INVESTIGATION INTO THE PRODUCTION AND EVALUATION OF MODERATELY RIPE PAWPAW FLUID AS MEAT TENDERIZER

BY

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MATRIC No. 2004/18413EA

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NIGERSTATE

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BEING A FINAL YEAR PROJECT REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF BACHELOR OF ENGINEERING (B.ENG.) DEGREE IN AGRICULTURAL & BIORESOURCES ENGINEERING, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE

FEBUARY, 2010

DECLARATION

I hereby declare that this project work is a record of a research work undertaken and written by me. It has not been presented before for any degree or diploma or certificate at any university or institution. Information derived from personal communications, published and unpublished work were duly referenced in the text.

8/02/2010

Oyedokun, Ganiyu Olayinka | Date

CERTIFICATION

This project entitled "Investigation into the Production and Evaluation of Moderately Ripe Pawpaw Fluid as Meat Tenderizer" by Oyedokun Ganiyu Olayinka, meets the regulations governing the award of the degree of Bachelor of Engineering (B. ENG.) of the Federal University of Technology, Minna, and it is approved for its contribution to scientific knowledge and literary presentation.

Engr. Dr. A.A Balami Supervisor	17/02/10 Date
Engr. Dr. A.A Balami HOD, Agricultural and Bioresources Engineering.	17/02/10 Date
External Examiner	10/2/2010 Date

DEDICATION

I dedicate this work to God almighty, the creator and giver of life.

ACKNOWLEDGEMENTS

My greatest appreciation goes to the Almighty God who has given me the grace by keeping me alive and enabled me to successfully complete this project.

I want to express my gratitude to my supervisor Dr. A.A Balami who took his time to encourage and review the work, may God grant you long life to enjoy the fruits of your labour (Amen).

To my able level adviser and all the Departmental staff whose contributions and suggestions were really helpful.

I want to say a big thank you to the management and staffs of Ebeboye consult limited for their cooperation and also to the staff of Animal production laboratory, and to Mr Isaac of the Biochemistry department.

Special thanks to my parent for their love, care, prayers and also for being there for me financially. Big thanks to my sister for her understanding and moral support. And to the Fellowship of Christian Students and also all maintenance unit members, God through you kept me going.

Appreciation goes to my course mate, may God reward you, to Adaeze 'Obim' and to my friends Olawunmi, Ali, Ola, Atolagbe, Gana, Omotainse, Ismail, Dare, Adetutu, Bulus, Oyinade, Dokun you are highly appreciated, God bless you all.

ABSTRACT

Fluid was extracted from moderately ripe pawpaw, analysed and used to treat meat at the Animal production and Food science Laboratory, Federal University of Technology Minna, Bosso Campus. To achieve this, three vertical cuts 1-2 mm were made on the fruit with a stainless steel blade, the latex flow is then collected in a polythene box with a close fitting led. The result of the analysis performed on the extract (moderately ripe pawpaw fluid) showed that the total dissolve solute (TDS), brix, pH, Alkalinity and Hardness values are 39.4, 3.8, 6.0, 25 ppm, 163 ppm respectively. The result obtained from the sensory evaluation of cooked meat treated with the fluid and ordinary cooked meat (control) shows that the aroma of the meat cooked with moderately ripe pawpaw fluid differed statistically ($p<0.05$) from that treated without the fluid. The texture of the meat cooked with moderately ripe pawpaw fluid differed statistically ($p<0.05$) from that treated the fluid but there is no difference in appearance, flavour and taste. Thus the use of moderately ripe pawpaw fluid can be encouraged as a tenderizing agent for meat consumption. More research work should be done in providing a means of preserving moderately ripe fluid, before drying as this determines its quality.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of study

Pawpaw (*Carica papaya*), common name for a small family of soft-wooded, sparsely branched trees of tropical America and West Africa, and for its representative genus. Four genera (set of closely related species) and about 30 species are placed in this family of dicots. They characteristically have palmately lobed or compound leaves; small, unisexual flowers; and separate male and female plants (dioeciously). All parts of the plants contain milky latex in special latex-producing cells. Papaya is the common name for the family *Caricacecae.* The representative genus is Carica. The common papaya is classified as *Carica* papaya (Microsoft Encarta, 2009).

The common papaya, also called papaw and pawpaw, is a native to tropical America, but its exact origin is unknown, it was cultivated in Mexico several centuries before the emergence of the Mesoamerican (region of Central America and southern North America that was occupied by several civilizations, especially the Maya, in pre-Columbian times) classic cultures. The earliest documentation of pawpaw is in 1541 report of the De Soto expedition, who found Native Americans cultivating it east of Mississippi river. The Lewis and Clark expedition depended and sometimes manage to live on pawpaw during their travels (Wikipedia, 2008). It is now widely cultivated in the tropics; many varieties have been developed. In the wild, the tree grows to about 1.8 m high, but cultivated trees may be about 7.6 m high. The fruits, which vary in shape from spherical to elongate and which may weigh as much as 9 kg, are eaten fresh as breakfast fruit or in salads or desserts (Microsoft Encarta, 2009). The northern, cold-tolerant common pawpaw (as mina trilobite) is deciduous, while the southern species are often evergreen. The leaves are alternate, simple ovate, 20 to 35cm long and 10 to 15cm broad wedge-shaped at base and primary veins prominent. The fetid flowers are produced singly or in clusters of up to eight together; they are large, 4 to 6cm across, perfect, with six sepals and petals (three large outer petals, three smaller inner petals). The petals color varies from white to purple or red-brown. The fruit is a large edible berry, 5 to 16cm long and 3 to 7 cm broad, weighing from 20 to 500g, with numerous seeds; it is green when unripe, maturing to yellow or brown. It has a flavor somewhat similar to both banana and mango, varying significantly by cultivar and has more protein than most fruits. The bark is dark brown, blotched with gray spots, sometimes covered with small growth that sticks out from it, divided by shallow fissures. Inner bark tough, fibrous. Its wood is pale, greenish yellow, sapwood lighter; light, soft, coarse-grained and spongy. The flower comes out in April, with leaves perfect solitary.

The pawpaw tree sheds its leaves in rainy season, is prone to producing root suckers a few feet from the trunk, and is a slow grower, particularly when it is young. However, under optimal greenhouse conditions, including photo-period extension light of approximately 16 hours, top growth of up to 1.52 m can be attained in three months. The dark green, obviate-oblong, drooping leaves grow up to 30.48 cm long, giving the pawpaw an interesting tropical appearance. The leaves turn yellow and begin to fall in mid-autumn and leaf out again in late spring after the tree has bloomed. Dormant, velvety, dark brown flower buds develop in the axils of the previous year's leaves. They produce maroon, upside-down flowers up to 5.08 cm across. The normal bloom period consist of about 6 weeks during March to May depending on variety, latitude and climatic conditions. The blossom consists of 2 whorls of 3 petals each, and the calyx has 3 sepals. Each flower contains several ovaries which explain why a single flower can produce multiple fruits. Individual fruits weigh 0.142 to 0.454 kg and are 7.6 to 15.24 cm in length. The larger sizes will appear plump, similar to mango. The fruit usually has 10 to 14 seeds in two rows. The brownish to blackish seeds are shaped like lima beans, with a length of ½ to1½ inches. Pawpaw fruits often occur as clusters of up to nine individual fruits. The ripe fruit is soft and thin skinned. Shelf-life of a tree-ripened fruit stored at room temperature is 2 to 3 days. With refrigeration, fruit can be held up to 3 weeks while maintaining good eating quality. Within the fruit, there are two rows of large, brown, bean shaped, laterally compressed seeds that may be up to 3cm long. Seeds contain alkaloids in the endosperm that are emetic. If chewed, seed poison may impair mammalian digestion but if swallowed whole, seeds may pass through the digestive tract intact. Pawpaw has tremendous potentials based on the following reasons:

1. Adaptation of trees to existing climatic and edaphic conditions.
2. Nutritional/cosmetic value of fruit.
3. Valuable natural compounds in plant.
4. Nursery wholesale and retail tree production.
5. As a component in residential 'edible' landscapes.

Pawpaw is an excellent food source. It exceeds apple, peach, and grape in most vitamins, minerals, amino acids, and food energy value. Papaya is exploited for its latex, which contains papain, a proteolytic (protein-digesting) enzyme used in meat tenderizers. The latex is obtained by making a longitudinal incision on the surface of the nearly mature fruit. The coagulated latex gives the protein hydrolyzing enzyme which is used in the manufacture of chewing gum and in the brewing industry to digest protein fragments of chilled beer so that it remains clear (James, 2006).

Papain is a protein-cleaving enzyme derived from papaya. Papaya latex is a proteolytic enzyme consisting of 4 enzymes: Chymopapaya A and B, Papain and Papaya peptidase A. The common name of these enzymes is papain. Enzymes are complex molecules produced in living organism to catalyze (speed up) chemical reaction within the cell. A number of digestive enzyme supplements are available. The simple ones are extracted from tropical fruits; brome lain from pineapple and papain from papayas, papain consists of 212 amino acids. Enzymes can be generally recognized by the ending –ase. This either indicates the nature of the substance affected by the enzyme (e.g.carbohydrase acts on carbohydrate material and proteases acts on proteins) or to indicate the nature of the reaction e.g. transferases catalyses the transfer of atoms or group of atoms within a substance. Enzymes occur naturally in foods and many traditional food processing technologies involve the use of enzymes. Today, with more advance knowledge of food science these enzymes can be extracted, concentrated and added to food during processing (e.g. meat tenderizers). One important group of enzymes is called proteases. These are enzymes which catalyses the breakdown of proteins. Chill proofing of beer, tenderization of meat. The most common of these proteases is papain (James, 2006).

One cannot over-emphasize the importance or use of pawpaw, traditionally pawpaw leave is washed and placed on meat when cooking (tenderizing) and can act as a clarifying agent in many food industry processes. It is a common ingredient in brewery and meat processing. The seeds also have insecticidal properties. They are dried, powdered and the powder can be applied to children's heads to control lice; specialized shampoos now use compounds from pawpaw for the same purpose. Papaya is the commercial source of the enzyme papain, which is distributed throughout the plant, but more concentrated in the latex of the fruit. It is used in the manufacturing of chewing gum and in the brewing industry to digest protein fragments of chilled beer so that it remains clear (James, 2006). Papain is used in the pharmaceutical industry, in medicine as well as in the food processing industry (e.g. in the preparation of vaccines and for the treatment of hard skin). It also has veterinary applications such as de-worming of cattle. Papain is also used in the tanning of leather and has applications in the paper and adhesive industries as well as in sewage disposal, aid digestion. Medical research includes plastic surgery on cleft palates using papain. Growers hope that potential use will eventually lead to increased market demand from the pharmaceutical industry. As a result of the above multi-traditional medicinal benefits of pawpaw leave, this research wok was carried out to investigate the production and evaluation of moderately ripe pawpaw fruit.

1.2 Statement of problem

In Nigeria, much time and energy is spent while cooking some parts of meat. This is because of the unavailability of readily made meat tenderizer. This research work is done in order to look for a solution to the problem of cooking meat without tenderizer.

1.3 Objectives of the project work

The objective of study includes:

(i) To produce fluid from moderately ripe but matured pawpaw fruit (*Carica papaya*).

(ii) To carry out chemical analysis on the fluid produced.

(iii) To carry out sensory evaluation on the meat that the fluid was used on.

1.4 Justification of this study

In Nigeria, several substances are used to tenderize meat, these substances reduce the nutritional value of meat. This research work tends to investigate a better alternative for tenderizing meat, which is the use of moderately ripe pawpaw fluid.

1.5 Scope of the study

The scope of this work includes only these:

(i) Chemical analysis of the extracted pawpaw fluid; Total dissolve solute (TDS), Brix (sugar content), p^{H} test, Alkalinity, Hardness.

(ii) Physical analysis of the meat that has been treated with the extracted fluid; flavour, taste, aroma, appearance, texture.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pawpaw

Pawpaw is the most economically important fruit in the *caricaceae* family. Brazil is the main producer and trader of pawpaw in the world. As trade has increased, new producers have appeared in the international market. With the development of better cultivation and post-harvest technologies, pawpaw is becoming a new star in the world tropical fruit market (FAO, 2008).

In 1998, the FAO reported an estimate of 5.1 millions of metric ton, which were harvested in the world, representing twice the harvest of 1980. The main consumer markets are usually supplied by one major supplier. In general, pawpaw crops have shown a continuous and stable growth and importers are confident of the future of this market. Nigeria is the main producer in the African continent with 748,000 metric ton, with a 90,000 ha devoted to the crop, South Africa, Mozambique and Congo are also important producers (FAO, 2008). Table 2.1 shows the major pawpaw fruit producing countries in the world.

Table 2.1 Major Papaya Fruit Producing Countries of the World

Country	Production,000 Tons
Brazil	1450
Nigeria	748
India	644
Mexico	613
Indonesia	470
China	152
Thailand.	119

FAO, 2008

The major pawpaw producing countries are represented graphically below

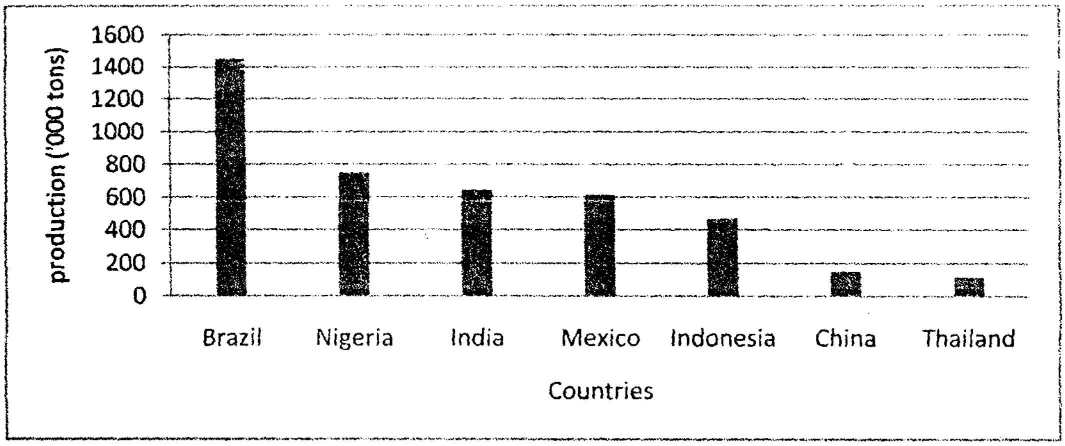

Fig. 2.1 Graphical representation of major pawpaw producing countries

Source: FAO, 2008

2.2 Chemical composition of pawpaw

Papaya is a common dessert, used in fruit salads and is enjoyed throughout the tropical countries. The raw fruit is also used as a vegetable for cooking. Papaya is a wholesome fruit, rich in sugars, and vitamins C, A, B1, and B2. The physicochemical quality of papaya fruits (such as, mean fruit weight, pulp yield, pulp–peel ratio, TSS as Brix, vitamin C, and total carotenoids) is influenced by various agronomic practices, planting time of the year being an important parameter. September planting produced heavier mean fruit weight (2.30 kg), maximum TSS (11.2 Brix), vitamin C (74.55 mg/100 g) and total carotenoids (1152.50 mg/100 g), higher pulp–peel ratio, than that of the fruits harvested from other months of planting (James, 2006).

Among the physicochemical determinants, pH and TSS (Brix) are very good indicators of sripening of the papaya fruit. Change of latex color from white to watery, is another index of maturity of papaya fruit. The chemical composition of papaya fruit with respect to sugars, organic acids, amino acids, vitamins, and minerals change during ripening. At harvest, water content varied from 87% to 97%, carbohydrates from 2% to 12%, protein content 0.6% fat content 0.1% and fiber content 1.8%. The DM, which was 7% at 15 days after pollination, increased to 13% at harvest. Alcohol-insoluble solids, starch and several minerals decreased, whereas total sugars increased during this time. The total and nonvolatile acidity decreased to a minimum at the fully ripe stage of harvest. The organoleptic qualities, volatile profiles, and lipid content of papaya have been shown to be highly dependent on the degree of fruit maturity. Soluble sugars accumulate mainly when papaya fruits are still attached to the plant. Sucrose synthesis still occurs even after harvest, as sucrose-phosphate synthase activity is highly correlated to sucrose content. Sugar content and sweet sensory savor are dissociated, while pulp softening has a strong correlation with sweetness, probably due to the easier release of cellular contents in fully ripe tissues (James, 2006). Table 2.2 shows the chemical composition of pawpaw fruits.

Table 2.2 Chemical composition of pawpaw fruits

Values	Per 100g
Moisture content	89.6
Protein content	0.5
Fat content	0.1
Fiber content	1.1
CHO	9.5
Ascorbic	57
Calcium	0.01
Phosphorus	0.01
Iron	0.04

The nutritional value of pawpaw is also shown in table 2.3

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The nutritional value of pawpaw is also shown in table 2.3

Table 2.3 Nutritional Value of Papaya (Per 100 g of Edible Portion of Raw Fruit)

Constituent	Content
Water	88.7g
Food energy	165kJ
Protein	0.6g
Fat	0.1g
Total carbohydrates	10g
Fiber	0.9g
Ash	0.6g
Calcium	20mg
Phosphorus	16mg
Iron	0.3mg
Sodium	3mg
Potassium	234mg
Vitamin A	1750IU
Thiamine	0.04mg
Riboflavin	0.4mg
Niacin	0.3mg
Ascorbic acid	56mg

Source: USDA, 1999.

2.3 By-Products from Papaya

2.3.1 Pectin

To make papaya cultivation and papain industry viable, the profitable use of scarred fruit is essential. The quality of such papaya fruits does not appear to be affected adversely but only the fruit appearance seems less attractive to the consumers. The green fruits, whether scarred or not, are rich source of pectin (10% pectin on dry basis), which can be extracted for use in food industry. Peel is shown to be higher in pectin content than the papaya pulp, and pectin content increases at a higher rate with fruit maturity up to a stage. The integrated processing of papaya fruits for the production of papain and pectin has been found to be economical. This process gives a papain yield of 0.25% and a pectin (jelly grade 200) yield of 1% on fresh fruit basis. The variety of the fruit, the growing conditions, and the stage of maturity of fruit are all known to influence the chemical composition of pectin (James, 2006).

2.3.2 Papain

Papain is the major by-product from dried latex derived from papaya fruit, which contains a protein hydrolyzing enzyme. This enzyme has a number of specific technological applications such as in food, meat tenderization, beverages, and animal feeds; pharmaceutical industry; textile industry and detergents; paper and adhesives; medical applications; sewage and effluent treatment; and research and analytical chemistry.

Papain is a hydrolytic enzyme, which digests proteins into amino acids. Papain in solution is easily oxidized by exposure to air, high temperature (above 70°C), or sunlight. Contact with metals such as iron, copper, zinc, and many others also inhibit this enzyme. For improving the stability of papain enzyme, a number of chemicals such as ascorbic acid, sodium ascorbate, erythorbic acid, sodium erythorbate, sodium metabisulfite, 4-hexylresorcinol, TBHQ, 55 °C for all chemicals except sucrose and trehalose, which gave their best performance at 40 °C. Among the food applications, the use of papain in chill haze removal during beer clarification as well as in the tenderization of meat has shown a steady increase over the past years. There is also a belief in some countries of Asia that eating papaya by pregnant ladies results in abortion. Based on rat feeding studies, they suggested that normal consumption of ripe papaya during pregnancy may not pose any significant risk but unripe or semi-ripe papaya may be unsafe in pregnancy, as the high concentration of latex produces marked uterine contractions (James, 2006).

2.4 Other tenderizing Enzymes

Tenderizing enzymes are used to treat tough cuts of meat and make them more acceptable to consumers. In addition to beef, pork and chicken, these enzymes can also be used on seafood such as squid or clams. The two most commonly used meat tenderizing enzymes are papain and bromelain. Both derived from plant sources. These are papaya fruit and the pineapple plant. To much lesser extent, ficin, derived from fig tree latex is also used.

2.4.1 Bromelain

Bromelain is prepared from the stump or root portion of the pineapple plant after harvest of the fruit. This stump or root portion is collected from the fields, peeled and crushed to extract the juice containing the soluble bromelain enzyme.

2.5 Papain production

Papain is a common enzyme obtained from the green papaya (pawpaw) fruit. At the end of an enzyme catalyzed reaction the enzyme itself is unchanged and is able to react again. Enzymes can be generally recognized by the ending *-ase*. This either indicates the nature of the substance affected by the enzyme (e.g. carbohydrates acts on carbohydrate material and proteases acts on proteins) or to indicate the nature of the reaction e.g. transferases catalyse the transfer of atoms or groups of atoms within a substance. Enzymes occur naturally in foods and many traditional foods. Processing technologies involve the use of enzymes. Today, with more advanced knowledge of food science these enzymes can be extracted, concentrated and added to foods during processing e.g. meat tenderizers (*Practical Answer.com*).

Production of papain requires considerable technical parameters starting from the plant. The latex is collected from the papaya green fruit after 2-3 months fruit is set. Different methods are used in lancing the fruit (Krishnaiah *et al.,* 2002). The collected latex is dried and treated with suitable solvents in order to validate the activity of papain enzyme to be used in the different applications. Yield of papain depends on cultivator, time of taping, nutritional status of plant and the region. It is reported that there is positive correlation between the fruit size, papain yield and rainfall in respect of papain enzyme yield. Papain is a cysteine protease (enzyme) present in papaya which is useful in tenderizing meat and other proteins. It consists of 212 amino acids. Papain is usually produced as crude.

Fruits grown for papain production are thinned on the plant so that each fruit hangs separately for easy collection of latex. Using plastic or stainless steel knives, fruits are lanced and the latex is collected in glass or porcelain containers. Four to five longitudinal cuts made during the morning hours gave the highest yield of latex. Over a period of 2 weeks, this process is repeated three or four times on the untapped portions of the fruits in 3–4 days intervals. The latex, which hardens within 15 min, is then precipitated with alcohol, washed with acetone, and dried in a vacuum drier for obtaining good quality crude papain. The addition of potassium metabisulfite (0.05 %) in small quantities to the liquid latex before drying acts as a preservative and improves the quality of crude papain powder. The dried papain is powdered and sieved through a 10-mesh sieve and stored in airtight containers. The yield of crude papain powder obtained from raw green papaya is reported to be usually around 0.025 % (James, 2006).

2.5.1 Factors that affects the production of papain.

The factors affecting the papain yield from papaya plants are fruit shape, stage of maturity, season of tapping, tapping time of the day, pattern of tapping, and frequency of tapping. Four repeated applications of the plant hormone, Ethephon in coconut oil (at 37 μM), have shown to increase papain yield.

2.5.2 Enzyme activity of Papain

If papain is to be exploited commercially for an export market or local food industry use, it is important to be able to determine the enzyme activity. The method is known as assaying. The assaying could be carried out by, for example, the National Standards office. Papain is used to hydrolyse (or breakdown) proteins. Therefore assays to measure papain activity are based on measuring a product of the hydrolysis.

There are two main assay methods. The first relies on the ability of papain to clot milk. It is a low cost method but is time consuming. Also the lack of a standard method to find the clotting point and variations in the milk powder used can introduce errors.

In this method a known amount of papain sample (made by dissolving a known weight of papain in a known volume of a solution of acetic acid) is added to a fixed amount of milk (made by dissolving a known weight of milk powder in a known volume of water) which has been warmed to 30°C in a water bath. The contents are thoroughly mixed and then observed until the first signs of clotting (formation of lumps) are detected. The time taken to reach this stage, from when the papain was added to the milk, is recorded. The experiment is then repeated using different known amounts of papain solutions. The different amounts of papain sample used should give a range of clotting times between 60 and 300 seconds for optimum results. The activity of the papain sample is then calculated by plotting a graph, finding the time taken to clot milk at an infinite concentration of papain and then using that value in a formula to calculate the activity. To introduce a measure of standardisation the amount of milk can be fixed at a certain known concentration. This is done by reacting a known concentration of high grade papain with the milk. The concentration of milk powder solution can then be adjusted to obtain the desired clotting time under fixed reaction conditions. The 'activity of pure papain' at this known amount of milk can then be calculated. Testing the sample papain under the same reaction conditions and same (known) amount of milk will then give an activity relative to the pure papain.

The second method is based on the science of the absorption of light known as absorptiometry. This is the analytical technique for measuring the amount of radiation (or 'colour' of light) absorbed by a chemical solution. It is known that, for example, a yellow-coloured solution will absorb blue light (Blue is the 'complement colour' to yellow). The greater the concentration of yellow in the solution the more the absorption of blue light. This is a useful discovery because certain products of chemical reactions are coloured. The more intense the colour, the greater the concentration of product. Therefore by directing the relevant complement colour through the sample liquid the amount of light absorbed can be related to the concentration of product.

Not all 'colours' (or radiations of light) are visible to the human eye. The technique used when the 'colours' extend beyond the visible spectrum is known as spectrophotometry and the instrument used is called a spectrophotometer. In the second method to determine the activity of a papain sample, a known amount of papain sample is mixed with a fixed amount of casein (the protein found in milk). The reaction is allowed to proceed for 60 minutes at 40°C. After this time, the reaction is stopped by the addition of a strong acid. The product of the reaction is known as tyrosine which is known to absorb ultra-violet light (invisible to the human eye). The solutions containing the tyrosine are prepared for analysis using the spectrophotometer. The amount of ultra-violet light absorbed by the solution can be related to the number of tyrosine units produced by the papain sample. Hence the greater this number, the greater the activity of the papain sample (*Practical Answers.com*).

2.5.3 Methods of collection and extraction

Moderately ripe pawpaw fluid is obtained by cutting the skin of the unripe but almost mature papaya and then collecting and drying the latex which flows from the cuts. Tapping of the fruit should start early in the morning and finish by mid-late morning (i.e. during periods of high humidity). At low humidity the flow of latex is low. Two or three vertical cuts (except the first cut, see below) 1-2mm deep are then made, meeting at the base of the fruit. The incisions are made using a stainless steel razor blade set into a piece of rubber attached to a long stick. The blade should not protrude more than about 2mm as cuts deeper than 2mm risk juices and starch from the fruit pulp mixing with the latex which lowers the quality. Fruits should be tapped at intervals of about 4-7 days and for the first tapping it is usually sufficient to make only one cut. On subsequent tapings the two or three cuts are spaced between earlier ones (as explained above). After about 4-6 minutes the flow of latex ceases. A dish is used to collect the latex and the latex is then scraped into a polythene lined box with a close fitting lid: such a box should be stored in the shade. The use of a close fitting lid and keeping the box in the shade are both important because they reduce the reactions which cause the loss of enzyme activity. Foreign matter such as dirt and insects in the latex should be avoided. Latex adhering to the fruit should be carefully scraped off and transferred to the collecting box. However, dried latex should not be mixed with fresh latex as this lowers the quality.

When handling fresh latex, care should be taken to ensure that it does not come into contact with skin as it will cause burning. Neither should it come into contact with heavy metals such as iron, copper or brass as this causes discolouration and loss of activity. Pots, knives and spoons should not be used unless they are made from plastic or stainless steel. Fresh latex does not keep well and should be dried to below 5% moisture (when it will have a dry and crumbly texture) as soon as possible. After two or three months the fruits are ripe and should be removed from the tree. The ripe fruits are edible but have very little sale value because of their scarred appearance. However, the skin of the green ripe papaya does contain about 10% pectin (dry weight) and the fruits could be processed to extract this (*Practical Answers.com*).

2.5.4 Drying of papaya latex

The method of drying is the main factor that determines the final quality of papain. There have been various grades used since papain became an international commodity. Up to the mid 1950s when papain from Sri Lanka dominated the market three grades were known:

1. Fine white powder,
2. White oven-dried crumb
3. Dark sun-dried crumb.

Up to the 1970s there were two grades:

1. First or high grade oven-dried papain in powder or crumb form usually creamy white in colour.
2. Second or low grade sun dried brown papain in crumb form.

Since 1970, as a result of new processing techniques papain has been re-classified into three groups:

1. Crude papain - ranging from first grade white down to second grade brown.
2. Crude papain in flake or powder form - sometimes referred to as semi-refined.
3. Spray dried crude papain -in powder form, referred to as refined papain.

2.5.5 Sun drying

Sun drying gives the lowest quality product as there is considerable loss of enzyme activity and the papain can easily turn brown. However, in many countries sun drying is still the most common processing technique for papain. The latex is simply spread on trays and left in the sun to dry.

2.5.6 Oven drying

Papain driers can be of simple construction. In Sri Lanka they are generally simple out-door stoves (about one metre high) made out of mud or clay bricks. Drying times vary but an approximate guide is 4-5 hours at a temperature of about 35-40°C. Drying is complete when the latex is crumbly and not sticky. A better quality product is obtained if the latex is sieved before drying. The dried product should be stored in air-tight and light-proof containers (e.g. sealed clay pots or metal cans) and kept in a cool place. Metal containers should be lined with polythene.

2.5.7 Spray drying

This is not possible at small-scale. A considerable investment in equipment (e.g. £10,000) is required. However, spray dried papain may be bought for the small-scale processing of foods.

Spray dried papain has a higher enzyme activity than other papain and is totally soluble in water. Extreme care must be taken when handling this form of papain because it can cause allergies and lung condition causing breathing impairment if inhaled. For this reason spray dried papain is often kept in a protective membrane.

2.5.8 Characteristics of papain

Papain is more temperature stable when compared to bromelain and can require a temperature as high as 77-85 °C to completely inactivate it. The pH optimum of papain is similar to that of meat itself. The general application of the finished papain product is 1 teaspoon or 3 grams per pound (500 grams) of meat.

2.5.9 World trade in papain

The principal producers of crude papain are Zaire, Tanzania, Uganda and Sri Lanka. Most of the spray-dried papain comes from Zaire. The principal importing countries are the United States, Japan, United Kingdom, Belgium and France. Almost all the best quality papain goes to the United States.

Crude papain is used, in Britain, in the brewing industry for chill-proofing beer and lager. However, the increasing trend for additive free beers initiated by other European countries is taking effect in Britain and so this market for papain is declining. Another use for papain is in the meat industry for the tenderisation of meat and the production of meat tenderising powders. (*Practical Answers.com*)

2.6 Meat

Man has satisfied his hunger with animal food from the earliest of times. The term meat refers to muscle of warm blooded terrestrial for legged animals, the chief ones being cattle, sheep and pigs. Meat also includes the glands and organs of these animals.

The annual consumption of meat of a country depends upon its economic status, affluent countries consuming more than others. The world demand for meat is growing up steadily because of preference based on palatability. Meat is rich in most of the nutrients required by man, this is to be expected since the tissues and body fluids of man are very similar to those of animals. Meat is rich in protein (15 to 20 percent) and contains all the essential amino acids. It is also rich in minerals and vitamins (Shadaksharaswamy and Shakuntala, 2006).

2.6.1 Composition of meat

Meat contains 15 to 20 percent proteins of outstanding nutritive value. The lean meat contains 20 to 22 percent proteins. Of the total nitrogen content of meat, approximately 95 percent is proteins and 5 percent is smaller peptides and amino acids. The amino acid make up of meat proteins is very good for the maintenance and growth of human (Shadaksharaswamy and Shakuntala, 2006).

2.6.2 Tenderizing meat

Tenderness, juiciness and flavour are components of meat palatability, although juiciness and flavour normally do not vary a great deal, tenderness can vary a considerably from one cut to the other and the most desired quality in meat. The amount and distribution of connective tissues and the size of both muscle fibres and bundles of fibres determine the tenderness of meat to various extents (Shadaksharaswamy and Shakuntala, 2006).

There are various opinions and approaches to the process of tenderizing meat. One is the ante-mortem use of meat tenderizing enzymes. This consists of the physical injection of a controlled solution of either papain or some other enzyme into the living animal. This practice has been discontinued and is no longer used. Post-mortem application is generally acceptable for the lesser quality cuts and a variety of application methods are available.

The major area of consumption of meat tenderizers that we see in the world is in the consumer households. This consumer use probably accounts for 90% of enzyme tenderizer sales, the consumer sprinkles the powder containing the standardized enzyme material on the meat and through a mechanical process called "forking" have the enzyme penetrate the meat cut and then immediately cook in order to produce a tenderized and highly palatable product *(enzymedevelopment.com).*

2.6.3 Factors involved in meat tenderness

There are certain factors that affect the tenderness of meat. These are genetics, species, age and feeding.

2.6.3.1 Genetics

The heritability of tenderness in beef is approximately 45percent, which means that 45percent of the observed variation in tenderness of cooked beef is due to the genetics or parents of the animal from which the beef came (Shadaksharaswamy and Shakuntala, 2006). A consumer can purchase certain quantity of meat (beef), cook it to a medium degree of doneness and end up with a very tender product. The same consumer can then go back to the same market buy the same type of meat (beef), cook it the same way and it could be less tender.

2.6.3.2 Species and age

Beef usually is the most variable in tenderness followed by lamb and pork. The tenderness variation from specie to species is due primarily to the age of the animal at the time of slaughter. Beef normally is processed at approximately 20 months of age, lamb at 8 months and pork at 5 months of age. Beef normally is slaughtered between 9 and 30 months of age. If a female beef animal has been used for breeding purposes, meat from such an animal becomes progressively less tender as the animal gets older. The decrease in tenderness with increasing age is due to the change of the connective tissue found in meat.

2.6.3.3 Feeding

Animals (beef) that are feed with grain tend to reach a given slaughter weight sooner than animals (beef) that are feed to the same slaughter weight on pasture. Thus grain-fed animals usually are slightly more tender because they are slaughtered at a slightly younger age.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The materials used are

- Moderately ripe pawpaw fluid
- Meat

The fluid was carefully extracted from moderately ripe pawpaw and taken to the laboratory for analysis.

Fresh meat sample (beef) was purchased from Minna central market, Minna, Niger state and taken to the animal production laboratory of the Federal University of Technology, Minna for chemical analysis and sensory evaluation.

3.2 Methods

3.2.1 Preparation of moderately ripe pawpaw fluid sample

The fluid was obtained by cutting the skin of the moderately ripe pawpaw fruit and then collecting and drying the latex which flows from the cuts. Tapping of the fruit started early in the morning and finish by mid-late morning (i.e. during periods of high humidity). At low humidity the flow of latex is low. Two or three vertical cuts (1-2 mm deep) are then made, meeting at the base of the fruit. The incisions are made using a stainless steel razor blade set into a piece of rubber attached to a long stick. After about 4-6 minutes the flow of latex ceases and the latex was then scraped into a polythene lined box with a close fitting lid. The flowchart for the production of pawpaw is shown in fig 3.1

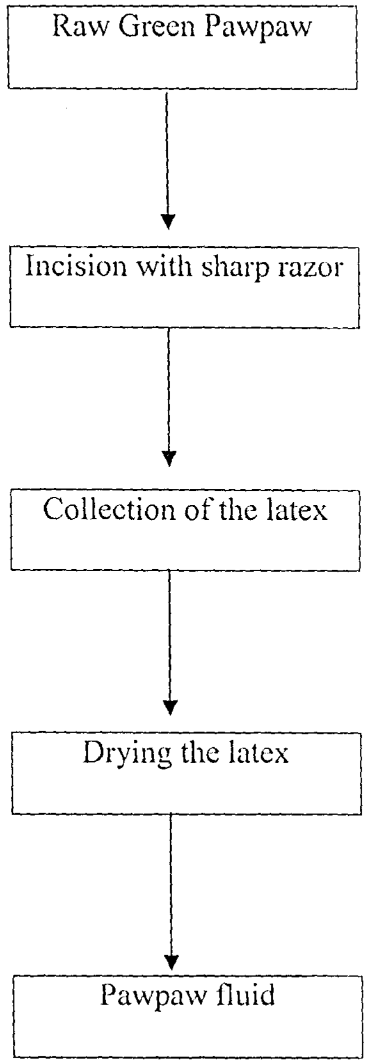

Figure 3.1: Flow Chart for the production of pawpaw fluid

3.2.2 Preparation of meat sample

Meat sample (beef) was collected from different distribution points at Minna central market. The meat cuts were from different parts of the animal. The sample was then taken to the animal/food science production department laboratory where functional analysis was carried out. Analysis of the sample was done after being treated with moderately ripe pawpaw fluid, so both the meat treated with moderately ripe pawpaw fluid and cooked and the one not treated with the fluid before cooking were analysed.

3.2.2.1 Meat sample treated without moderately ripe pawpaw fluid

200g of meat sample was washed with clean water, cut into stripes (pieces) and was placed in a pot containing $1\frac{1}{2}$ (one and half) litres of water and heated for 20 minutes (Woods, 1985).

3.2.2.2 Meat sample treated with moderately ripe pawpaw fluid

The meat sample was washed with clean water, cut into stripes (pieces) and was placed in a pot containing $1\frac{1}{2}$ (one and half) litres of water then $\frac{1}{2}$ (half) teaspoon of pawpaw fluid was added to it and heated for 20 minutes (Woods, 1985).

3.2.3 Chemical analysis of moderately ripe pawpaw fluid

3.2.3.1 Determination of Total Dissolve Solute (TDS)

Total dissolve solid can have different meanings depending on the analytical method used. In water quality, it refers to the amount of residue remaining after evaporation of water from the sample. Materials required for the experiment is dish and lid, (porcelain dish), oven at 100 °C, desiccator. The empty dish was heated in the oven, cooled in a dessicator, and then weighed. 4 g of the fluid was put in the dish and weighed again. The dish was then placed in the oven at 102 °C until the fluid is dry (Lee, 1987).

$$\%\, Total\ solid = \frac{Weight\ of\ residue}{Weight\ of\ milk} \times 100$$

3.2.3.2 Determination of Brix (sugar content) using the Refractometer

The measurement of the approximate amount of sugar to water mass ratio of a liquid. It is measured with the digital refractometer (modern brix meters); it calculates the brix value based on refractive index. The refractometer lens was cleaned with distilled water and a drop of the fluid sample was introduced on the lens.

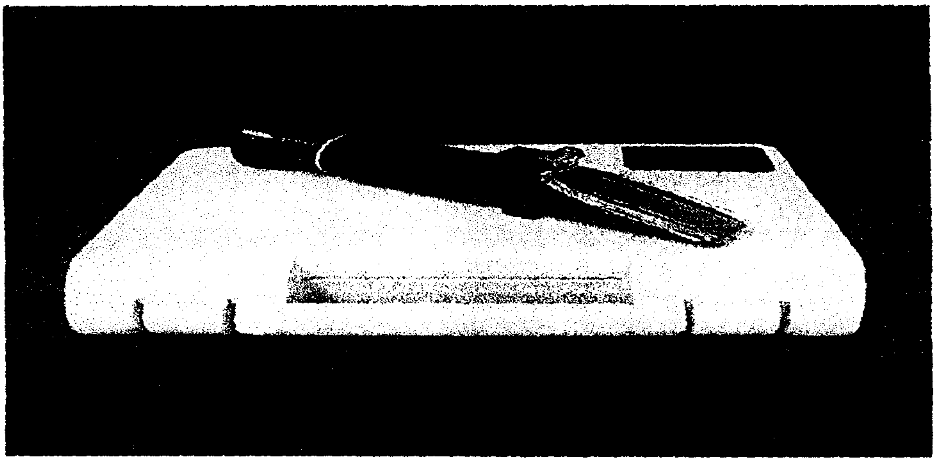

Plate 3.1 Digital Refractometer

3.2.3.3 Determination of pH

The term pH is used to measure the amount of hydrogen ion concentration [H^+] of a solution. It is therefore described as a measure of the acidity or alkalinity of the solution. $pH = \log[H^+]^{-1}$. A digital pH meter was used to determine the pH of fluid, a digital pH meter has a glass electrode probe connected to it, the probe was placed into the fluid sample and the pH of the fluid sample was digitally displaced (Lee, 1987).

3.2.3.4 Determination of Alkalinity

The alkalinity of fluid was determined by titration with a standard solution of an acid to end point. This end point are usually detected with methyl orange (pH 4.5) indicator. 2 g of fluid sample was diluted with distilled water; the mixture was then stirred vigorously, allowed to settle and then filtered. 100 cm^3 of the diluted sample was measured out, 3 drops of methyl orange indicator was added to it, and this was then titrated against 0.02 N of tetraoxosulphate (vi) acid, slowly stirring at the same time with a glass rod until the end point was reached (Lee, 1987)

$$Alkalinity = \frac{Vol.\ of\ 0.02N H_2SO_4}{Vol.\ of\ sample\ used} \times 1000 = \ ppm\ in\ terms\ of\ CaCO_3$$

3.2.3.5 Determination of Hardness

Hardness of fluid was determined by titrating a sample at the correct pH value with the disodium salt of EDTA in the presence of a suitable indicator. Under these conditions, the indicator has a red colour in the presence of Ca and Mg. On addition of EDTA (ethylene diamine tetra-acetate) to the red colour solution the Ca and Mg are converted to their EDTA complexes and when this process is completed the colour of the indicator reverts to blue. 10 cm^3 of the sample was measured, 2 cm^3 of ammonia buffer solution was added, and this was then titrated against 0.02 N EDTA (ethylene diamine tetra-acetate) until the solution in the dish has lost all traces of real colour (Bolaji, 1979).

$$Total\ Hardness = \frac{Vol.of\ 0.02N\ EDTA}{Vol.of\ sample\ used} \times 1000 = \ ppm\ in\ terms\ of\ CaCO_3$$

3.2.4 Functional analysis of meat sample and meat sample treated with fluid.

3.2.4.1 Determination of pH

pH is a measure of the acidity or basicity of a solution. To determine the pH of meat, the sample was grinded; 2g of the grinded sample was then weighed and dissolved in 100ml of distilled water, a digital pH meter was then used to determine the pH of the mixture (Lee, 1987).

3.2.4.2 Determination of crude protein content

Protein content was determined using the macro kjeldahl method as described by the Association of Official Analytical Chemist (AOAC, 2002). The crude protein value was obtained by multiplying the nitrogen value by 6.25. The process involve three (3) processes; digestion, distillation and titration.

i. Digestion: The organic matter was oxidized by concentrated Hydrogen tetraoxosulphate (vi) acid (H_2SO_4) in the presence of catalyst (selenium oxide) and nitrogen is converted to ammonium sulphate.

$$N_2 + H_2SO_4 \xrightarrow{catalyst} NH_4SO_4$$

ii. Distillation: The ammonium sulphate was treated with concentrated sodium hydroxide. Ammonium is liberated and distilled into a standard quality of dilute acid H_2SO_4.

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2NH_3 + 2H_2O$$

iii. Titration: The ammonia in acid is then back titrated with H_2SO_4.

$$Total\ nitrogen\ \%\ by\ weight = \frac{A \times 0.0014 \times 100}{W}$$

Where, $A = Vol.of\ acid\ used\ in\ titration$

$W = Weight\ of\ sample\ used$

$\%\ Crude\ protein = Total\ Nitrogen \times 6.25$

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3.2.5 Sensory Evaluation

Sensory analysis involves the assessment of quality of food products by four of the human senses. Meat cooked with moderately ripe pawpaw fluid and that cooked without the fluid were evaluated for colour, appearance, aroma, texture and overall acceptability using a 7-point descriptive hedonic scale (7 = excellent and 1 poor) as described by Simo, (2008). A panel of 10 untrained judges consisting of randomly selected students of the food science and nutrition Department of Federal University of Technology Minna was used for evaluation. The samples were cooked under the same conditions and equal amounts of spices added to each. The order of presentation of the sample to the judges was randomized and the samples to the judges were randomized and the samples were coded to cover their identity.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Presentation of Result

The results of the chemical composition of moderately ripe pawpaw fluid, meat and meat treated with moderately ripe pawpaw fluid are shown in table 4.1 --- 4.4

Table 4.1: Chemical analysis of moderately ripe pawpaw fluid

S/n	Parameters	% composition
1	Total dissolve solute (TDS)	39.4
2	Brix (Sugar content)	3.8
3	pH	6.0
4	Alkalinity	25ppm (part per million)
5	Hardness	163ppm (part per million).

Table 4.2 Functional analysis of meat

S/n	Parameters	% composition
1	pH	6.11
2	Crude protein content	15.77

Table 4.3 Functional analysis of meat treated with moderately ripe pawpaw fluid

S/n	Parameters	% composition
1	pH	6.12
2	Crude protein content	31.5

Table 4.4: Sensory evaluation of meat (control) and meat treated with moderately ripe pawpaw fluid using t-test

Sample	Sensory Evaluation				
	Appearance	Flavour	Taste	Aroma	Texture
Meat treated with Papain	5.80±0.422[a]	4.90±0.316[a]	5.00±0.667[a]	6.00±0.000[a]	6.80±0.422[a]
Meat treated without papain (Control)	5.60±0.516[a]	5.00±0.817[a]	5.10±0.737[a]	4.60±0.843[b]	4.90±0.738[b]

Values with the same subscript are not significantly different

The Aroma of the meat cooked with moderately ripe pawpaw fluid differed statistically ($p<0.05$) from that treated without moderately ripe pawpaw fluid.

The texture of the meat cooked with moderately ripe pawpaw fluid differed statistically ($p<0.05$) from that treated without the fluid.

No difference in appearance, flavour and taste.

4.2 Discussion of Results

From the experiment carried out, moderately ripe pawpaw fluid has a Brix (sugar content) content of 3.8%, which is relatively fair depending on the thickness of the unripe pawpaw fluid juice and is comparable with papaya Brix content (11.2); amounts vary considerably depending on the cultivar and agronomic conditions. The average African cultivars have higher sugar content (10–10.2% TSS) than the papaya cultivars being grown in the Nigeria (5.65–7.1%) (James, 2006).

The Total dissolve solid (TDS) of moderately ripe pawpaw fluid is 39.4% which is relatively high when compared to the TDS of local tenderizing agent in Nigeria, i.e. the solid content that is soluble and is measured as total solids content minus the suspended solids. Moderately ripe pawpaw fluid dissolves faster in water compared lime. Total dissolved solids are normally only discussed for freshwater systems; the principal application of total dissolve solids is in the study of water quality.

The pH optimum of moderately ripe pawpaw fluid varies with the nature of the substrate. In general, the pH optimum range for moderately ripe pawpaw fluid concentrate is pH 5.0 to 7.0, moderately ripe pawpaw fluid concentrate demonstrates optimum stability over the range of pH 5.0 to 9.0 at 60 to 70°C. In solution, values below pH 3.5 and above pH 10.0 rapidly inactivate the enzyme (Shadaksharaswamy and Shakuntala, 2006). The pH obtained (from moderately ripe pawpaw fluid) is 6.0.

Alkalinity is the measure of alkali, i.e. the concentration of alkali in a solution, measured in terms of pH. The alkalinity of moderately ripe pawpaw fluid is 25ppm; in the natural environment carbonate alkalinity tends to make up most of the total alkalinity due to common occurrence and dissolution of carbon dioxide in the atmosphere.

Hard water is water that has high mineral content (mainly calcium and magnesium ions) and sometimes other dissolve compounds such as bicarbonates and sulphate. The degree of hardness of moderately ripe pawpaw fluid is 163ppm,

The pH obtained (from meat) is 6.11 compared to 6.12 (meat treated with moderately ripe pawpaw fluid), the pH value of meat provides evidence as to: how long it will keep; technical processing characteristics. The flesh of animals prior to slaughter has a pH value of 7.1, after slaughtering some of the glycogen in the meat turns into lactic acid. As a result, the pH is lowered. Beef normally reaches its lowest pH value of 5.4 to 5.7 at 18-24 hrs after slaughter. After the lowest pH level is reached, the pH starts to rise again slowly but steady. By the time it reaches pH of 6.5 it starts to decompose.

Meat has a protein content of 15.77% compared to 31.5% (meat treated with unripe pawpaw fluid) determined from the experiment, protein is an important nutritional parameter needed and essential to man and animal. Proteins are made of amino acids Essential Amino Acids (EAA) cannot be synthesized by humans and must be supplied by the diet.

Pioneer nutritionists noticed very early in their studies on rats that the administration of ertain combinations of these proteins kept the animals in good health. On the other hand, rotein utilization or protein efficiency can be evaluated by standard procedures of nitrogen alance, growth and chemical score. Nitrogen balance refers to the established concept that trogen intake approximates nitrogen elimination in healthy adults animals and that such intake ightly exceeds output in developing animals, growth in context with dietary protein evaluation, fers to the ratio of grams gained in to a procedure whereby the quality of a protein is judged in rms of its ability to provide an organism with essential amino acids. The amount of protein eeded by the body depends primarily upon the particular need of the cells for the essential mino acids.

From the questionnaire disibuted for the sensory evaluation of meat cooked without ıoderately ripe pawpaw fluid and 't cooked with unripe pawpaw fluid it was observed that the verall acceptability of meat cod without moderately ripe pawpaw fluid was average. owever, the overall acceptabilityneat cooked with moderately ripe pawpaw fluid was above erage, i.e. higher than that coordinarily without moderately ripe pawpaw fluid.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In conclusion, the research study showed that the brix (sugar content) of moderately ripe pawpaw fluid is on the average '3.8'; likewise it's pH, alkalinity, hardness and the rate at which it dissolves in universal solvent (water) are 6.0, 25 ppm, 163 ppm and 39.4 respectively. The study also showed that meat that has been cooked with moderately ripe pawpaw fluid is generally more acceptable than the meat that was cooked ordinarily without moderately ripe pawpaw fluid.

5.2 Recommendations

The results obtained after the research work necessitate the following recommendations

1. Although there was no significant difference statistically in appearance, flavour and taste of meat treated with moderately ripe pawpaw fluid and meat treated without moderately ripe pawpaw fluid, the use of moderately ripe pawpaw fluid as a meat tenderizer should be encourage.
2. More research work should be done in providing a means of preserving moderately ripe pawpaw fluid, before drying as this determines it's quality
3. Laboratory equipments should also be provided in the department to reduce the cost of carrying out experiment in other laboratory within and outside the school.

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