaves bunches together near the apex on long, hallow, soft petioles (Department of Agriculture, 1898).

1.1.2 Papaya fruit

Generally, the fruit is melon-like, oval to nearly round, somewhat pyriform, or elongated club-shaped, 15-50 cm long and 10-20 cm thick; weighing up to 9 kg. Semi-wild (naturalized) plants bear miniature fruits 2.5-15 cm long. The skin is waxy and thin but fairly tough. When the fruit is green and hard it is rich in white latex. As it ripens, it becomes light- or deep-yellow externally and the thick wall of succulent flesh becomes aromatic, yellow, orange or various shades of salmon or red. It is then juicy, sweetish and somewhat like a cantaloupe in flavor; in some types quite musky. Attached lightly to the wall by soft, white, fibrous tissue, are usually numerous small, black, ovoid, corrugated, peppery seeds about 5 mm long, each coated with a transparent, gelatinous aril (MARDI, 2008).

The papaya has been regarded as one of the most valuable of tropical fruits. It is a large, fleshy. hollow berry up to 50-60 cm. in diameter and usually weighs from 1/2 kg to 2 kg. It is cylindrical or pear-shaped. The central cavity is surrounded by hundreds of small seeds, though sometimes seedless varieties of the fruit are also found. The fruit has a thin smooth skin. It is dark green in color at first, but as the papaya ripens, it changes to bright yellowish or orange. Inside, the thick juicy flesh has a soft melting quality, and may be yellow or pink. It has a delicate aroma and delicious flavor (MARDI, 2009).

Inside the inner cavity of papaya fruit are black, round seeds encased in a gelatinous-like substance. Papaya's seeds are edible, although their peppery flavor is somewhat bitter. The fruit, as well as the other parts of the papaya tree, contain papain, an enzyme that helps digest proteins. This enzyme is especially concentrated in the fruit when it is unripe. Papain is extracted to make digestive enzyme dietary supplements and is also used as an ingredient in some chewing gums (Department of Agriculture, 1898).

Papayas offer not only the luscious taste and sunlit color of the tropics, but are rich sources of antioxidant nutrients such as carotenes, vitamin C and flavonoids; the B vitamins, folate and pantothenic acid; and the minerals, potassium and magnesium; and fiber. Together, these nutrients promote the health of the cardiovascular system and also provide protection against colon cancer.

1.2 Papain enzyme

Papaya is the main source of papain enzyme. Enzyme is a protein with catalytic properties due to its power of specific activation. Enzymes are noted for their ability as biological catalyst. Papain enzyme does not instantly indicate protease activity. The name papain simply deriving from papaya, which is the source of enzyme.

This enzyme has a multitude of commercial uses. Besides its use as a meat tenderizer, it is used to treat wool products to prevent shrinkage. It is also used in products that remove stains from fine fabrics. It is used in antidotes for insect and jellyfish stings. It is used in making toothpaste, beer and cosmetic products. It is used in cleaners for soft contact lenses. But my research is basically is about the effect of papain enzyme in meat tenderization.

The latex of *papaya* is a rich source of the cysteine endopeptidases, including papain, glycyl endopeptidase, chymopapain and caricain, which constitute more than 80% of the whole enzyme fraction. Papain is a minor constituent (5–8%) among the papaya endopeptidase. The enzyme is used widely as meat tenderizer, and has also several other applications, e.g. for defibrinating wounds, treatment of edemas, shrink proofing of wool, etc. Purification of papain from papaya latex has traditionally been achieved by precipitation methods. However, the purified enzyme still remains contaminated with other proteases. An alternative purification strategy has involved the use of various chromatographic techniques including ion exchange, covalent, or affinity chromatography but here the initial processing of the latex is essential before samples can be applied on a chromatography column (Sarote, 2006).

Papain is the most temperature stable and can require a temperature as high as 170-185°F to completely inactivate it. This has certain advantages and certain disadvantages. The main disadvantage is that a piece of meat cooked to what we call "medium rare" will not reach a temperature high enough to inactivate the papain. Thus, subsequent storage of the meat will allow the enzyme to continue to tenderize and if extended over too long a period will produce a mushy unpalatable texture. Papain should be used in very controlled processes where each step and cut of meat is under controlled time and temperature and served properly to the consumer. This is the best process for large scale highly organized restaurant chains where the process is thoroughly outlined and adhered to. The pH optimum of papain is typically similar to that of meat itself. The connective tissue in the meat breaks down when it comes in contact with Papain (Krishnaiah *et al.*, 2002).

¼ riped ½ riped ¾ riped 100% riped

(Mardi,2008)

1.2.1 Other uses of papain enzyme

Papaya has many other benefits too. Papayas may be very helpful for the prevention of atherosclerosis and diabetic heart disease. Papayas are an excellent source of vitamin C as well as a good source of vitamin E and vitamin A (through their concentration of pro-vitamin A carotenoid phytonutrients), three very powerful antioxidants (Mateljan, 2001).

These nutrients help prevent the oxidation of cholesterol. Only when cholesterol becomes oxidized is it able to stick to and build up in blood vessel walls, forming dangerous plaques that can eventually cause heart attacks or strokes. One way in which dietary vitamin E and vitamin C may exert this effect is through their suggested association with a compound called paraoxonase, an enzyme that inhibits LDL cholesterol and HDL cholesterol oxidation (Mateljan, 2001).

Papayas are also a good source of fiber, which has been shown to lower high cholesterol levels. The folic acid found in papayas is needed for the conversion of a substance called

homocysteine into benign amino acids such as cysteine or methionine. If unconverted, homocysteine can directly damage blood vessel walls and, if levels get too high, is considered a significant risk factor for a heart attack or stroke (Mateljan, 2001).

The nutrients in papaya have also been shown to be helpful in the prevention of colon cancer. Papaya's fiber is able to bind to cancer-causing toxins in the colon and keep them away from the healthy colon cells. In addition, papaya's folate, vitamin C, beta-carotene, and vitamin E have each been associated with a reduced risk of colon cancer (Mateljan, 2001).

These nutrients provide synergistic protection for colon cells from free radical damage to their DNA. Increasing your intake of these nutrients by enjoying papaya is an especially good idea for individuals at risk of colon cancer (Mateljan, 2001).

Papaya contains several unique protein-digesting enzymes including *papain* and *chymopapain*. These enzymes have been shown to help lower inflammation and to improve healing from burns. In addition, the antioxidant nutrients found in papaya, including vitamin C, vitamin E, and beta-carotene, are also very good at reducing inflammation. This may explain why people with diseases that are worsened by inflammation, such as asthma, osteoarthritis, and rheumatoid arthritis, find that the severity of their condition is reduced when they get more of these nutrients (Sarote, 2006).

Vitamin C and vitamin A, which is made in the body from the beta-carotene in papaya, are both needed for the proper function of a healthy immune system. Papaya may therefore be a healthy fruit choice for preventing such illnesses as recurrent ear infections, colds and flu.

Like avocados and bananas, papayas contain substances called chitinases that are associated with the latex-fruit allergy syndrome. There is strong evidence of the cross-reaction between latex and these foods. If you have a latex allergy, you may very likely be allergic to these foods as well. Processing the fruit with ethylene gas increases these enzymes; organic produce not treated with gas will have fewer allergy-causing compounds. In addition, cooking the food may deactivate the enzymes.

1.2.3 Extraction of papain enzyme

The papaya fruit is very rich in papain. The greener the fruit the more active is the papin. This protein digestion enzyme is found in the latex and is very abundant in green, unripe fruits. The extraction of papaya enzyme was carried out by collecting the latex from green papaya from a suitable size and age having 2 to 3 months after the fruit is set. Different methods are employed in getting the latex. A suitable method established by using the fine razor blade or with a stainless steel knife for lancing the fruit. Four longitudinal skin deep incision on fruit from the end to the fruit tip are given. A plastic container is useful for collecting the latex (Krishnaiah *et al.*, 2002 and Sarote, 2006).

1.3 Retired Chicken (Aged Chicken)

Retired chicken is at approximately 1.5 - 2 years of age, some chickens begin to slow down their egg production eventually producing nothing. However, they can live between 5 and 7 years. The aging of chicken is highly related to changes of AMP breakdown in chicken heart. The aging affect the characteristic of meat especially tenderization. The hardness of meat will result in difficulty in chewing digesting and less preferable by people as compared to normal broiler chicken meat. Therefore there must be a technique on how to overcome this problem. The treated aged chicken meat can improve the acceptability of consumer. Protein chemistry is playing an increasingly important role in development of methods for selective tenderization of muscle used as food.

Physical incorporation of tenderizers into meat is an important factor influencing their effectiveness. Papain is a plant enzyme obtained from the papaya is the active ingredient in most commercial tenderizers. The proteolytic action of the enzyme cleaves or breaks apart the muscle fiber proteins and connective tissue of meat by hydrolysis in a manner similar to that of digestion which makes food more absorbable (Tappel, 1956).

Therefore this study was carried out to:

1) Determine the effectiveness of the papain enzyme in tenderizing the aged chicken meat.
2) Select the most effective variety of papaya that acts as the best towards tenderizing the aged chicken meat.
3) Select the most suitable combination of marinating temperature and time for papain enzyme activation towards tenderizing the aged chicken meat.

CHAPTER 2

LITERATURE REVIEW

2.1 Papaya Plantation

2.1.1 *Sekaki* Papaya

Sekaki variety is also known as the Hong Kong Variety. This variety is the second most popular in Malaysia after Exotica. Sekaki papaya plant is quite short. The weight of this fruit is abour 1.5 to 2.0 kg (Harizamry, 2007).

Figure 2.1(a); Sekaki Papain Plant

2.1.2 Exotica Papaya

According to a report published by MARDI, exotica is a hybrid which is invented by Mardi. Recently this type of hybrid is successfully entered the world market because the attractiveness in the size which is smaller compared to any other papaya fruit, not only that the taste is also much more sweeter comparing to other papaya fruit. The success of this invention is seen clearly in the year of 2001 when this fuit became popular and the export value became higher compare to other fruits generally.

There is another report by the Malaysian Agricultural Research and Development Institute (2009) says that papayas, before the advent of Exotica, were very inconsistent in yield and generally had very poor eating qualities. Popular varieties then were Sitiawan, Batu Arang and Subang and their fruit size were large and inconvenient to handle and serve. Papayas were grown mainly for domestic consumption and export was insignificant.

In 1972, MARDI started a backcross breeding programme for improving papayas. The Sunrise Solo which has excellent eating qualities but with poor yield and small fruit, was introduced from Hawaii. It was crossed with the locally adapted, large-fruited Subang 6. Subsequent progenies underwent a series of 'self-pollination' and backcrossing to Sunrise Solo to reconstitute its excellent eating qualities while selecting for larger fruit size of the Subang 6. After 15 years of breeding and selection, a line called 'Backcross Solo' with the features of Sunrise Solo but with increased fruit size and local adaptability of Subang 6 was selected. In1987, it was released as the 'Eksotika'. The Eksotika had shortcomings in fruit freckles, soft texture and sensitivity to environmental stress. Eksotika was crossed with its sister line which was resistant to freckles and had better keeping qualities. The resultant of this hybrid was more robust, higher yielding and had much improved fruit cosmetics and keeping quality. This hybrid named 'Eksotika II' was released in 1991.

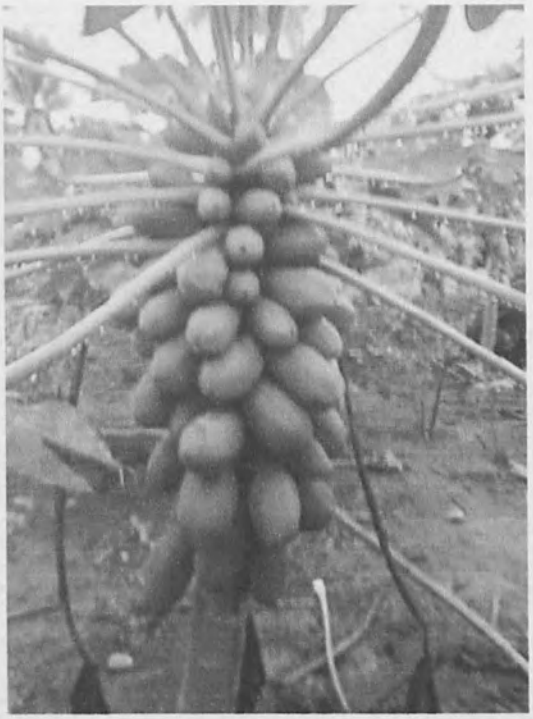

Figure 2.1 (b); Picture of Exotica papaya plant

2.1.3 Stages of Ripening of Papain Enzyme

Colour	Stage of ripeness	Ripening Time at Room Temperature
Green, with slight yellow tinge at larger end.	¼ ripe	5 – 7 days at room temperature
1/3 yellow, 2/3 green	½ ripe	2 – 4 days at room temperature
1/2 yellow, 1/2 green	¾ ripe	1 -2 days at room temperature
Mostly yellow or yellow-orange	100% ripe	Ready to eat.

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