Studies on the production of value-added products from the pineapple plant (*Ananas comosus*) agro-waste

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in

Biochemistry

Submitted by

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Under the guidance of

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on the production of value-added products from the pineapple plant (*Ananas comosus*) agro-waste**" submitted to University of Calicut, as partial fulfilment of the requirement for the award of the degree of Doctor of Philosophy in Biochemistry by **Rinju R**., embodies the results of bonafide research work carried out by her under my guidance and supervision at the Department of Life Sciences, University of Calicut. This thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Calicut University   **Dr.B.S.Harikumaran Thampi**
Date: (Research Supervisor)

DECLARATION

I hereby declare that the work presented in the thesis entitled "**Studies on the production of value-added products from the pineapple plant (*Ananas comosus*) agro-waste"** submitted to the University of Calicut, as partial fulfilment of the requirement for the award of the degree of Doctor of Philosophy in Biochemistry, is original work carried out by me under the supervision of Dr. B.S.Harikumaran Thampi, Associate Professor in Biochemistry, Department of Life Sciences, University of Calicut. This has not been submitted earlier either in part or full for any degree or diploma of any university.

Calicut University   **Rinju R**
Date:

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*Above all, I am forever indebted to my dearest **parents** for their love, sacrifice and care throughout my carrier and for giving me the freedom to realize my own interests. This accomplishment would not have been possible without them. Words fall short to thank my **siblings** and their family for their unconditional love and encouragements. I thank my **parents-in-law** for their cooperation and support especially in the last stages of my work.*

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Rinju R

Dedicated to
My Family

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LIST OF ABBREVIATIONS

ANOVA	:	Analysis of Variance
BAPNA	:	Benzoyl-DL-Arginine-P-Nitroanilide
C^{13} CP/MAS NMR	:	^{13}C Cross-Polarization, Magic Angle Spinning Nuclear Magnetic Resonance
DSC	:	Differential Scanning Calorimeter
FTIR	:	Fourier Transform Infrared Spectrometer
GC-ECD	:	Gas Chromatography with Electron Capture Detector
GC-FPD	:	Gas Chromatography with Flame Photometric Detector
HCN	:	Hydrogen Cyanide
RBC	:	Red Blood Cells
rpm	:	Revolutions per Minute
SEM	:	Scanning Electron Microscopy
TBS	:	Tris Buffered Saline
UV	:	Ultra Violet
WAXS	:	Wide-angle X-ray Scattering
XRD	:	X-ray Diffraction

Chapter 1

INTRODUCTION

1. INTRODUCTION

Food can be defined as any nutritious substance that people or animals can eat or drink in order to maintain their life and growth. Food may be from plant or animal origin and primarily consist of protein, carbohydrate, fat, vitamins and minerals for generating energy essential for the vital processes, building blocks and protection of the living beings. Food production is the transformation of raw ingredients into food for the consumption of living beings, mainly for human use. It involves several scientific approaches including the selection of crops, their cultivation, harvesting, nutritional screening, preservation, adding ingredients in correct proportions if needed and presenting. Food distribution is the process of supplying of food materials to the consumers. Distribution of food is a difficult task compared to other materials as the quality of food products are continuously changing throughout the supply chain. So we must care about the quality, health, and safety requirements of food materials [1]. Food insecurity refers to the lack of reliable access to sufficient quantity of safe, affordable and nutritious food for normal growth and development of all life forms. People will be food secure only when the food is regularly available in sufficient quantities. That may be homegrown, locally grown or imported from elsewhere. Availability, access, utilisation and stability are known as the four pillars of food security. The first three must be maintained consistently and is called the 'stability' which is the fourth pillar [2]

Global report on food crises 2018 reported that in 2017, almost 124 million people across 51 countries and territories faced food crisis and required urgent humanitarian action. In 2016, it was 108 million across 48 countries. More than 3 million food-insecure people lived in Latin America and the Caribbean while 3 million in South Asia [2]. Gabriella Nunes da Costa in 2017 reported that around 800 million people are still affected by hunger across the world. This situation will be more severe in the future as the world's population is projected to reach 9.6 billion people by 2050 [3]. So it is clear that the population growth is the main reason for food crisis in the world. Next reason is the price of crude oil in the international market. Price of crude oil and essential commodities are closely related. Crude oil price significantly affects the transportation and production processes. As a result of this, the food price will increase. Crude oil price will lead to the use of bio-fuel as an alternative source. It is produced from the oil seeds, food grains and also from food products. It was reported that in 2007 the US utilized 20 % of corn for the production of bio-fuel and in 2016, it was 32 %. In China, rice and wheat were also used apart from the corn for ethanol production. As a result of this, the supply of food grains in the global market has been reduced markedly and the food prices increased gradually. Industrialization also causes food crisis by decreasing the food grain production as the agricultural lands are widely used for non-agricultural purposes. Excessive agricultural practices also have an adverse effect. It reduces the fertility of the land, and the excessive use of pesticides, chemical fertilizers, insecticides, adds to existing problems. Natural calamities are also

responsible for the food crisis at the global level. Food security of the present situation cannot be maintained by destructing the environment by excessive agricultural practices, as it affects the food security of future generations. For surviving these situations, there is a tendency to introduce genetically modified crops to the developing countries by multinational corporations. But they are harmful to the environment and eventually lead to the hampering of the agricultural developments in developing countries. Water scarcity is also a major problem for the food crisis as a huge amount of water is needed for various agricultural practices [4].

The situation in India is also alarming; the main reason for the food crisis in India is population increase. It was reported that, since 2003-2008, India's population increased by 8 % while the increase in food grain production was only by 5 % [4].
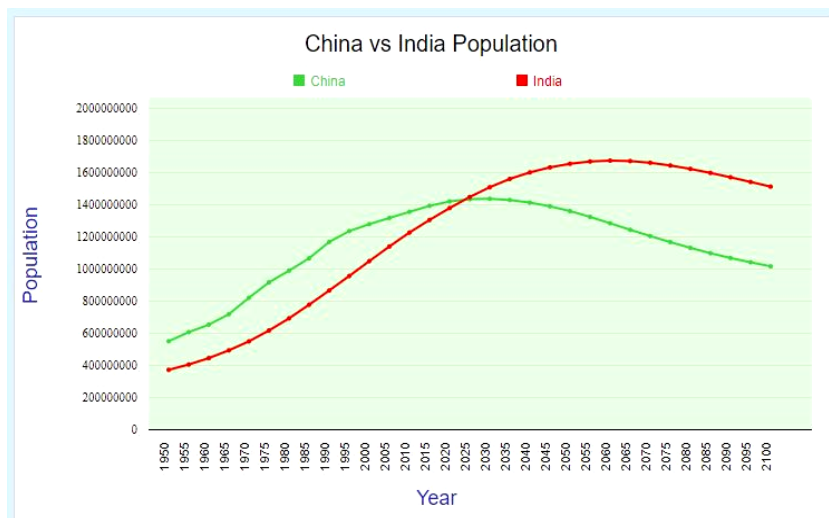
Figure 1.1: Comparison of population hike in India with China from year 1950-2100 [6].

It is estimated that the population will increase to 1.3 billion in 2020. To feed the large population we need millions of tons of food grain. It was reported that by 2020, India would require 343.0 million metric tons of food grains for feeding its whole population [5].

1.1 Food-feed-fuel competition-a major reason for food insecurity?

The impact of the "food-feed-fuel competition" on food scarcity and hunger has been a major concern worldwide[3]. The reasons are, increase in purchasing power, which will lead to an increase in meat consumption, resulting in the rise of demand for animal feed for more meat production. Urbanization and modern lifestyle will also cause higher demands for meat products[7]. Environmental impacts caused by an increase in the production of meat and meat products are more challenging [3].

Further, demand for meat products will increase the market price of meat products, which will lead to higher demand for animal feed and cause high grain price and will shift the use of grains from food to feed which in turn leads to a situation like the use of food grains for feed use, which will in turn increases the food price. This situation will affect more to weaker sections in the society than middle and upper parts because they use more grain directly as food compared to the other classes[7]. Considering the increasing demands for feed, food crops are diverted for the production of animal feeds (Figure 1.2).

Figure 1.2: Situation leading to the conversion of food crops into animal feeds

Ethanol and biodiesel-the two primary bio-fuels are mainly derived from grain, sugar and oilseeds. It was reported that the consumption of agricultural commodities for the production of bio-fuels is expected to continue for the next ten years [8]. There is a tendency of changing the utilization of grain crops. To substitute fossil fuels with bio-fuels, grain utilization in this direction increased and it

makes new level of competition between food and fuel [7]. United States, world's largest producer of ethanol, produced around 16 billion gallons (around 60 billion litre) in 2017. In United States, corn is the main source of ethanol production [9].

Food vs. Fuel debate has actually emerged after the food crisis of 2007 and 2008, which generated the fear of the competition made by the bio fuel production from food crops [10]. Yotopoulos reported in his article, that there is a relationship between grain demands, population, income and demand for food and expressed in an equation

$$D = N + e.y$$

*(*where D= the growth rates of the total demand for grains, N= population *and* y=per capita real income over time, e=elasticity of food demand with respect to income) [11,7].

The Food-Feed Competition
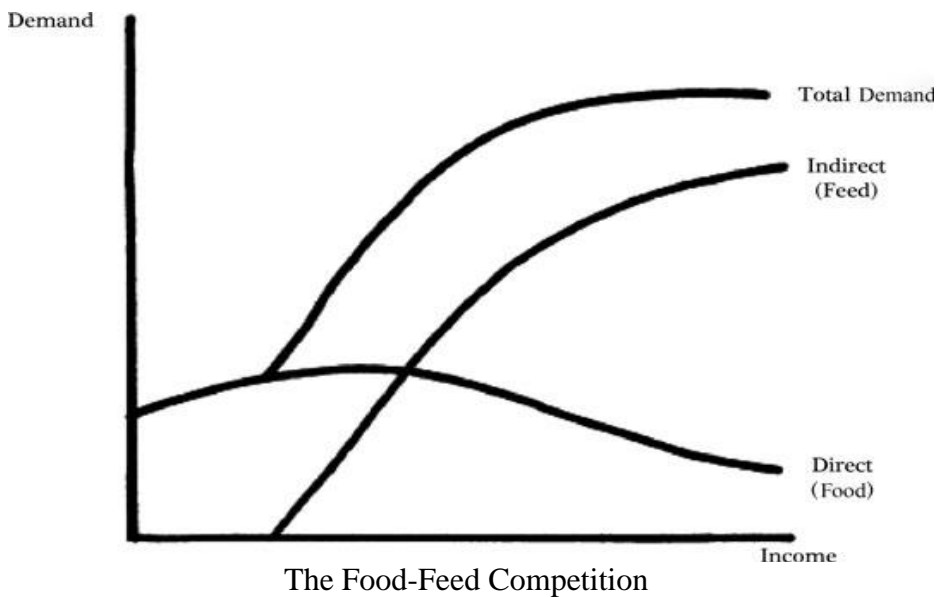
Figure 1.3: Food-feed competition with respect to demand [11,7].

Banerjee in 2011 elaborately explained multiple levels of competition to food from bio-fuels. It can be observed in the stage of bio-fuel production. Higher demand for bio-fuel can generate competition for arable land for the production of grain as well as non-grain crops. Utilization of more land for crop cultivation will lead to the degradation of natural habitat and loss of forests. This issue already happened in the case of Brazilian natural forests in Cerrado and in the Southeast Asian rainforests, which is at the risk of being destroyed. The destruction of natural habitat can also adversely affect the food security of ethnic communities. Their lives are depending on forest resources. Article of Banerjee mainly focused on the competition for food crops (mainly corn) caused by the extensive production of bio-fuels from grain crops [7].

Worldwide production of primary bio-fuels is given in table 1.1 and table 1.2.

Table 1.1: Worldwide ethanol production in 2019 [12].

Country	Ethanol Production (Billion Litres)
United States	59.80
Brazil	32.63
European Union	5.45
China	3.41
India	2
Canada	1.89
Thailand	1.6
Argentina	1.1
Rest of world	2.27

Table 1.2: Worldwide biodiesel production in 2018 [13].

Country	Biodiesel Production (Billion Litres)
United States	6.9
Brazil	5.4
Indonesia	4
Germany	3.5
Argentina	2.8
France	2.2
Spain	2
Thailand	1.6
Italy	1.4
China	1
Poland	1
Netherlands	0.7
United Kingdom	0.5
Canada	0.4
India	0.2

1.2 Sustainable food production

The issue of food-feed-fuel competition has been carefully assessed. Several reports are explaining the use of by-product sources for the animal feed industry. Sindhu et al., in 2002, reported the use of agro-industrial by-products as a protein supply for livestock. They have used sugar industry by-products, animal by-products, fruit and vegetable by-products., forests by-products., marine waste and aquatic plants by-products. These by-products contain several anti-nutrient

factors, and they have explained the ways to reduce them also [14]. Ajila et al. in 2012 studied the use of agricultural and food-industry residues for the production of animal feed after processing. These by-products constitute a significant proportion of agricultural output [15].

In contrary to this, an appreciable step by researchers, aiming with a sustainable approach for the production of bio-fuels from non-food crops or from agro-waste has emerged. It was reported that several studies are showing the use of non-food resources for the production of biofuel. In 2016, Fradj et al. explained the potential of perennial crops like miscanthus (a grass plant) for the production of bio-fuels instead of food or feed crops [10].

Considering the alarming threat to food security, the world needs to increase the agricultural productivity, either by increasing the crop area, using scientific methods of agriculture or maximum utilization of existing food crops by reducing the loss. But in reality, we cannot increase land under agriculture due to the high demand for property for other anthropogenic activities. Next is adopting new scientific methods in agriculture to boost productivity. But it may not be cost-effective for small and medium level farmers who are the primary food grain producers of the world. In this situation, it is clear that the maximum utilization of existing crops and agro-waste will be a sustainable solution.

Agro-waste is the material left after each agricultural activity. A large amount of such waste is produced after cultivation of various crops. In earlier times, they were either burnt or naturally converted

into organic fertilizers. Nowadays, the situation is changing, and researchers realized the potential of agro-waste for making value-added products. There are several studies reported on the effective utilization of agricultural residues. Daifullah et al., in 2003, reported that the agro-waste, the rice husk could be effectively utilized as sorbent materials to absorb toxic metals from the environment [16]. Suthar in 2008 communicated the utilization of the post-harvest residues from wheat, millets and pulse crops for vermicomposting by using the earthworm-*Eudrilus eugeniae* Kinberg, and explained that crop residues act as an efficient culture media for the production of this earthworm. This method would be useful for keeping the fertility of agricultural lands [17]. Chaudhary et al. 2012 explained the potential utilization of agro-waste for the production of composite boards by a flat press process. According to them, husk and shell of coconut, hull, husk, the ground of coffee, stalks, and leaves of corn, stalks of cotton, hulls of nuts, shells of peanuts, hull/husk, stalks and straw of rice, bagasse of sugarcane could be utilized for this purpose [18].

Lim and Matu, 2015 reported a study related to the production of biofertilizer from agro-waste. They have explained a cost-effective solid-state fermentation method to produce biofertilizer using agro-waste from watermelon, papaya, pineapple, orange, and banana [19]. Harshwardhan and Upadhyay communicated that it is necessary to develop new technology to increase the utilization and economic values of agricultural waste products. They have reported that there are several studies relating to the use of biomass in the manufacture of brick making, in the biogas industry and as filler in asphalt mixing [20].

Omo-Okoro et al., 2018 thoroughly reviewed and studied the use of agricultural waste like wood bark, sawdust (powdery particles of wood produced by sawing), nuts, shells and husks of crops and plants, chitosan, starch and cellulosic fibres of crops and plants for the adsorption of pollutants (per- and poly-fluoroalkyl substances (PFAS) from aqueous solutions [21].

Zamzuri and Abd-Aziz, 2013 reported the production of biovanillin from agro-waste like cereal bran, sugar beet pulp, rice bran oil, and palm oil biomass which contains ferulic acid [22]. Biovanillin is a widely used flavour compound in the foods, beverages and pharmaceutical industries. Ferulic acid is a potential precursor of biovanillin, and microbial fermentation of ferulic acid could yield biovanillin [22]. Coffee pulp and husks could be bio converted into livestock feed and other products like enzymes, organic acids, flavour and aroma products. Solid-state fermentation is the method widely used for this bioconversion. Some anti-nutritional factors like caffeine and tannin are limiting the use of coffee pulp as animal feed. Removal or reduction of these factors could make coffee pulp as the best feed ingredient [23]. Pandey et al. 2000 reported the production of animal feed, organic acids, amino acids, enzymes, and the compounds of pharmaceutical importance from the sugarcane bagasse. In this review, they explained that the sugar cane bagasse could make as a substrate for enzyme production and protein enrichment using microorganisms. Pre-treatment of the sample would enhance the microbial action. Pre-treatment of sugar cane bagasse include gamma radiation, treatment with alkali, hydrogen peroxide and solvents [24].
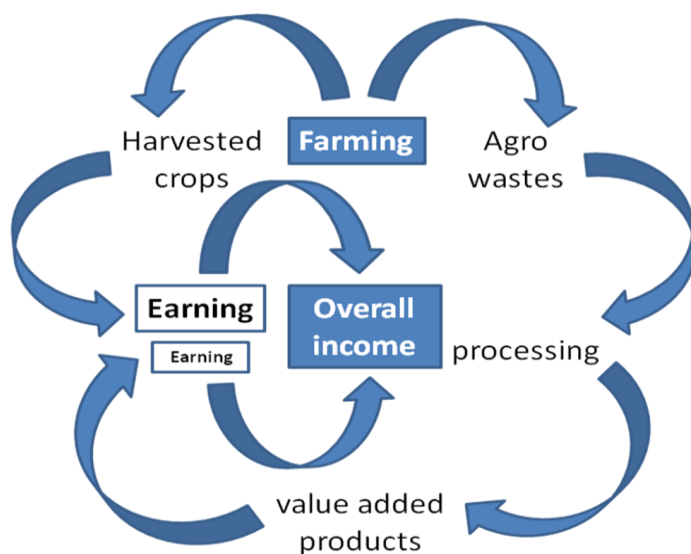
Figure 1.4: Flowchart showing sustainable agriculture by effective utilization of agro-waste

1.3 The pineapple plant

The pineapple plant *(Ananas comosus)* is a herbaceous perennial plant, and the main pineapple growing countries are Brazil, Thailand, Philippines, Costa Rica, China and India. Pineapple is cultivated for about 9,09,840 hectares in the world, in India, it is 89,000 hectares, and in Kerala, it is 10,200 hectares [25].

Pineapple cultivation originated in Brazil and spread to other parts. In AD 1548, Portuguese started it's farming in India. The planting is suitable in the humid tropical areas with an optimum temperature of 22 °C-32 °C. The high temperature at night is harmful to its growth. Pineapples can be cultivated in high rainfall and humid

coastal regions of south India as well as in hilly regions of north-eastern areas of India. Mainly cultivated varieties are Kew, Queen, and Mauritius. Sucker, slip, and crown portions are the main planting materials. Pineapple produced by tissue culture can also be used. Planting season varies with each state. The planting seasons are August-October is in Assam, April-June in Kerala and Karnataka, October-November in the northern part of West Bengal, and June-July for other regions. Planting density is also differed according to the climatic conditions. Planting density of 63,400 plants/ha is suitable for subtropical and humid conditions, 53,300 plants/ha in hot and humid conditions, 31,000 plants/ha in hilly areas in north-eastern states. Dipping the suckers in Bordeaux mixture can be used to control pests before planting. Pineapple can be harvested during May-August under normal environmental conditions [26]. Those solid waste that result from agricultural practices of pineapple plant are the pineapple agro-waste. The fruit is the only used part of the pineapple plant and other portions mainly stem and leaf remains as waste in the farm field.

Upadhyay et al. in 2010 reported the importance of pineapple waste for the production of useful products like bromelain, dietary fibres and phenolic antioxidants. They also explained that with the help of novel scientific and technological methods, we could produce many valuable products from pineapple fruit waste [27]. Adrizal et al. in 2017 studied the use of pineapple waste (fruit waste) as poultry feed. They enhanced the nutritive value of pineapple waste by fermentation [28]. Large amounts of waste are generated from pineapple farm through agro-industrial activities each year, and these materials were

underutilized and disposed off in the environment without any treatment, or burnt, leading to environmental pollution problems. This agricultural waste can be bio-converted into value-added products like starch and animal feed. For assessing the specific application of starch in various industries, we have to characterize the isolated starch.

1.4   Characterization of starch

The starch industry is a major one among the various technological developments in the world, and its use and demand continue to expand in many areas with substantial economic value. In India, the demand for starch is continuously growing. It is mainly used in food, textile, paper and pharma sectors as sweeteners, thickener, binder, adhesive, stiffening agent and texture maintainer. Among the various starch application sectors, the food sector is the dominant one [29].

In India, the main starch source is the corn. Wheat, cassava, sweet potato, and potato are the other sources. It is a widely available, naturally occurring carbohydrate reserve in plants, and considerably significant biopolymer for humans. Starch is deposited in plant tissues as insoluble, semi-crystalline granules, with size ranging from 1-100 μm [30]. It consists of glucose units linked through α-D-(1–4) glycosidic bonds. Starch consists of two molecules – the linear amylose (usually 10–38 %) and the highly branched amylopectin. The proportion of amylose and amylopectin varies according to the botanical source of the starch. Starch in its natural form is inert, white in colour, tasteless, odourless, and insoluble in solvents like cold water, ethanol, ether and

acetone [31]. Starches can differ with respect to the amylose content depending on their origin or can be structurally modified [32].

Functional properties like water absorption, gelatinization, pasting, retrogradation, and the susceptibility of enzyme attack largely depend upon the amylose content, branch length and position of branches in amylopectin. These properties influence the overall starch behaviour during processing stages. Native starches are highly variable depending upon the botanical source used. This variability can be seen in granule morphology, crystallinity, the content of amylose and amylopectin and amylopectin architecture. Starch structure variability is due to the diversity in genes that encode the enzymes of starch biosynthesis. Environmental factors also influence it [30].

Recently more attention has been focused on the production of starches from different botanical sources for meeting the increasing demands of starches in various industries. There are reviews explaining the properties of starches from new sources like tubers and roots (yams, sweet potato, cassava, different types of arrowroots, ginger, cocoyam, yam bean, taro starch and tacca starch), grains and cereals (*Digitaria* genus, millet, sorghum and pigeon pea) and fruits (plantain starch, *Ensete ventricosum*, breadfruit and sago palm) [31]. But there were a few studies focused on the production of starches from farm waste like pineapple plant parts. High potentials are expected in this agro-waste for the production of starches and starch-based products. The focus of this work is also to check whether starch from new sources can provide starch with unique properties for specific applications.

This type of research can add value on these neglected, underutilized crop parts and will be very useful for minimising the overexploitation of food crops for the production of starch-based products and the efficient utility of croplands. Starch can be extracted from the plant parts by simple grinding and wet separation. Then the sediment starch can be washed with water to obtain the clean starch [31].

Modification of extracted starch is generally including chemical treatments like acid or enzymatic hydrolysis and oxidation of starch or physical treatment of starch using heat or moisture. Chemical modifications markedly altered physicochemical properties-profoundly alters their gelatinization, pasting and retrogradation behaviour [33]. Starch modifications are also used to eliminate undesirable starch properties and making them more suitable for particular demands [31]. The starch modification will enhance its versatility and satisfy consumer demands. Thus, the various chemically modified starch derivatives can develop a variety of fabricated food products having varied texture and mouthfeel. The modified starch derivatives are the products of either glycosidic bond cleavage or forming new functional groups or substitution of free available hydroxyl groups or bridging of molecular chains by cross-linking reactions [34].

Characters of starch from genetically modified potatoes were studied by Kortstee et al. 1998 and observed that branches in starch structure could affect their physicochemical properties [35]. Characteristic features of native and differently modified (oxidised, acetylated and acid-thinned) cocoyam (*Xanthosoma sagittifolium*) starch were studied by Lawal in 2004 [36]. Characteristics of genetically

modified potatoes, having different amylose/amylopectin ratios were studied by Karlsson et al. in 2007 [37]. Diop et al. 2011 studied the acetyl modification on starches and observed that starch properties were improved by this modification [38]. A study on the influence of moisture content on the degradation of waxy and normal corn starches treated by acid in methanol was carried out by Lin et al. in 2012 and their result indicated that moisture content has stronger influence on the functional properties of both starches [39]. Odeku and Akinwande, 2012 communicated that acid modification of yam starches increased their disintegrative efficiency in paracetamol tablet formulations [40].

Starch is a macro-constituent of several foods and has many industrial applications [30]. There is a need for developing new starch-based products with suitable properties to meet the demands of novel formulations. In food and pharmaceutical industries, starches are used as stabilizers, fillers, glidants, thickeners, binders, disintegrants, gelling, bulking, texturizing material, film-forming, encapsulation, shelf life extension material and as water retention agents. Starch is considered as the most used excipient in pharmaceutical formulations [41]. Odeku in 2012 reviewed the current knowledge on the use of starches as pharmaceutical excipients which were extracted from tropical plants (yams, sweet potato, xanthosoma, cassava, cocoyam, taro starch, yam bean, canna, Indian arrowroot, West Indian arrowroot, tacca starch, sago palm and breadfruit starch) and concluded that, there was an increasing demand for starches in drug industries as excipients with suitable properties [31]. Starch has a significant role in providing texture and sensory properties of processed foods [34]. Non-food

applications include in the textile industry (wrap-sizing), paper industry (surface sizing material), as adhesives and for the production of biodegradable plastics [42].

Characterization studies of starch include molecular, granular, thermal and rheological levels.

1.5 Molecular characterization of starch

Svensson and Eliasson, 1995 investigated the crystalline changes in cereal and tuber starches using WAXS (wide-angle X-ray scattering) and observed that the crystalline properties of starches are strongly affected by the amount of water available during gelatinization [43]. X-ray diffraction (XRD) data of starches from three cultivars of Chinese Baizhi (*Angelica dahurica*) were studied by Zhou et al. in 2010 and reported that these Baizhi starches exhibited B-type XRD pattern in their granular structure [44]. Yu et al. 2013 reported that the starch present in the rhizome of lotus cultivar ''Meirenhong'' exhibited C-type crystalline structure, while starch in the rhizome of ''Wawalian'' cultivar showed A-type crystalline nature [45]. Molecular organization in starches (rice, maize, waxy maize, amylomaize and potato starches) were studied using the ^{13}C cross-polarization, magic-angle spinning nuclear magnetic resonance (C13 CP/MAS NMR) technique by Gidley and Bociek, 1985 and concluded that this technique is an important tool in studies of starch structures which will help to find out the specific application of starches [46]. Graaf et al. 1995 published a paper regarding the ^{1}H-NMR spectroscopy of acetylated and hydroxypropylated starch and commented that the ^{1}H-NMR

spectroscopy is a time saving analytical method for starch characterization [47].

Characterization of irradiated starches by using Fourier Transform Raman (FT-Raman) and Fourier Transform Infrared (FTIR) spectroscopy were studied by Kizil et al. in 2002. These methods can be used for rapid characterization of irradiated starch samples. Their results showed that irradiation of starch leads to the breakage of glycosidic linkages. These depolymerised starches can be effectively studied using FTIR and FT-Raman spectroscopy [48]. Properties of starch like retrogradation can be effectively studied by FTIR technique. Soest et al. 1994 communicated the use of this technique in the study of starch retrogradation. Their observations indicated that the structural changes in starch occurred by retrogradation were easily distinguished by the FTIR technique [49].

1.6 Granular characterization of starch

Starch granules from different botanical sources have different granule morphology. Scanning electron microscopy (SEM), light microscopy and the particle size analyser can be effectively used to study the granular properties of starch molecules. Several studies have reported about this granular property. Singh et al. 2004 reported the properties of corn and potato starch granules and explained that these starches have different granule morphology. In potato starch, there is a wide range of granule size observed (15-20 μm for small and 20-45 μm for large potato granules). Corn starch granules were smaller than potato starch granules (5-7 μm for small 15-18 μm for large granules).

Granule surface of potato starch was smooth, oval, irregular in shape and corn starch have more rigid granules compared to potato starch granules [42].

Lawal in 2004 studied the granular properties of native and modified cocoyam starch using scanning electron microscopy and light microscopy. This study observed that the cocoyam starch has round and polygonal-shaped granules with the size of 15-40 μm [36]. Stevensson et al. in 2007 have reported a study dealing with the structural changes of starches by dispersing them in the ionic liquid-1-butyl-3-methylimidazolium chloride. Scanning electron microscopic analysis of these modified samples showed clumps of particles with a smaller particle size (<1 μm in diameter) [50]. Scanning electron microscopic studies of starches from Chinese Baizhi (*Angelica dahurica*) cultivars were studied by Zhou et al. in 2010 and communicated that these Baizhi starch granules have an irregular polygonal shape with the granular size of 1-10 μm [44].

Morphological analysis of *Anemone altaica* starch (a Chinese medicinal plant-rhizome variety) was reported by Man et al. in 2012 and observed that the granules of *Anemone altaica* starch were mainly oval-shaped, with a size of 6.25 μm long axis length and 5.24 μm short axis length [51]. Pascol et al. in 2013 communicated the granular characterization of *Solanum lycocarpum* fruit starch using scanning electron microscopy. The granule surface of that fruit starch was smooth, heterogeneous in size with conical appearance [52]. Granular properties of starch present in rhizomes of two lotus cultivars were reported by Yu et al. in 2013. According to them, Meirenhong sample

has granule size ranged from 33.3-70.1 μm in length and 16.9-28.2 μm in width. Wawalian sample ranged from 33.9-92.7 μm in length and 14.6 to 29.3 μm in width [45]. Wei et al. 2018 isolated and characterized starch from the stem of the cassava plant and reported that the granular properties of cassava stem starch were similar to cassava root starch. Both have oval-shaped granules with a flat surface on one side [53].

1.7 Thermal and rheological characterization

Differential scanning calorimetric analysis (DSC) was used to study the gelatinization behaviour of corn, potato, acid modified corn, smooth pea, and various legume starches, which was reported by Biliaderis et al. 1980 [54]. Hoover et al. in 2003 published the differential scanning calorimetric analysis of starch from six cultivars of oat grains and observed that those starches were significantly varied in their thermal properties [55]. Singh et al. in 2003 studied the rheological properties of corn, rice, wheat, and potato starches and reported that the rheological properties-storage modulus (G') and loss modulus (G") increase to a maximum and then drop during the heating process. Potato starch showed the highest peak for G', G" than other starches during the heating cycle [56].

Thermal characteristics of normal, waxy and high amylose wheat starches were determined and compared by using a differential scanning calorimeter. The result indicated that the waxy wheat starch (amylose-free) has high gelatinization temperature [57]. Properties of corn starch were studied using several techniques, including thermal properties after the modification (acid hydrolysis). A considerable

decrease in gelatinization enthalpy was observed, which was reported by Beninca et al. in 2008 [58]. Liu et al. 2010 communicated that the progress of retrogradation could effectively be studied by a differential scanning calorimeter, and they have reported a study on waxy and normal corn starches. They observed that the amylose content gets interfered with the reformation of amylopectin double helices and decreased the retrogradation process [59].

Ahmed and Auras in 2011 observed the effect of acid hydrolysis on the thermal and rheological properties of lentil starch. According to them, the peak gelatinization temperature shifted to a higher value, and the gelatinization enthalpy is not changed by acid hydrolysis. Rheological properties were greatly affected as the gel strength of lentil starch become weaker by acid hydrolysis [60]. Physicochemical, thermal, and rheological properties of acid-hydrolyzed sago (*Metroxylon sagu*) starch was studied by Abdorreza et al. in 2012 and explained specific applications of hydrolyzed sago starch [61]. Man et al. 2012 reported the DSC analysis of a rhizome variety (*A. Altaica*) starch and observed that this particular starch has the lowest gelatinization temperature which indicated that less energy was required to initiate the gelatinization of *A. Altaica* starch. They also reported that the gelatinization enthalpy is affected much by the granule shape and relative granule crystallinity [51]. Yu et al. 2013 have done the gelatinization studies on starch isolated from the rhizome of different lotus cultivars. They have observed that the gelatinization temperatures of starch in Meirenhong and Wawalian cultivars were 330.5 and 342.4 K, respectively and the gelatinization temperature

range for Meirenhong sample was 20.8 K, while that of Wawalian sample was 13.0 K [45].

As agricultural waste management, in addition to starch, research is required to use this pineapple agro-waste for the production of value-added products, for example, as a component of cattle feed. For this, we have to assess the proximate and anti-nutrient content present in it.

1.8 Proximate and anti-nutrient analysis

According to H. Bennett, proximate analysis can be defined as the "determination of a group of closely related components together, e.g. total protein, fat." [62]. Proximate analysis generally includes the determination of moisture, ash, crude protein, crude fat, crude fibre, and total carbohydrates. The proximate and anti-nutrient contents of plants are highly variable as it highly depends upon the variety, climate, soil, processing methods, pesticides, fertilizers, and other environmental conditions [63].

Anti-nutritional factors are naturally occurring chemical compounds generated in plants by the normal metabolism and act to reduce nutrient intake, digestion, absorption and utilization. Many such plant substances have the potential to make harmful effects on the productivity of farm animals [64]. The higher level of anti-nutrient factors will reduce the full utilization of such plants. Anti-nutrient factors either directly or indirectly (through their metabolic products) adversely affects the feed utilization and nutrient uptake and their proper digestion and absorption. These factors are largely present in

many plants that are used as feed or feed ingredients. They can also be called as plant secondary metabolites and are highly biologically active and very harmful [64]. Plants produce them for their defence mechanisms against fungi, insects and predators. The toxicity due to these factors is reported to be prevalent in farm animals[64, 65]. Anti-nutrient factors decrease animal productivity and will be dangerous when animals consumed the feed rich in these factors in large quantity [66] [63]. To use a plant part for livestock nutrition, proper assessment of these anti-nutrient factors, their concentration, nature, and the availability of nutrients are necessary. If their concentration is enormous, we can reduce the level by proper processing methods, or we can add supplementary materials like minerals, amino acids and vitamins to reduce or neutralize the adverse effect of anti-nutrient factors [67].

By using efficient processing techniques like soaking, sprouting, cooking and gamma irradiation, we can enhance the nutritive value of foods and feeds. Soetan and Oyewole, 2009 reported that there were several processing techniques to reduce the level of anti-nutrient factors. Gamma irradiation significantly reduced the amounts of protease inhibitors, phytohaemagglutinins and tannins [63]. Heat treatment almost wholly eliminated the trypsin inhibitor activity. Germination method was less effective than cooking treatments for trypsin inhibitor, hemagglutinin activity, tannins and saponins. This method was more effective for phytic acid, stachyose and raffinose. Microwave treatments effectively reduced the anti-nutritional factors in green vegetables. De-hulling greatly reduced the condensed tannin

and polyphenol levels in legumes. Extrusion was the best method to minimize haemagglutinating activity without modifying the protein content. Due to steam treatment, the anti-nutritional factors broke down, and the nutrients such as fats became better accessible, which increased the nutritional value of the final animal feed. Boiling or roasting reduced the levels of cyano glycosides in sweet potato, yellow yam and cocoyam. Roasting lowered the level of trypsin inhibitor activity than the boiling method. Cooking and fermentation reduced the cyanide level and tannin levels largely. In short, boiling and roasting were effective ways to reduce the level of anti-nutritional factors [63].

Mohan and Janardhanan, 1993 studied the nutritional and anti-nutritional factors of the seeds collected from the *Vigna capensis* and *V. sinensis,* and observed that most of the harmful anti-nutritional factors are eliminated by moist heat treatments and cooking processes [68]. Effect of processing on anti-nutritional factors of lentils was studied by Vidal-Valverde et al. 1994 and communicated that cooking and germination were effective methods for the removal of anti-nutritional factors from lentil flour in both domestic and industrial scales [69]. The nutritional and anti-nutritional analysis of underutilized legume varieties was studied by Siddhuraju et al. 1995 [70]. Benevides et al. 1998 communicated a study on proximate analysis and anti-nutritional factors of ten Brazilian marine algae and reported that the algae contained the toxic anti-nutritional factors that may cause problems in both humans and animals [71]. Akwaowo et al. 2000 studied the biochemical composition of fluted pumpkin at different growth

stages. They have reported that there was a change in biochemicals with plant age. Moisture, carbohydrate, crude protein, crude fat and elemental analysis were done as a part of nutrients and cyanide, tannin, oxalates and phytate contents were examined for anti-nutrients. Their results showed that anti-nutrients in the leaves were above the acceptable limit, but proper cooking methods can reduce it [72].

Vadivel and Janardhanan in 2004 reported the nutritional and anti-nutritional level of an under-utilized tribal pulse-sword bean [*Canavalia gladiata* (Jacq.) DC.] [73]. Nutrient and anti-nutrient analysis of thirteen underutilized green leafy vegetables were extensively studied by Gupta et al. 2005. They have examined the level of moisture, ash, ether extract, iron, calcium, phosphorus, ascorbic acid, thiamine, total carotene, oxalate and tannin in green leafy vegetables. Their study reported that the green leafy vegetables could be recommended to irradiate micronutrient malnutrition in developing countries [74]. Antia et al. in 2006 evaluated the nutritional and anti-nutritional composition of sweet potatoes (*Ipomoea batatas*) leaves and communicated that this leaf contained low levels of anti-nutritional factors. The oxalate level was comparatively high and was lowered by cooking processes [75].

Anti-nutrient contents present in the seeds of some common spices in Nigeria-*Uapaca guineense*, *Zanthoxyllus zanthoxyloides, Parinari excelsa, Afromomum danielli* and *Syzygium aromaticum* were evaluated by Nnoka and Mepba in 2008 and reported that boiling and dehulling of seeds were efficient methods for the reduction of toxic compounds and it enhanced the nutritional quality of that samples [76].

Fathima and Mohan in 2009 analysed the nutritional and anti-nutritional content of *Mucuna atropurpurea* DC-an underutilized tribal pulse variety [77]. Nutritional and anti-nutritional assessment of another underutilized pulse variety (*Mucuna pruriens* (L.) DC *var. Pruriens*) was studied by Fathima et al. in 2010 and reported that the presence of anti-nutritional factors was not a problem if the samples were adequately processed [78, 77, 73]. Wobeto et al. in 2007 examined the anti-nutrients contents in the leaves of cassava and reported that the concentration is minimal in leaves of twelve months old plant. So they recommended that leaf powder prepared from the twelve months old plant is safe for consumption [79]. Nutrient and anti-nutrient analysis on edible flowers of some Mexican wild plants were studied by Sotelo et al. in 2007, and reported that they contain a considerable amount of nutrients. The anti-nutritional analysis revealed that their concentration was very low and eliminated by cooking processes [80]. Nutrient and anti-nutrient levels of eight different types of fruit and vegetables were studied by Ali Aberoumand in 2009 [81].

Nutritional and anti-nutritional evaluation of some unconventional wild edible plants was evaluated by Mohan and Kalidass in 2010 and they reported that harmful anti-nutritional factors could be removed by moist heat treatments, soaking and cooking [82]. Ogbe and Affiku, in 2011, communicated the proximate and anti-nutrient analysis of *Moringa oleifera* leaves and reported that there was a considerable amount of carbohydrate, protein and minerals. Anti-nutrient factors (tannins, phytates, trypsin inhibitors, saponins, oxalates and cyanide content) were also present and could be reduced by heat

treatments. So this plant leaves were suitable as a feed supplement in poultry farms [83]. Anti-nutritive compounds in twelve *Camelina sativa* genotypes were analysed by Russo and Reggiani in 2012 and studied the possibilities for utilizing particular genotypes in the animal feed industry [84].

Kala and Mohan in 2012 investigated the role of microwave treatment in eliminating the anti-nutritional factors in the seeds of velvet bean. Their study concluded that the microwave treatment is a useful technique for reducing both heat-stable and heat-labile anti-nutrients [85]. Tresina and Mohan in 2012, studied the physicochemical and anti-nutritional attributes of gamma-irradiated *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* seeds and reported that the irradiation processing significantly reduced the levels of anti-nutritional factors like L-DOPA, phytic acid, hydrogen cyanide, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity [86]. Effect of processing on the level of anti-nutritional factors present in an underutilized legume variety was studied by Kalpanadevi and Mohan, 2013. Their results showed that germination significantly reduced the level of phytic acid, tannins and oligosaccharides while cooking and autoclaving were adequate methods for trypsin inhibitors and phytoheamagglutinating activity [87]. Shimelis and Rakshit, 2007 investigated the effects of processing methods on the reduction or elimination of anti-nutritional factors in kidney beans. Hydration, autoclaving, germination, cooking and their combinations, were used as processing methods. They concluded that there was no single method that could remove or eliminate most of the anti-nutrients

factors. The combination of germination and autoclaving was an effective method to be used [88, 87].

Level of anti-nutrient factors of some selected fermented foods was studied by Mohite et al. in 2013. Their results showed that the fermentation technology lowered the level of anti-nutritional elements and enhanced the nutritional value of foods [89]. Tresina and Mohan, in 2013 analysed the nutritional and anti-nutritional factors present in the underutilized legume variety of the genus *Mucuna* [90]. Anti-nutrient factors-enzyme inhibitors, lectins, phytates, oligosaccharides, and phenolic compounds, present in cereals and pulses were communicated by Rehman et al. 2014 and explained that they have a positive role in human health like antioxidant properties, boosting of the immune system and the regulation of metabolism. Even though they are important for human health, their toxic effects should also be considered. Various processing techniques like soaking, boiling and steeping in water could be applied to reduce their deleterious effects [91]. Sango et al. in 2016 studied the phytochemical and anti-nutrient analysis (phytates, oxalates, saponins, alkaloids and tannins) of common vegetables (*C. gynandra and S. Nigrum*) in Zimbabwe and concluded that they were safe for human consumption [92]. Sinha and Khare in 2017 reviewed the nature and effects of anti-nutritional factors (Cyanogens, Alkaloids, Protease Inhibitors, Lectins, Phytates, Saponins, Oxalates and Lathrogens) present in plants mainly in vegetables like carrot, spinach, sweet potato, watermelon, legumes, tomato and potato. They reported that we could safely consume these vegetables using proper cooking methods [64]. From these studies, it is

clear that we can utilize the plants with anti-nutritional factors after reducing their level by appropriate processing methods.

In the pineapple farm, the usual practice is after the harvest, the sucker portion is taken for planting a new crop, and the other parts are discarded as waste (Figure 1.5 & 1.6). The stem and the leaf are the main agro-waste from pineapple farm (Figure 1.7). After harvesting, tonnes of such agro-waste have been produced every year. The management of this agro-waste is a major challenge for the pineapple farmers. Among different ways of agro-waste management, value-addition is one of the sustainable processes for food and feed production. This work mainly explored the possibilities of food and feed production from the stem portion of pineapple agro-waste and the details are classified in different chapters.

Figure 1.5: Pineapple farm located at Kalikavu, Malappuram

Figure 1.6: The experimental pineapple farm

Figure 1.7: The agro-waste from the pineapple farms (stem and leaf)

Chapter 2
REVIEW OF LITERATURE

2. REVIEW OF LITERTURE

2.1 Agro-waste utilization

The effective utilization of agro-industrial waste was extensively studied and reported by several researchers. Sadh et al., in 2018 reviewed the use of agro-waste (molasses, husks, bagasse, seeds, leaves, stem, straw, stalk, shell, pulp, stubble, peel and roots) and industrial waste (from juice, chips, meat, confectionery, and fruit industries) for the production of value-added products using solid state fermentation. These products include bio-fuels, some valuable enzymes, vitamins, antioxidants, animal feed, antibiotics, single-cell protein and xanthan production. [93]. Truong et al. in 2019 studied the use of meat, vegetable, fruit, and bread waste for animal feed production, especially as poultry feed. This type of utilization will reduce the food waste, could meet the growing demand for poultry production, and also could replace the corn and soy products, which are mainly used for poultry diet [94].

Jose et al. 2016 reviewed the use of pineapple leaf fibre in textile industries and the present difficulties. According to them, the potential of pineapple leaf fibre was not fully utilized due to the lack of proper methodologies. The development of such technologies may lead to the production of diversified textile products from this agro-waste [95]. Adrizal et al. 2017 studied the utilization of pineapple fruit waste as poultry feed by reducing their fibre content using fermentation by microorganisms from different sources like bamboo sprouts, banana corms, and fixed fruit waste. They have reported that

the fermentation for one week with microorganisms from bamboo sprouts could significantly reduce the fibre content of pineapple waste. Thus, it could be effectively used as a poultry feed [28].

Nakthong et al. in 2017 studied the utilization of the pineapple stem waste as a starch source, and the starch was extracted using the mechanical method using water [96]. Ilyas et al., in 2019 extracted the underutilized stem starch and fibres from the sugar palm tree and explored their potential as packing materials. Water was used for extracting the starch, and the extracted starch was air-dried, then by hot air oven at 120 °C for 24 h [97].

2.2 Extraction of starch

Different methods for the extraction and characterization of starch from a plant source have been developed. Lim et al., in 1992, used alkaline pH for extracting the starch from oat flour. For this, they mixed the flour with sodium hydroxide, centrifuged, residue mixed with water, filtered by a nylon cloth, filtrate neutralized by hydrochloric acid, repeated centrifugation and washing steps, then dried in an oven at 40 °C [98]. Kortstee and their co-workers in 1998 used sodium metabisulfite for extracting the starch from genetically modified potato. They then washed the sample with water, filtered with filter cloth, paper, sieved, and kept at room temperature [35]. In 2004, Kaur et al. reported the isolation of starch from mango kernels using sodium hydrogen sulfite ($NaHSO_3$) with subsequent filtration and washing with distilled water [99].

Zhang et al., in 2005, reviewed the extraction and properties of banana starch. They have used alkaline method (Cut and macerated with sodium hydroxide, filtered with cheesecloth and bolting cloth, washed with water and dried) and non-alkaline method extraction (blended with water, centrifuged, removed the dark upper layer, again washed with water, decanted the supernatant, and allowed to stand for the settlement of starch) for the banana starch extraction [100].

L. A. Bello-Perez et al. 2006 isolated the starch from pinhao seeds (*Araucaria angustifolia*), by mixing the sample with cold water and then filtered, then kept in a refrigerator overnight, again centrifuged. Precipitated starch was then washed repeatedly with water, then dried in an oven at 37 °C for one night [101].

Li et al. 2008 communicated the extraction of starch from maize kernels by wet milling method (steeped in sodium metabisulfite then milled in a micro blender, ground, filtered, washing with water, centrifuged, suspended in NaCl containing toluene solution and stirred. Then the purified starch was washed with water, then with 100 % ethanol, and dried at 30 °C for 48 h) [102]. Torres et al., in 2011, extracted the starch from Andean crops by chopping and homogenizing in distilled water. Collected the precipitate and degreased by the mixture of methanol and water. Then collected the precipitated starch and dried at 40 °C for 48 h and kept in a desiccator. Protein-rich samples were subjected to an extra step of soaking with NaOH solutions [103].

Singh and Kaur in 2017 extracted the starch from oat cultivars by homogenized with water at 50 °C, filtered by a nylon cloth and centrifuged. Then the sediment was mixed with NaOH and removed the supernatant, repeated this step, and the final sediment was mixed with distilled water and centrifuged. Then scrapped off the upper non-white layer and suspended in distilled water and centrifuged again. The final starch residue was collected and dried using an oven at 40 °C for 12 h [104]. Wijaya et al., in 2019, isolated the starch from a flowering plant-*Limnophila aromatic* using alkaline method (soaking and rinsing using NaOH then water washing followed by centrifugation, and drying) [105].

2.3 Characterization studies on starch

Cael et al. 1974 reported a study about the molecular characterization of carbohydrates using the Infrared and Raman spectroscopy. They observed that none of the molecular vibrational modes in carbohydrates are formed due to a single type of vibration [106]. Huang et al. 1990 studied the granular character of potato starch after heating the sample in a microwave oven and boiling water. Results revealed that the microwave heated sample was less reticulated, less hydrated, and more compact and observed to be denser than the sample from conventional heating. They concluded that the microwave heated sample would be more suitable for producing commercial products [107]. Garbow and Schaefer, in 1991, used the cross-polarization, magic-angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) spectra for measuring the relative analysis of protein present in wheat flours [108]. Baik et al. 2003 used ^{13}C CP/MAS NMR techniques to study

the aging of white bread starch. According to them, some NMR peaks became stronger by aging, and the formation of duplex peaks was also observed during aging [109].

Characterization of starches isolated from the kernels of some Indian mango cultivars (*Mangifera indica L.*) was studied and reported by Kaur et al., in 2004. Their scanning electron microscopic (SEM) studies revealed that these mango kernel starches have a varying size (15.8–21.7 and 8.7–14.1 µm) and shape (oval-to elliptical), which was similar to legume starches. Some of the kernel starch showed surface pores in their SEM analysis. Differential scanning colorimetric (DSC) analysis revealed that they possess transition temperature, which was higher than that of other commonly used starches like corn, rice, wheat, and potato. Rheological properties indicated that the storage and loss moduli of them were higher than that of rice starches and lower than that of potato starches [99]. The thermal properties of corn starch using DSC was studied and reported by Liu et al., in 2005 and observed that the change in the moisture content of the sample influenced the gelatinization enthalpy and the number of endotherms obtained. The gelatinization enthalpy increased with increased moisture content [110].

Starch characterization after different types of heating (microwave heating and moist heating) was studied by Palav and Seetharaman in 2007 and reported that different types of heating causes the formation of starches with different properties. It happened due to the different heat and mass transfer during microwave and moist heating. Lack of granular swelling and soft gel formation were

observed with the starch samples heated in a microwave oven. During the microwave heating, loss of birefringence occurred much earlier than the gelatinization temperature. It was due to the vibrational motion of water molecules and the rapid increase in the temperature by the microwave heating [111].

Singh et al. 2010 studied the nature of starches extracted from eighteen Indian wheat varieties. They communicated that the granular size and the chain length of amylopectin have a crucial role in the rheological properties of wheat starches. Amylopectin with short-chain length showed an inverse relationship with the gelatinization temperatures (To-onset, Tp-peak, and Tc-conclusion temperatures). They have also performed the X-ray diffraction studies on wheat starches and observed that they possessed a typical A-type starch crystal with strong reflections at 15, 17, 18 and 23 two θ angles [112].

Structural changes occurred in rice starch granule by different types of heating were also observed by Fan et al., and their co-workers in 2012 and studied the changes by FTIR and Raman spectroscopic techniques. The result indicated that these heating processes didn't produce any new chemical groups and didn't alter the existing chemical groups [113]. Singh and Kaur in 2017 characterized the starch from eight oat cultivars and communicated that these starches have polygonal and irregular shaped starch granules with A-type X-ray diffraction pattern. Rheological properties revealed that the oat starches showed increased storage and loss moduli initially. Then it decreased after the gelatinization peak. It was also reported that the studied oat starches were highly elastic [104].

Kowsik and Mazumder in 2018 studied the structural and chemical changes that occurred in both rice and potato starches due to α- amylase treatment. They used the FTIR and X-ray diffraction (XRD) techniques for monitoring the changes. The results indicated that the potato starch granules were more stable than the rice starch granules [114]. In the same year, Decastro et al. conducted a characterization study on the starch extracted from a non-conventional source-the seeds of pitomba (*Talisia esculenta*). They concluded that this starch could successfully be used as a thickening agent and stabilizer, as an ingredient for edible films [115]. In 2019 Siroha et al. studied the characters of a novel starch from *Pongamia pinnata* seeds and reported that this starch contained round to oval-shaped granules with an A-type diffraction pattern. DSC analysis showed the To, Tp and Tc of 61.5 °C, 72.1 °C, and 82.9 °C, respectively [116].

2.4 The pesticide residue analysis

Diepens et al. in 2013 studied the effect of organophosphate pesticides like ethoprophos and chlorpyrifos on a cladoceran-Daphnia ambigua and a fish-Parachromis dovii. Ethoprophos and chlorpyrifos were used in both banana and pineapple farms of Costa Rica and observed that both the pesticides were toxic, and chlorpyrifos exert more harmful effects than ethoprophos on these species [117]. Munoz-Quezada et al., in 2016, reviewed the impact of chronic exposure to organophosphate pesticides on the neuropsychological functioning of the farmers. The study reported that exposure caused difficulties in memory, attention, processing speed and coordination [118]. In the same year, the toxic effects of organochlorine pesticides were reviewed by

Jayaraj and co-workers. Their paper explained that pesticides could alter the ecosystem. They have also proposed that covering the weeds with plastic, removal of the pest breeding sites, and increase of consumer awareness could effectively control their impacts [119].

Darnaudery et al., in 2016, observed the effect of organic farming on pineapple cultivation. For that, they studied three sets of samples. One with chemical fertilizers other with a mixture of both chemical and organic fertilizers and the third was completely organic fertilizers. They concluded that the organic fertilization provided very encouraging results which can lead to the production of certified organic pineapples [120].

2.5 Proximate and anti-nutritional analysis of pineapple stem and leaf

Adnan et al. in 2010 studied the proximate content of five medicinal plants collected from the humid and sub-humid regions of Pakistan. Results indicated that the moisture, ash, and fat were observed to higher in species from humid areas. The carbohydrates and protein content were higher in the sub-humid species [121]. Ilodibia et al., in 2014, studied the proximate composition of various parts (leaf, stem, root, and fruit) of Dracaena species. They observed that the carbohydrate, protein, crude fat and moisture contents were higher in the leaves and the crude fibre and ash contents were higher in the root and stem parts compared to other regions [122]. The proximate and mineral composition of *Myristica fragrans* seeds were studied by Rancy Ann Thomas and S Krishnakumari in 2015 and reported that

those plant seeds were a good source of moisture, ash, fats, fibre and minerals [123].

Monago and Akhidue in 2000 studied the anti-nutritional factors present in a flowering plant *Garcinia kola* and communicated that the oxalate, tannin and cyanogenic glycoside levels were in minute quantities. But the saponin level was observed to be high compared to standard values [124]. Jain et al., in 2009, reviewed the anti-nutritional factors present in pulses. They concluded that the soaking, cooking, and germinating will enhance the nutritive values of pulses by reducing the anti-nutritional factors present in them [125]. Olajide et al., in 2011 studied the anti-nutritional content of wild cocoyam and reported that soaking, cooking, and fermentation techniques increased in the nutritional availability and decreased the level of anti-nutritional factors [126]. Soetan, 2008 in their review paper, explained the pharmacological and medicinal effects of anti-nutritional factors in plants. They explained that by the use of tissue culture, genetic manipulation, and other modern plant breeding methods, we could exploit the beneficial effects of anti-nutritional factors [127].

Bolanle et al., in 2014 studied the proximate and anti-nutritional content of seeds collected from the two plant families- *Brachystegia eurycoma harms* and *Pipper guineense schum and thonn* and reported that the tannins, phytate, and cyanide levels were in limited quantities while the carbohydrate content was in appreciable amount [128]. Gemede and Ratta, in the same year, published a review paper regarding the beneficial and adverse effects of anti-nutritional factors. They have explained that the beneficial and harmful effects of

these factors depend upon their concentration in plants [129]. Sowbaghya et al., in 2019, tested the trypsin inhibitory activities of ten selected plant seeds and concluded that all the ten plant seeds had the activity. Among them, red lucky seed (*A. pavonina*), bitter gourd (*M.charantia*), and babul seed (*A. nilotica*) seeds were found to be rich sources of trypsin inhibitor [130].

OBJECTIVES OF THE STUDY

1. To isolate and characterize the starch present in the stem and leaf of pineapple plant.

2. To explore the possibilities of making value-added products like cattle feed from the pineapple plant agro-waste by assessing the proximate and the anti-animal nutrients as a preliminary step.

Chapter 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Collection of plant material

The stem and leaf of the pineapple plant *(Ananas comosus)* of Mauritius variety were collected from pineapple farms located in Kozhikode (Perumbally), Wayanad (Ambalawayal), Malappuram (Kalikavu) districts of Kerala (State), India. The samples were then washed with water and mild acid to remove soil and other debris.

3.2 Setting of an experimental plantation

A small farm of the pineapple plant has been formed using the pineapple saplings (Mauritius variety) brought from Regional Agricultural Research Station, Ambalavayal, Wayanad. Stem from this plantation was used for the studies on stem starch at different growth stages.

3.3 Isolation of starch

Samples were cut into small pieces, added an adequate amount of distilled water and ground in a mixer grinder. The slurries were filtered through double-layered cheese cloth. The steps were repeated several times until the milkiness of the slurry disappeared or it became minimal, and then the slurry was centrifuged and discarded the supernatant. The residues obtained were pooled and washed with 60 % alcohol, 0.1N NaOH, and distilled water. The centrifuged residues were dried in an incubator at 40 °C, powdered and passed through a standard sieve (75 µm), collected and stored in desiccators.

3.4 Molecular characterization of pineapple stem starch

3.4.1 Fourier Transform Infrared Spectroscopic (FTIR) Analysis

FTIR spectra were recorded using an FTIR 4100 JASCO model instrument and compared with corn starch [131]. FTIR analysis was performed after different heat treatments as follows using the method reported by Fan et al., 2012 with slight modifications. The pineapple plant stem starch (3 g) was dispersed in deionized water (made up the volume to 50 ml) and heated in a water bath at different temperatures (40 °C, 60 °C, 80 °C, and 100 °C). Another set of sample was boiled for 10 seconds using a microwave oven (900W-SHARP R32BST-frequency 2450 MHz). All samples were cooled and lyophilized. Lyophilized samples were dried, sieved and used for the analysis. Commercial corn starch was used for comparison, which was treated in the same way [113].

3.4.2 Nuclear Magnetic Resonance Spectroscopic (NMR) Study

Solid-state ^{13}C CP/MAS spectra were collected at x frequency of 100.5 MHz on a DELTA2_NMR spectrometer (JNM-ECX400II) operating at 25 °C. 9.38976 [T] field strength was used and a spin set at 15 Hz, x 90 pulse width was 2.8 µs with a recycle time of 5 s. A contact time of 3500 µs was used for all samples; the filter width was 18 kHz. Total scans were 1028 with dimension 1.

3.4.3 X-ray diffraction study

X-ray diffraction (XRD) studies were carried out by an X-ray diffractometer (XRD-RigakuMiniflex 600) operating under the

conditions-voltage used 40 mV, current 15 mA, scan rate 10 deg/min and source was CuKα radiation (1.54 Å) and compared the result with corn starch. The % crystallinity of samples was measured using the method of Nara and Komiya, 1983 [132].

3.5 Granular characterization of pineapple stem starch using scanning electron microscopy (SEM)

The surface and structure of starch granules were observed using a scanning electron microscope (Carl-ZEISS Gemini SEM 300), using a secondary electron detector with 2.00 kV of acceleration and compared with corn starch.

3.5.1 Granular characterization with different growth stages of the plant.

Granular changes with age were observed after extracting the starch from the plant at different growth stages-three months, six months, nine months, twelve months, fifteen months and eighteen months.

3.5.2 Granular characterization after microwave heating and moist heating

SEM analysis was also done after different heat treatments, the same as in FTIR heat treatments (Section 3.4.1).

3.5.3 Granular characterization after dispersing in an ionic liquid- '1-butyl-3-methylimidazolium chloride'

Morphological changes were observed after dispersed the starch samples in an ionic liquid (1-butyl-3-methylimidazolium

chloride) and deionized water using the procedure explained by Stevenson et al. 2007 with slight modifications [50]. Stem starch (100 mg) was heated from 25-80 °C for 15 minutes with an ionic liquid, 1-butyl-3-methylimidazolium chloride, then heated to 100 °C for 1 h. Then the starch from the ionic liquid treated samples was precipitated using 95 % ethanol, centrifuged at 2500 rpm for 15 minutes, washed the pellets with deionized water and the starch precipitation using ethanol was repeated. Filtered, then the pellets were dried using an oven at 40 °C for 72 h. The same treatment was performed on another sample of stem starch using deionized water also. All the data were compared with corn starch treated in the same manner.

3.6 Thermal characterization of pineapple stem starch

Thermal properties were studied by a differential scanning calorimeter (DSC) (Perkin Elmer DSC 4000) using 10 % of starch suspension. Onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), enthalpy change of gelatinization (ΔH) and the transition temperature interval (ΔT) were calculated with the scanning temperature range of 40–120 ℃ (heating rate-10 ℃/min) [133], [57]. An empty pan was used as a reference [55] and compared the result with commercial corn starch.

3.7 Rheological studies of pineapple stem starch

A dynamic rheological measurement was made with a rotational rheometer (Physica MCR 51). One g (10 %) of the sample was used, the frequency was set from 0.1 to 10 Hz and the dynamic rheological properties-storage modulus (G'), loss modulus (G''),

complex viscosity (η*) and phase angle (δ) were determined and compared with commercial corn starch.

3.8 Pesticide residue analysis of pineapple stem starch

The pesticide residue analysis was performed by gas chromatography (GC-Model No. Agilent 7890A). GC-FPD (gas chromatography with flame photometric detector) was used for organophosphate pesticides, and GC-ECD (gas chromatography with electron capture detector) was used for organochlorine pesticides.

3.9 Proximate analysis of pineapple stem and leaf

The dried and powdered samples of the pineapple plant stem and leaf were used for the proximate and anti-nutrient analysis. The proximate compositions for nutrients were determined using the AOAC (1995) method in dry weight basis [134]. The moisture content was determined by drying the sample to a constant weight at 135 °C in a hot air oven for 2 h. Protein was determined by the Kjeldahl method. Crude fat was estimated by extracting a known weight of sample with petroleum ether (boiling point 60 °C) using a soxhlet apparatus. Ash content was determined by ignition in a muffle furnace for four h at 600 °C. Crude fibre content was estimated using an automatic fibre analyser (Model: FES 4 R TS) after defatting the samples by hexane (1:2 ratio) from the loss in weight of the crucible and its content on ignition. Carbohydrate was determined using the difference method [100-(% of ash+% of moisture+% of crude protein+% of crude fat)] [135].

3.10 Anti-nutrient analysis of pineapple stem and leaf

3.10.1 Phytate Phosphorus (Phytate P)

The powdered samples were extracted with 3 % TCA. To the 10 ml of extract, 4 ml of $FeCl_3$ was added, and the mixture was heated in a boiling water bath for 45 minutes. Centrifuged for 15 minutes and decanted the supernatant. The precipitate was washed with 3 % TCA, heated in a water bath and again centrifuged. Repeated the washing with water and dispersed the precipitate in a few ml of water and added 3 ml of 1.5 N NaOH. Made up the volume to approximately 30 ml with water and again heated for 30 minutes. Filtered, washed the precipitate with hot water and discarded the supernatant. Dissolved the precipitate with 40 ml of hot 3.2 N HNO_3 into a 100 ml volumetric flask, cooled the flask to room temperature and diluted the volume with water. Then transferred 5 ml of aliquot to another volumetric flask of 100 ml volume and diluted to 70 ml. Added 20 ml of 1.5 M KSCN diluted to volume, and read the colour immediately (within 1 minute) at 480 nm.

A reagent blank was used simultaneously with each set of samples. Fe $(NO_3)_3$ was used as the standard, and the μg Fe present in the sample was obtained from the standard curve. Using the following equation phytate P was calculated.

$$\text{Phytate P mg/100 g sample} = \frac{\mu g\ Fe \times 15}{\text{Weight of sample (g)}}\ ^{[136]}.$$

3.10.2 Cyanogenic glycosides

One gram of the powdered sample was homogenized with 25 ml of distilled water and 3-4 drops of chloroform. Kept the homogenate in 500 ml conical flask and cyanogenic glycosides were determined by using the alkaline picrate paper method. Hanged the paper inside the flask and incubated for 24 h. The sodium picrate present in the filter paper was reduced to a reddish compound by the amount of hydrocyanic acid evolved from the sample. Then eluted the colour by using 10 ml of distilled water and read at 625 nm using water as blank. 5 ml of alkaline picrate and potassium cyanide solutions were mixed, heated for 5 minutes in a boiling water bath and used as a standard solution [136].

3.10.3 Oxalic acid

Oxalate was determined by using the method of Vasant Naik et al. 2014. For this, 0.5 g of the powdered samples were mixed with 30 ml 0.25 N HCl and kept in boiling water bath for about 15 minutes, cooled to room temperature and made up to 50 ml in a volumetric flask with 0.25 N HCl. Assay mixtures contained 1 ml of the sample, 5 ml of 2 N H_2SO_4 and 2 ml of 0.003 M $KMnO_4$. This mixture was incubated for 10 minutes at room temperature. After that the absorbance was recorded at 528 nm on a UV-Visible spectrophotometer. Reagent blank was prepared with distilled water, and oxalic acid was used as the standard solution. Absorbance for the blank solution was recorded as Ab, and for sample, it was As. The

oxalic acid concentration was obtained in mg/ml from the calibration curve and regression equation [137].

3.10.4 Tannins

Tannins were estimated using tannic acid as the standard. It consisted of water extraction of 0.5 g ground sample, heated for 30 minutes, centrifuged (2000 rpm for 20 minutes) and collected the supernatant, made up the volume to 100 ml. Then, 1 ml aliquot was added to a 100 ml volumetric flask containing 75 ml water. Added 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate and diluted to 100 ml with distilled water. Shaken well, and absorbance at 700 nm was read. The determination used a standard curve of tannic acid, and the results were expressed in % [136].

3.10.5 Saponins

A gravimetric method was used with the use of a soxhlet extractor. Extract 2 g of the powdered sample with acetone in a 250 ml round bottom flask for 3 h, after which the extraction was repeated for another 3 h by using 250 ml of methanol. At the end of second extraction, the methanol was recovered by distillation and the flask oven dried, allowed to cool in a desiccator and then weighed. The saponin content was calculated in percentage [138, 139].

3.10.6 Trypsin inhibitor activity

Trypsin inhibitor activity was determined using BAPNA (benzoyl-DL-arginine-p-nitroanilide) as substrate. One g of powdered sample was defatted using hexane and extracted with 0.1 M phosphate

buffer (pH: 7.6) for 1 h, centrifuged at 10,000 rpm for 30 minutes at 4 °C. The clear supernatant (crude extract) obtained was assayed for trypsin inhibitor activity. 0.5 ml of the extract and 0.5 ml of trypsin solution (4 mg of trypsin dissolved in 0.001 M HCl) were mixed with 1.25 ml of the substrate-BAPNA. After the incubation, the reaction was stopped by the addition of 0.25 ml of 30 % acetic acid. Blank and control were also prepared accordingly. The reaction mixture was filtered through Whatman No. 3 filter paper and the absorbance read at 410 nm (The activity was calculated as the increment of 0.01 units of absorbance at 410 nm for 10 ml of reaction mixture). Trypsin inhibitor activity was expressed in terms of trypsin units inhibited per g of dried sample [140, 141].

3.10.7 Lectin/Haemaglutinating activity

In this study, we used haemagglutination property of lectin to quantify them. For this, the dried and powdered samples (5 g) were mixed with 25 ml of Tris-HCl extraction buffer (20 mM Tris-HCl at pH 7.2, containing 150 mM NaCl). The suspension was stirred at 4 °C and centrifuged at 10,000 rpm for 20 minutes. The clear supernatant was used for the haemagglutination assay. Trypsin treated erythrocytes were used here as it enhances the susceptibility of erythrocytes to agglutination by lectins [142].

Human blood was collected, centrifuged at 2000 rpm for 10 minutes (at 5 °C) and washed with TBS (10 mM Tris HCl buffer pH 7.2 containing 150 mM NaCl). Washing repeated till the colour of the supernatant became colourless. The RBC suspension (3 %) was then

incubated with 0.05 % (w/v) trypsin at 37 °C for 1 h. Then the erythrocytes were repeatedly washed with TBS to remove trypsin and finally make 3 % erythrocyte suspension in TBS. The samples were serially diluted in a 96 microtiter plate, using TBS, and incubated at 37 °C for 1 h. Then 25 µl of trypsinized red blood cells were added to each well and incubated again at 37 °C for 1 h for haemagglutination to occur. The plates were then tilted about 45 degrees to check the agglutination. The samples with red blood cells moved in a tear drop fashion were considered negative. Lectin standard was used as a positive control and lectin free sample as negative control [143, 144]. The RBCs treated samples were also viewed under a fluorescent microscope (Leica DM6 B).

3.11 Statistical analysis

IBM SPSS Software v 21 (student's t-test and one-way ANOVA with 5 % level of significance), Microsoft Office Excel 2007 and OriginPro 8.0 were used to analyse the experimental data.

Chapter 4

ISOLATION OF STARCH FROM THE STEM AND LEAF OF PINEAPPLE PLANT

4. ISOLATION OF STARCH FROM THE STEM AND LEAF OF PINEAPPLE PLANT

Starch is the most widespread carbohydrate, which forms the basis of human nutrition. The yield and properties of starches are specific for each source. Several methods can be used for the extraction of starch from the plant sources.

4.1 Isolation of starch from the stem and leaf after the harvest

In this study, the starch was isolated from the pineapple stem and leaf (Figure 4.2 and 4.3) using conventional method (washed with water, ground, centrifuged, washed with alkali and ethanol, again washed with distilled water, dried and kept in desiccators) (Figure 4.1) and the yield was 11.08±0.77 % for stem and 0.57±0.01 % for leaf in wet basis (Table 4.1).
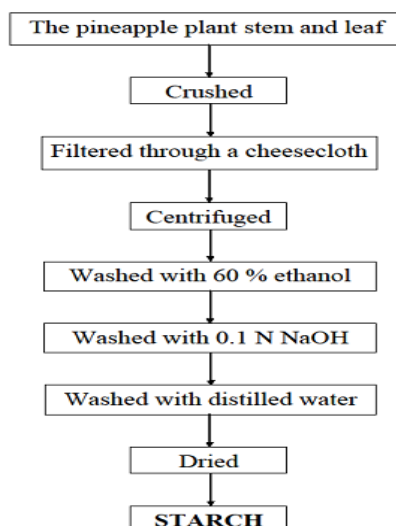
Figure 4.1: Flow chart showing the isolation of starch from the pineapple stem and leaf

Figure 4.2: The starch isolated from the stem of the pineapple plant

Figure 4.3: The starch isolated from the leaf of the pineapple plant

Table 4.1 Starch yield from the stem and leaf of the pineapple plant

Sample	Starch yield (%)
Stem	11.08±0.77
Leaf	0.57±0.01

The results are comparable to that of yellow banana, pinto bean and peanut bean with the starch content of 9.7 %, 9.6 %, 13.8 % respectively [100, 145]. Seeds of the fruit, Shahi Litchi are another nonconventional source of starch. This seeds yield 11 % starch when used the acidic media and 12.6 % starch when used alkaline media for extraction [146].

The average weight of pineapple stem is estimated as 497.32±21 g. On 11 % starch content, 54.71 g of starch is present in one plant. From this data, it can be calculated that there would be 3.47 tonnes of starch yield per hectare of pineapple farms (considering the plant density of 63400 plants/ha.) [26]. This value is comparable to the yield of sago starch (2.2-3.3 tons per hectare) [145].

Consider 1 kg of rice that will yield 0.9 kg of starch (The starch content of rice is taken as 90 %) [147]. From the above data, we can calculate that 8.18 kg of the stem will yield 0.9 kg of starch which means 8.18 kg of pineapple stem will be equal to 1 kg of rice.

Rice equivalent yield (REY) is calculated to compare system performance by converting the yield of non-rice crops into equivalent rice yield on a price basis, using the formula,

$$REY = Yx(\frac{Px}{Pr})$$

(Yx is the yield of non-rice crops (kg/ha.), Px is the price of non-rice crops (Rs./kg), and Pr is the price of rice (Rs./kg). (Prices were assumed to be stable during the experimental period) [148]. The market price of corn starch is Rs. 39/kg [149]. From this, we assume that the pineapple stem starch will cost roughly Rs. 30/kg. Starch yield from the stem was calculated as 3466.078 kg/ha. (considering the stem weight and the planting density [26]). The market price of rice starch is Rs. 45/kg [150]. From these data, we can calculate the REY of the stem starch as,

$$REY = Yx(\frac{Px}{Pr})$$
$$REY = 3466.078 \times (\frac{30}{45}) = 2310.718 \text{ kg/ha.} = 2.31 \text{ tonnes/ha.}$$

As the starch content in the pineapple leaf was very low, leaf starch was excluded from further studies. The leaves of wheat, oat and barley also contain negligible amount of starch [151].

4.2 Isolation of starch from the stem of pineapple plant at different growth stages

To study the yield of starch with the growth stages of the plant, starch was isolated from three months, six months, nine months, twelve months, fifteen months and eighteen months old plant stem. The maximum starch yield obtained from the stem at nine months age (Table 4.2). After that the yield decreased and then there was almost constant starch content observed.

Table 4.2 Starch yield at different growth stages of the pineapple plant

Sl. No.	Age of the plant	Starch yield (%)
1	Three months	3.93±0.52
2	Six months	8.4±0.68
3	Nine months	16.03±0.84
4	Twelve months	11.56±0.53
5	Fifteen months	11.58±0.44
6	Eighteen months	11.08±0.77

Starch is formed in the leaf chloroplast in higher plants and can be classified into transitory starch granules and reserve starch granules. Transitory starch granules are stored for a short period of time. Reserve starch granules are stored for the long term use. Synthesis of starch granules occurs at the time of development and maturation of plant storage organs. ie, the level will be maximum at the time of maturation.

After that the starch degradation occurs at the sprouting stage or at the germination stage or at the stage of fruit ripening. The released metabolites from this degradation will be further used by the plants for their carbon and energy needs [152].

Because of shoot extension growth, utilization of photosynthate increased, which caused lower carbohydrate availability for storage, and starch content, would decrease [153]. The amount of starch content varies with the age of the plant and also with the environment. Starch content would decrease when they used for rapid root and shoot formation. At that condition, the sugar consumption for the rapid growth of the plant exceeds the photosynthetic production of sugars [154, 155].

From the results obtained, it can be concluded that the stem starch can be easily extracted using simple conventional method. The yield was 11.08±0.77 %, which is comparable with the starch content of pinto bean, peanut bean and the seeds of Shahi Litchi. The starch extraction from the different growth stages of the plant revealed that the content was increased up to the nine months old stage. The starch granules are synthesized during the developmental and maturation stages of the plants. After the nine months stage, the yield decreased. After maturation, starch degradation occurs and the metabolites produced from this degradation are used for the rapid shoot and root growth of the plant.

Chapter 5

MOLECULAR CHARACTERIZATION OF STEM STARCH

5. MOLECULAR CHARACTERIZATION OF STEM STARCH

Starch properties are highly variable, and it depends on factors like the environment, genotype, and botanical sources. An insight into the structure and properties of stem starch is a starting step for finding their particular use in starch industries [53]. New findings in the molecular level characterization of starches would be essential for the increased use of them in diversified applications [156].

In this study, fourier transform infrared spectroscopy (FTIR) technique, nuclear magnetic resonance spectroscopy (NMR) and the X-ray diffraction method (XRD) were used for the molecular characterization of pineapple stem starch. FTIR spectroscopy is the technique that can be used for studying the short-range ordering and interactions of starches [157]. It helps for the rapid and accurate observation of structural and group changes that are occurring in starch molecule [158], while the NMR technique can be used for analysing the long-range interactions and the formation of helices in starches. In XRD studies, we can observe the packing of these helices [157]. Thus the observations using these techniques together can contribute an overall picture of the starch structure at the molecular level as evidenced by previous reports.

5.1 Fourier Transform Infrared Spectroscopic (FTIR) Studies

FTIR spectroscopy is a valuable tool for starch characterization as it creates a molecular fingerprint of that molecule. This technique helps us to identify the primary functional group present in the extracted starch sample [52]. FTIR spectra obtained for stem starch are

given (Figure 5.1 and 5.2) and peak assignments are summarised in Table 5.1.
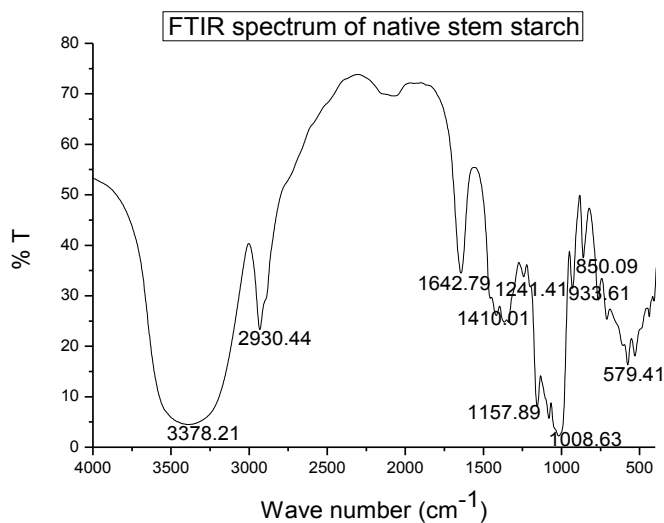
Figure 5.1 FTIR spectrum of native stem starch
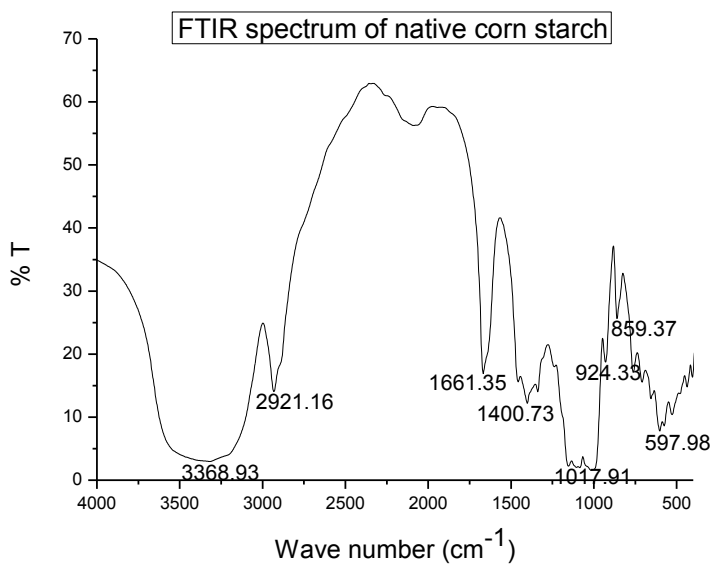
Figure 5.2 FTIR spectrum of native corn starch

Table 5.1: FTIR peak assignments of native pineapple stem starch

Wavelength (cm^{-1})	FTIR Peak assignment
3378.21	-OH stretching vibration (peaks around 3300 cm^{-1}) [159]
2930.44	CH-stretch (peaks between 2800 and 3000) [159]
1642.79	O=C (carbonyl stretching) (near 1640 cm^{-1}) [159, 131]
1410.01	CH$_2$ bending, C-O-O stretch (near 1415 cm^{-1}) [48]
1157.89	C-O, C-C and C-O-H stretching and C-O-H bending [157]
1008.63	Skeletal vibrations of the glycosidic bond (1000-1200 cm^{-1}) [160]
933.61	Skeletal mode vibrations of α(1,4) glycosidic linkage, (C-O-C) (near 930 cm^{-1}) [48]
850.09	C(1)-H, CH$_2$ deformation (near 840 cm^{-1}) [48]
579.41	Peaks specific for starches (Peaks below 800) [159]

Native stem starch showed a broad, large band at 3378.21 cm^{-1} and a small peak at 2930.44 cm^{-1}. Peak obtained at 3378.21 cm^{-1} was due to –OH stretching vibration and the other peak at 2930.44 cm^{-1} was due to the stretching of the CH bond present in the glucose molecules. Peak obtained at 1642.79 cm^{-1} was due to the O=C bond stretching (carbonyl stretching). Other peaks around 1157 cm^{-1}, 1082 cm^{-1}, and 1017 cm^{-1} were reported to be the characteristic feature of polysaccharides [52]. Warren et al. 2016 reported that the major peaks from the starch molecules could be observed in the 1200-1000 cm^{-1} region [157]. FTIR spectra of stem starch were characteristic of native starches and comparable with that of corn starch.

Vibrational spectroscopic techniques like the FTIR method can easily be used to investigate the process-induced changes like

microwave heating and moist heating (commonly used in food industries) to measure the quality of food products [113].

5.2 FTIR analysis of heat-treated starch samples

In moist heating and microwave heating, the heating mechanism is different. In microwave heating, both the electromagnetic effect and the thermal effect applied to the starch sample, which leads to a rapid increase in the sample temperature [113]. Microwave heating has many advantages like saving energy and time, acceptability of consumers and improving the nutritional quality [161, 111]. FTIR can easily be used for the structural characterization of heat-treated samples.

Figure 5.3: FTIR spectra of stem starch heated at 40 °C

Figure 5.4: FTIR spectra of stem starch heated at 60 °C
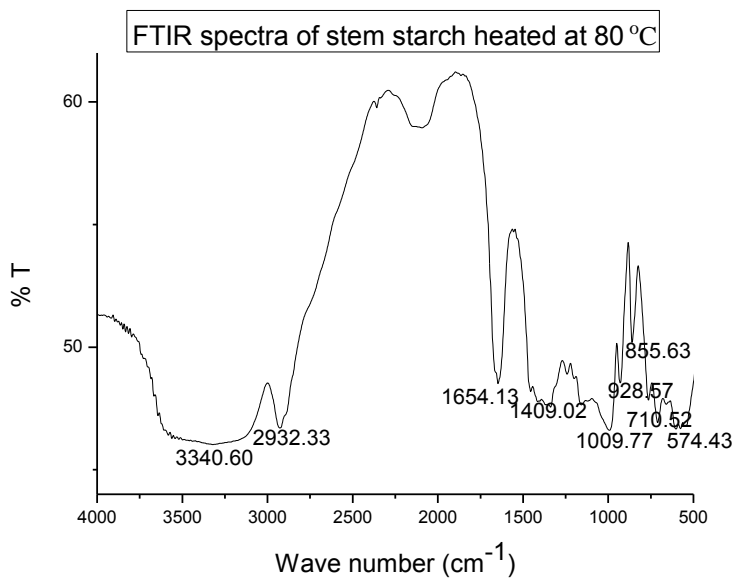
Figure 5.5: FTIR spectra of stem starch heated at 80 °C
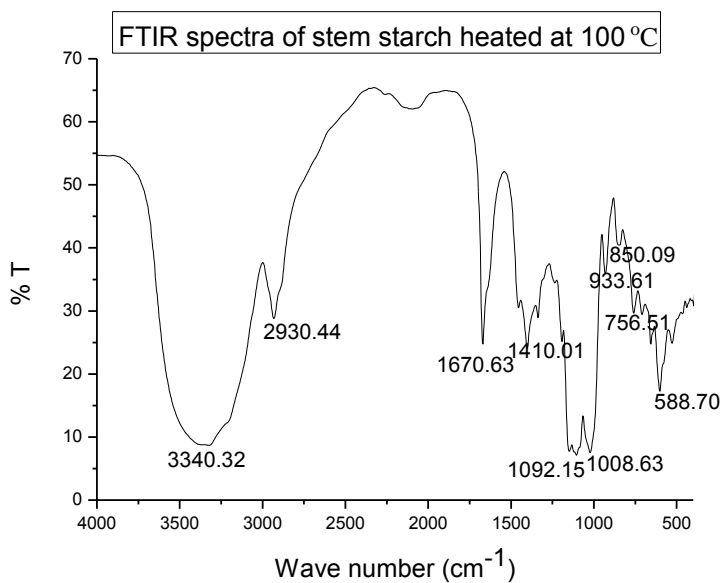
Figure 5.6: FTIR spectra of stem starch heated at 100 °C
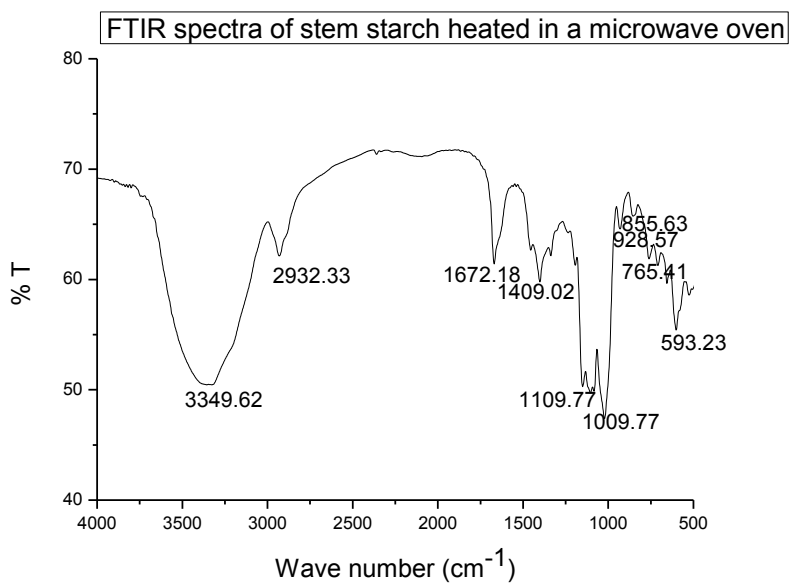
Figure 5.7: FTIR spectra of stem starch heated in a microwave oven

FTIR spectra after different heat treatments gave the same peak position and the same number of major peaks. It was observed that there was a band position shift from 3378.21 to 3340.60 at 60 °C, 80 °C and in 100 °C heated samples. In the case of microwave heating, the change was from 3378.21 to 3349.62. The wavelength 3000-3600 was the indication of the O-H bond stretching vibrations [48]. The peak obtained at 1642.79 was changed to 1654.13 in both 60 °C and 80 °C. 1670.63 were in the case of a sample heated at 100 °C. In microwave heating, it was 1672.18. This peak was due to the O=C bond stretching (carbonyl stretching) [160]. There was only a slight peak shift observed for other peak positions, and the peak assignments (C-H stretch, C-O-O stretch, CH_2 bending, skeletal vibrations of glycosidic bond and CH_2 deformation) were observed to be same. Results indicated that the different heat treatments (moist heating and microwave heating) did not have any effect on changing the chemical groups already present in the stem starch molecule. And they did not produce any new chemical groups (Figure 5.3-5.7).

Results were in agreement with the previous observation on rice starch [113] and were comparable with commercial corn starch (Figure 5.8-5.12).
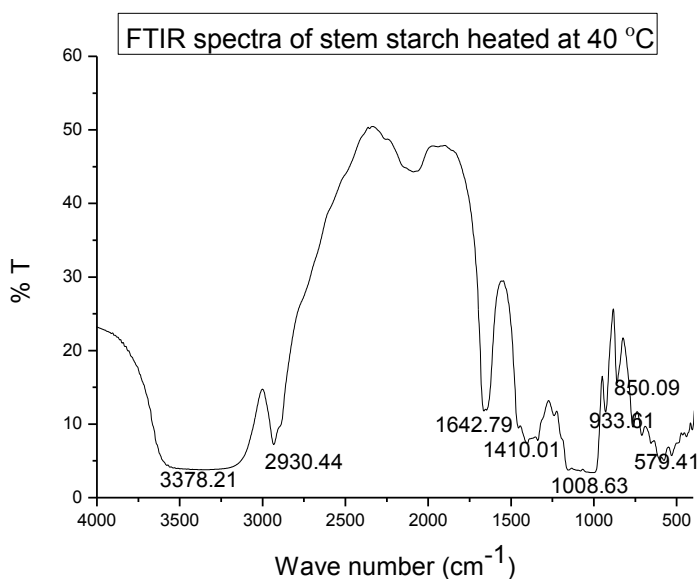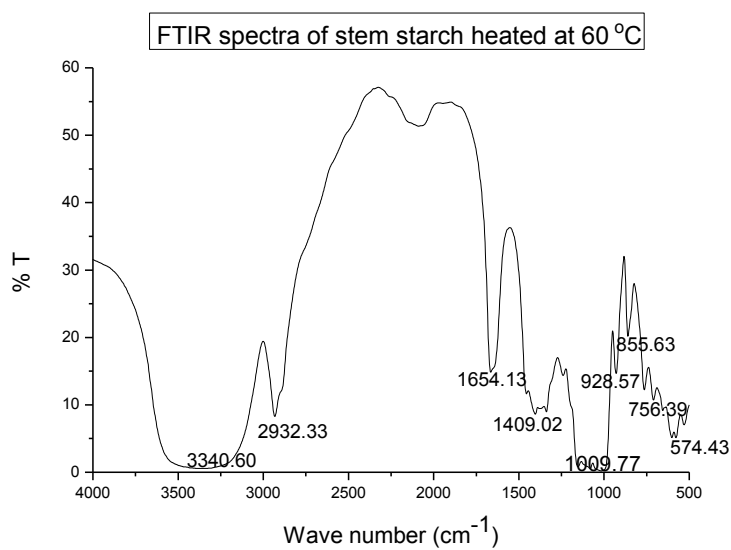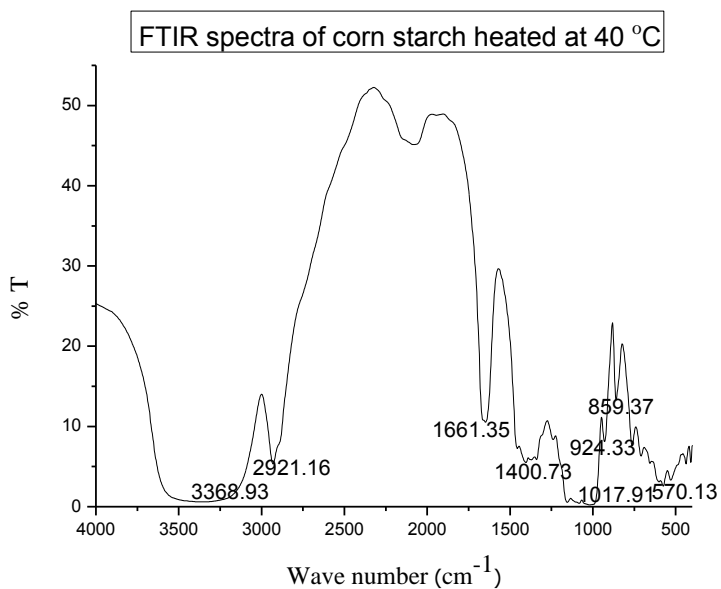
Figure 5.8: FTIR spectra of corn starch heated at 40 °C
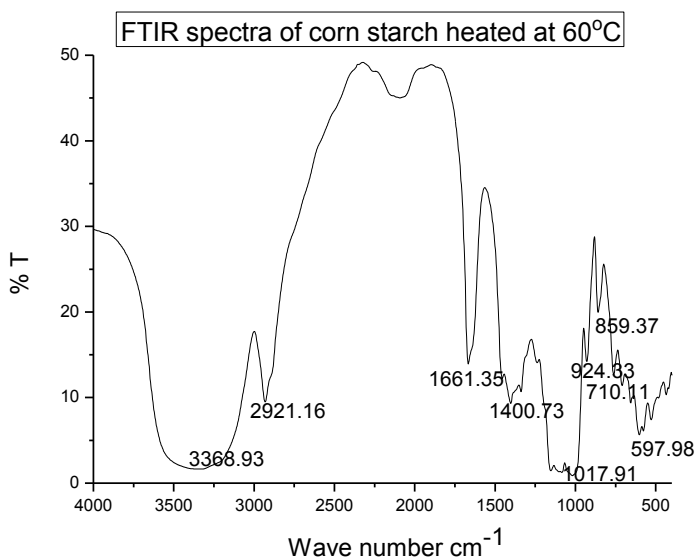
Figure 5.9: FTIR spectra of corn starch heated at 60 °C
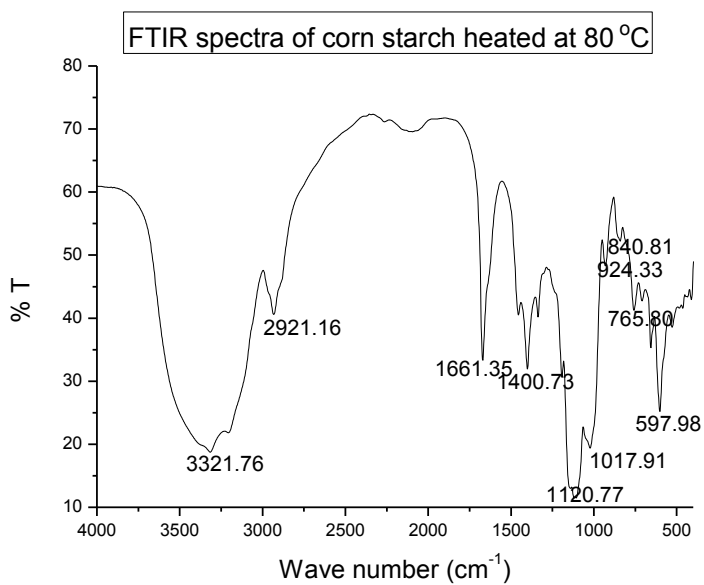
Figure 5.10: FTIR spectra of corn starch heated at 80 °C
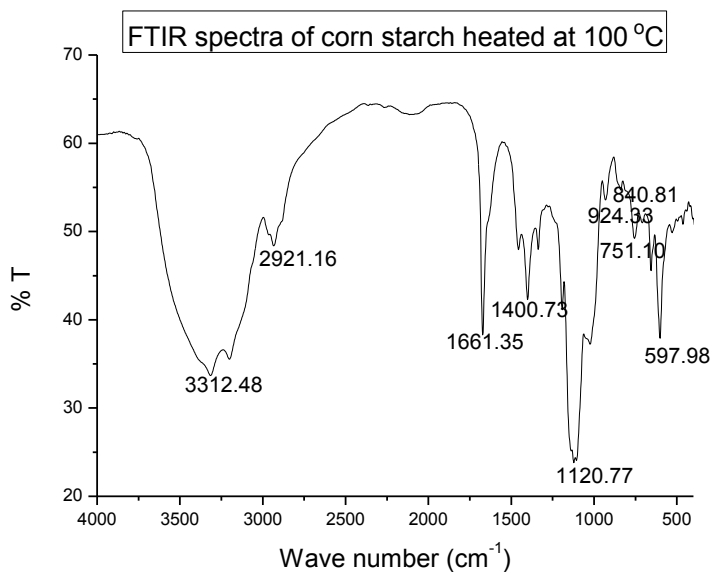
Figure 5.11: FTIR spectra of corn starch heated at 100 °C
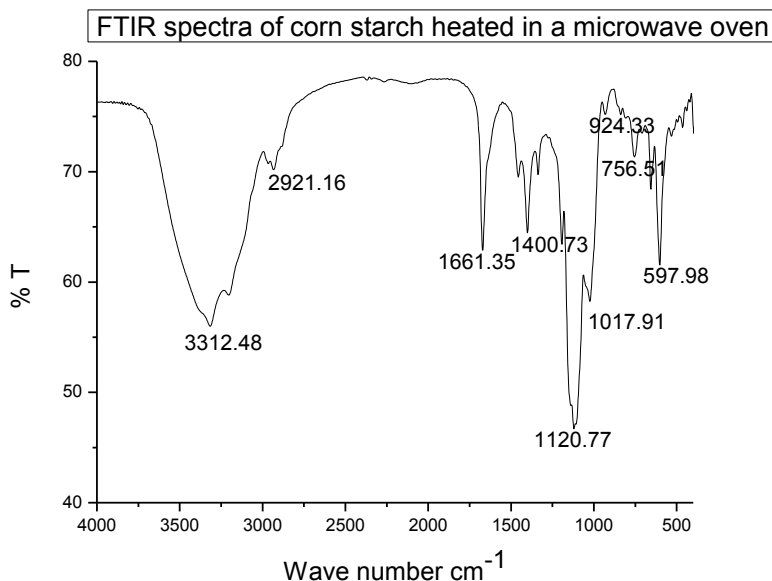
Figure 5.12: FTIR spectra of corn starch heated in a microwave oven

Some band narrowing and intensity changes were also observed after the heat treatments. This band narrowing occurs due to the ordering of polymer structure and thus the decrease in the number of conformations that they can form. Intensity changes are due to the changes in the specific conformation of starch molecules. The heating process causes the destruction and formation of hydrogen bonds, which will also affect the vibrational intensity [113]. The result could be used to increase the application of microwave heating of starch in food industries and the structural studies of modified starches in many industries like pharmaceutical industries.

5.3 Nuclear Magnetic Resonance Spectroscopic (NMR) Studies

Nuclear magnetic resonance spectroscopy provides atomic-level structural information and can successfully be used for complex biomolecules like starch [162]. NMR studies allow the quantitative analysis of molecular structures within the starch granules. It depends mainly on the degree of branching, orientations of molecules and the crystallinity of starch granules [163, 46]. NMR can effectively study structural changes that occurred by the modification of starches. The changes are observed as new peak formation and peak broadening [164]. This technique can also successfully used for finding the presence and quantify other molecules like the protein molecules in starch flours [108].
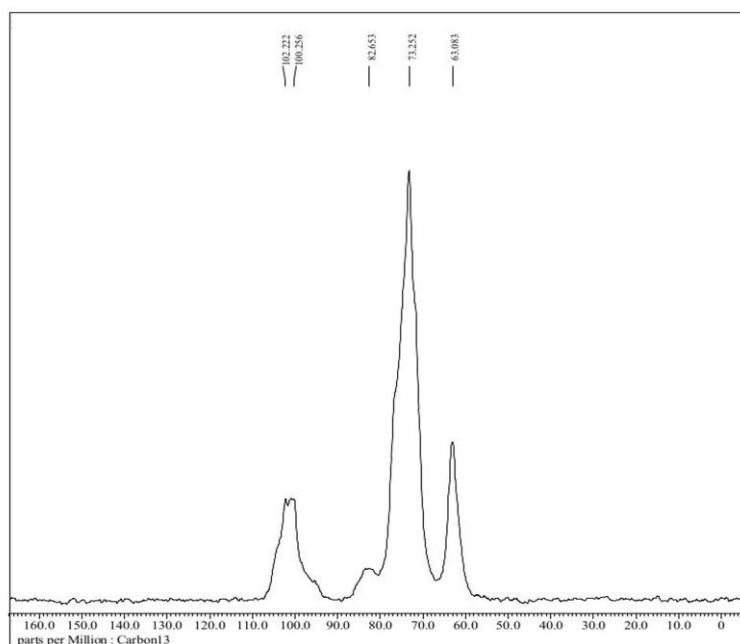
Figure 5.13: NMR spectra of stem starch

Table 5.2: NMR resonance assignment of stem starch

Chemical shifts of ^{13}C resonance (ppm)	NMR Resonance assignments
102.222	C1[109, 165, 166]
100.256	C1[165, 164]
82.653	C4 [109, 166]
73.252	C3 of oligosaccharides and large amylopectin fragments[163], C2 and C5[109]
63.083	C6[109]

In this study, the stem starch showed a chemical shift of 102.222 ppm, 100.256 ppm, 82.653 ppm, 73.252 ppm and 63.083 ppm (Figure 5.13). The peak assignments are given in table 5.2. The chemical shift positions obtained were the characteristic feature of native starches. This can be successfully used for studying the long-range helical interactions and further modification studies on stem starch.

5.4 X-ray diffraction (XRD) studies

Starch molecules are composed of amorphous and ordered (crystalline) areas. The ordered regions are composed of the short chains of amylopectin molecules that are arranged in clusters. The short range order and the long range order packing of amylopectin molecules can be seen in these ordered regions. According to the long range order of amylopectin double helix packing, the starch granules are of two crystal types-A and B. In A-type, the double helixes are closely packed (orthogonal packing) with small number of water

molecules inside the unit cell. In the case of B-type, the packing is more open (hexagonal) and contains more number of water molecules. Another type of starch-the C-type contains both of these A and B type polymorphs in their crystal structure [167]. In diffraction studies, the crystalline samples give reflections from their crystal planes according to their nature [168].

X-ray diffraction is a technique thus used for the molecular structural elucidation of starch granules and is used to study the crystalline properties of starches. Starch crystallinity is due to the formation and packing of double helix between the chains of amylopectin molecules as already mentioned [38, 36].

The A-type starch granules show peaks around 15º, 17º, 18º, 20º and 23º 2θ angles, B-type granules show around 5º, 15º, 17º, 20º, 22º and 24º 2θ angles and C-type starch has the mixture of A and B [44, 133]. A-type starches are mostly found in cereal starches. B-type in tuber and root starches and C-type (mixture of A and B), in particular root, legume, and seed starches. The packing of the helix in A-type starch is more compact and less hydrated than B-type as mentioned above [30, 169, 170].

In this study, the stem starch showed main peaks at 2θ angles 15.4º, 17.26º and 23.21º and corn starch showed at 14.73º, 17.85º and 23º (Figure 5.14). Stem starch showed the peaks of A-type granules, a characteristic feature of cereal starches. The result was in agreement with those reported by Nakthong et al. in 2017 [96].
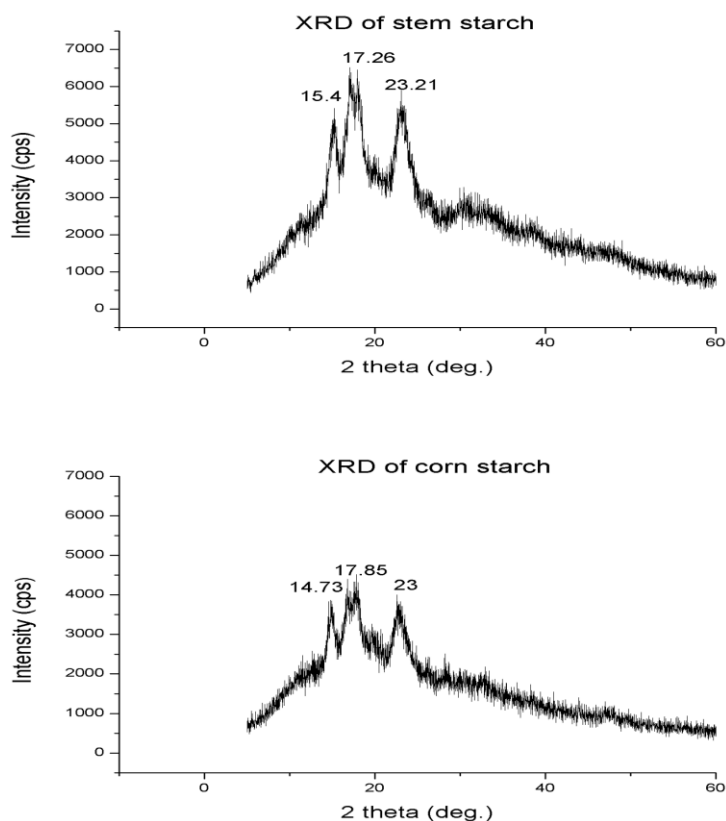
Figure 5.14: XRD of stem and corn starches

The relative crystallinity of stem starch was found to be 25.12±0.50 %, and that of corn starch was 22.34±0.67 %. Higher crystallinity is the indication of higher structural stability of stem starch than corn starch [96]. The increased proportion of crystalline fraction makes the granular bonding stronger, which leads to an increased gelatinization temperature [171]. It was also reported that the native starch granules have a crystallinity range of 15 to 45 % [172].

In order to explore the presence of any difference in the packing of the double helix in the starch structure (crystallinity change) with plant growth, XRD analysis was done with the starch isolated from the pineapple plant stem at different growth stages.

5.5 X-ray diffraction study of stem starch extracted from different growth stages

The X-ray diffraction studies was done on the starch extracted from the pineapple stem at different growth stages of three months, six months, nine months, twelve months, fifteen months and eighteen months intervals.
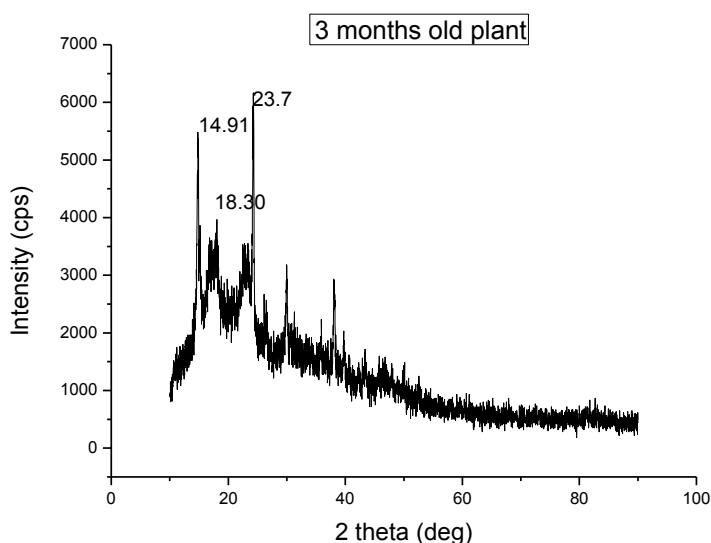
Figure 5.15: X-ray diffractogram of stem starch extracted from three months old pineapple plant
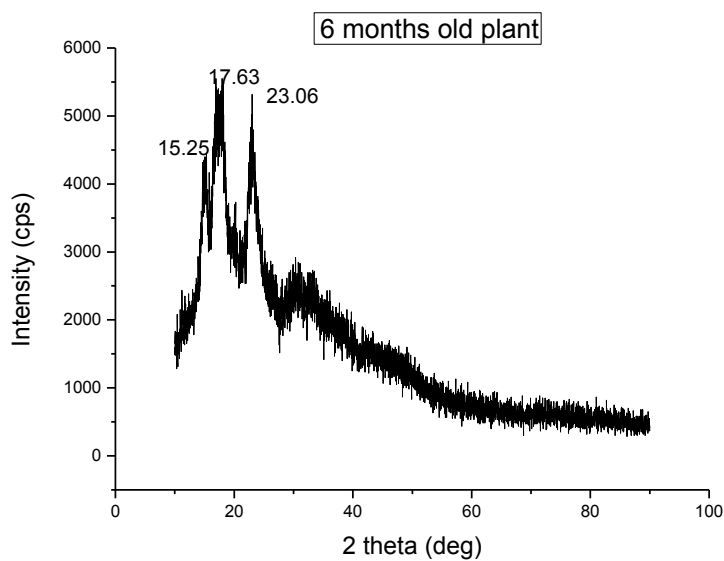
Figure 5.16: X-ray diffractogram of stem starch extracted from six months old pineapple plant
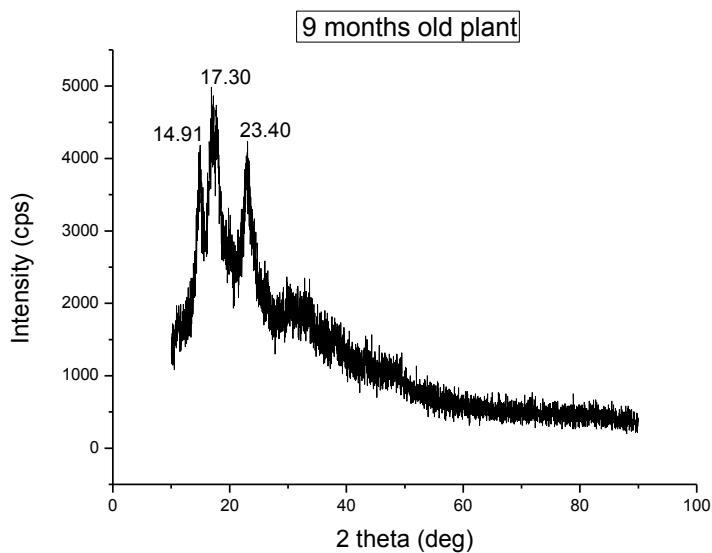
Figure 5.17: X-ray diffractogram of stem starch extracted from nine months old pineapple plant
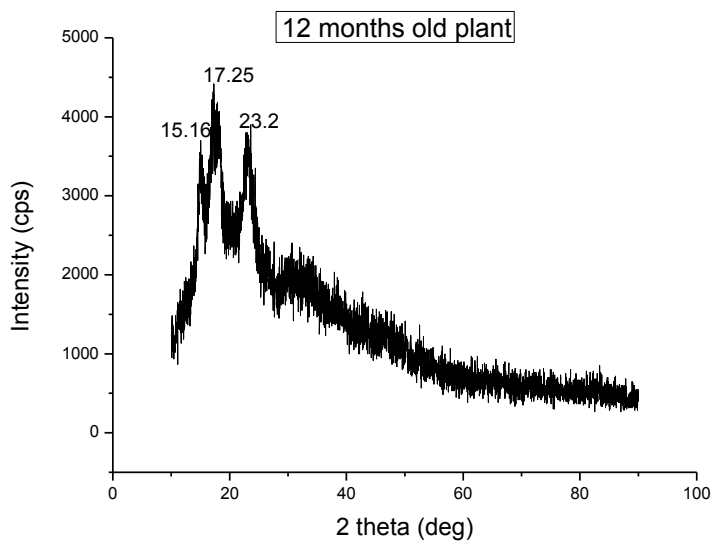
Figure 5.18: X-ray diffractogram of stem starch extracted from twelve months old pineapple plant

Figure 5.19: X-ray diffractogram of stem starch extracted from fifteen months old pineapple plant

Figure 5.20: X-ray diffractogram of stem starch extracted from eighteen months old pineapple plant

Three months old plant showed peaks at 2θ angles 14.91, 18.3 and 23.7 (Figure 5.15), six months old plant had peaks at 2θ angles 15.25, 17.63 and 23.06 (Figure 5.16), nine months old plant showed at 14.91, 17.30 and 23.40 (Figure 5.17). Starch from 12 months old plant showed major peaks at 2θ angles 15.16, 17.25 and 23.2 (Figure 5.18). Fifteen months had peaks at 14.91, 17.30 and 23.06 (Figure 5.19) and the eighteen months showed main peaks at 2θ angles 15.4º, 17.26º and 23.21º (Figure 5.20). From the result obtained, it can be concluded that this stem starch possesses A-type crystals, and throughout all the growth stages studied.

This chapter revealed that the FTIR spectra obtained for stem starch were characteristic of native starches and comparable to that of corn starch. It was observed that the different heat treatments (moist heating and microwave heating) did not make any change in the chemical groups already present in the stem starch molecule and did not produce any new chemical groups. NMR spectra obtained was similar to those obtained for other native starches and can be used for studying the long-range helical interactions that may occur in stem starch during the modification studies. XRD analyses revealed that the stem starch possessed A-type granules, which was a characteristic feature of cereal starches and the same crystal structure was maintained throughout in all the growth stages studied.

Chapter 6

GRANULAR CHARACTERIZATION OF STEM STARCH USING SCANNING ELECTRON MICROSCOPY (SEM)

6. GRANULAR CHARACTERIZATION OF STEM STARCH USING SCANNING ELECTRON MICROSCOPY (SEM)

Starch is synthesized and stored as granules in the leaves and accumulated in the plant storage sites. Starch granules are well organized complex structures and the size vary from 1-100 μm [173, 174]. Scanning electron microscopy (SEM) is a frequently used technique for granular characterization of starches. It can be used for studying the granule morphology more accurately than light microscopy [175, 176]. Surface characteristics of granules have many practical applications as it influences the enzyme actions. The enzyme action on starch granules largely determines the quality of starch-based food products [114]. Viscosity, one of the main physical properties of starches, is influenced mainly by their granule morphology [177]. The granular properties of starch are essential for understanding other starch properties like rheology, thermal and pasting properties [173, 178, 179, 177].

6.1 Granular properties of pineapple stem starch

SEM studies revealed that the granular size of stem starch was comparatively smaller than the corn starch granules and was mainly polyhedral with sharp angles and edges and surfaces were smooth with no surface pores (Figure 6.1).
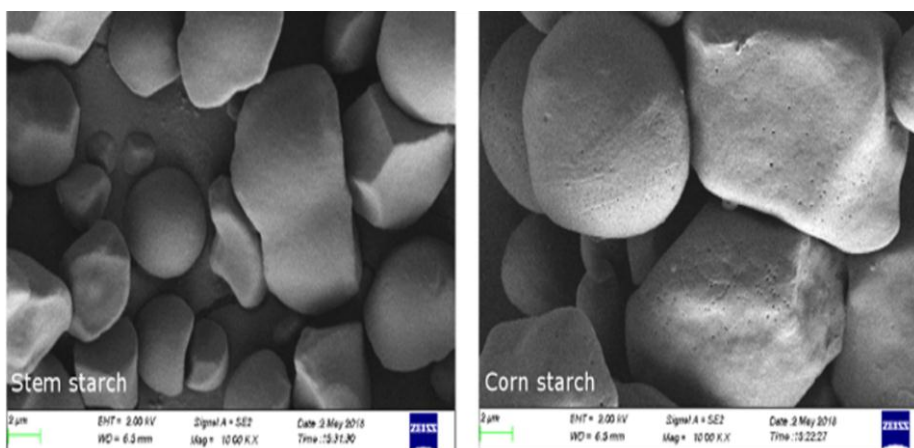
Figure 6.1: The SEM images of the pineapple stem starch and corn starch

Nakthong et al. 2017 reported that the size of pineapple stem starch granule is smaller than corn, rice and cassava starch granules. Our finding was in agreement with that reported by Nakthong et al. 2017 [96] and Jane et al. 1994 [180]. Wei et al. 2018 reported that another stem starch-cassava stem starch granules have a size of 5.65-7.64 µm [53]. Small and medium-sized starch can be used as a fat substituent, stabilizers in baking powder, stiffening agent in laundry and in the manufacture of biodegradable plastics. Small sized granules are also gaining interest in food applications [181, 182].

6.2 Morphological properties of the stem starch granules with different growth stages of the pineapple plant

Starch morphology is highly varied with the botanical source (Table 6.1). This morphological variation has a significant influence in determining the physicochemical and functional properties of a particular starch [180, 183, 177].

Table 6.1: Granule morphology of starches extracted from different
sources [180]

Sl No.	Source	Size (μm)	Shape
1	Water chestnut , sweet potato	5-30	round, polygonal, irregularly shaped, intermediate size
2	Sorghum	5-30	irregularly shaped
3	Oat starch and millet starch	2-15	smaller irregularly shaped, polygonal granules
4	Normal maize	5-20	irregularly shaped granules with a number of faces (polyhedric) and relatively sharp edges
5	Mung bean	10-27	oval and irregularly shaped granules
6	Banana	15-45 (long axis) 10-20 (short axis)	extended irregularly shaped granules that are disk shaped
7	Tapioca	5-40	Smooth, irregular and intermediate size

Figure 6.2 to 6.7 showed the SEM photographs of the stem starch at
different growth stages of the plant.
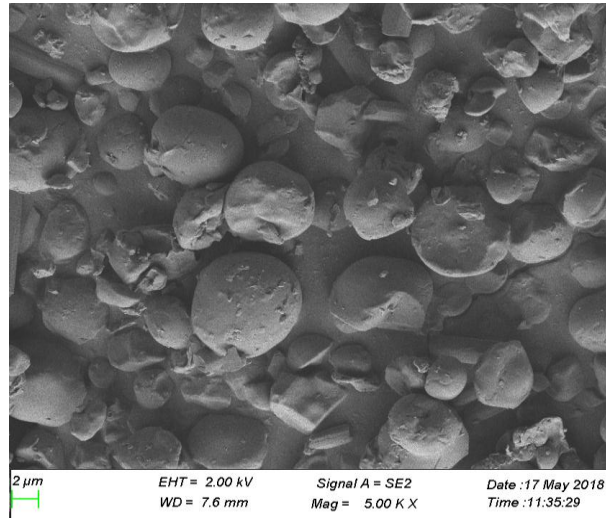
Figure 6.2: SEM image of stem starch extracted from three months old pineapple plant
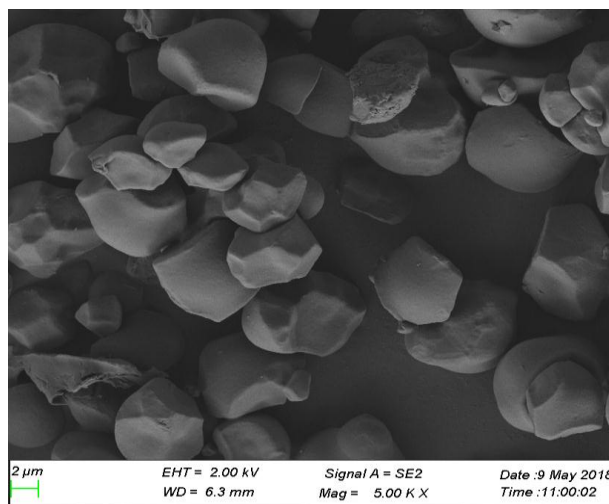
Figure 6.3: SEM image of stem starch extracted from six months old pineapple plant
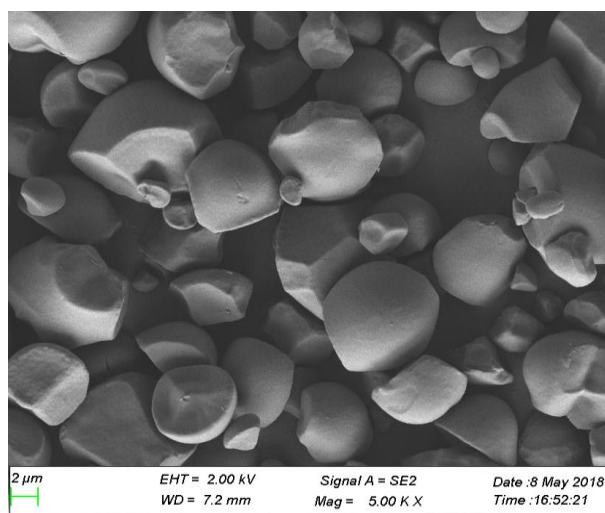
Figure 6.4: SEM image of stem starch extracted from nine months old pineapple plant
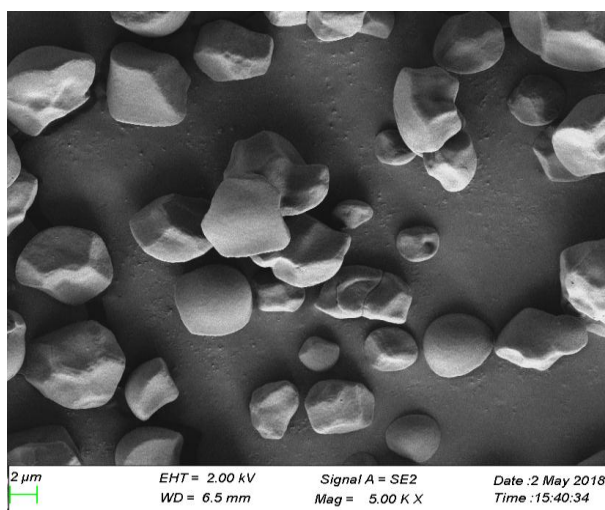
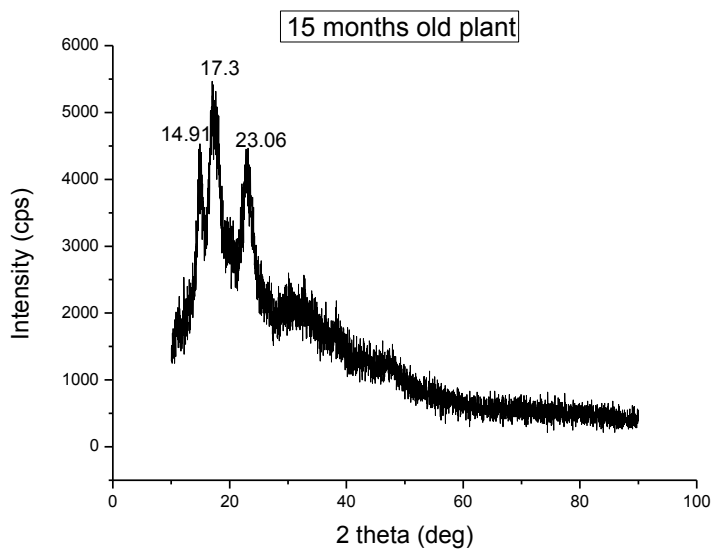Figure 6.5: SEM image of stem starch extracted from twelve months old pineapple plant

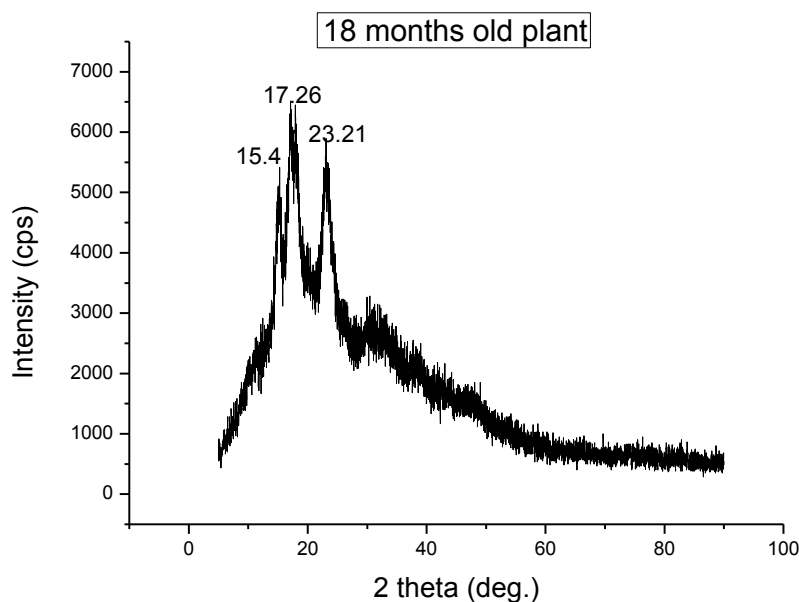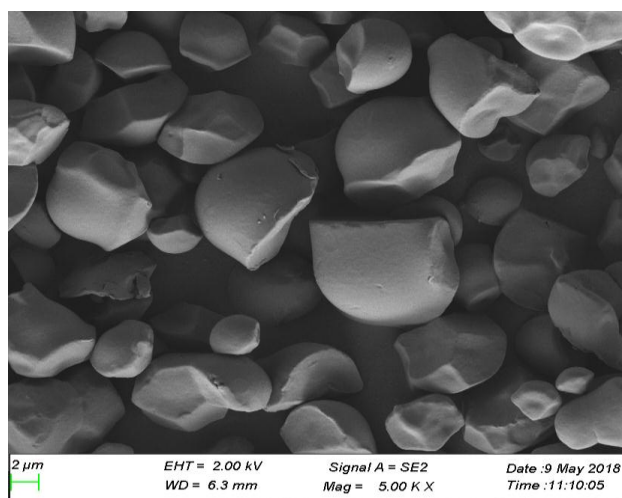Figure 6.6: SEM image of stem starch extracted from fifteen months old pineapple plant
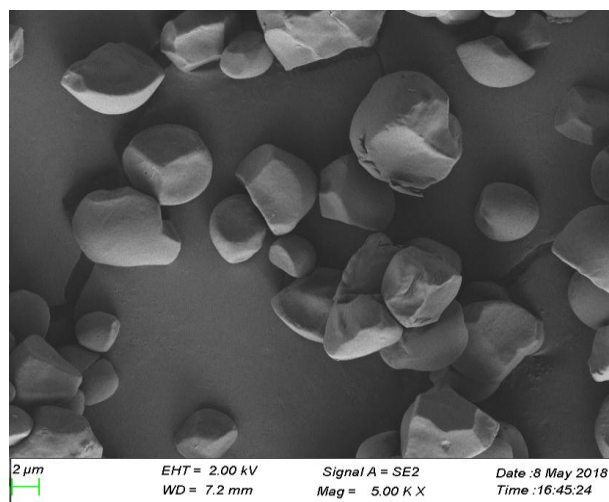
Figure 6.7: SEM image of stem starch extracted from eighteen months old pineapple plant

The analysis revealed that there was no uniform distribution in granule size and shape. An increasing trend was observed in granule size with age (Table 6.2).

Table 6.2: Granule size variation in the stem starch at different growth stages of the plant

Age of the plant	Size of the starch granules (μm)
Three months	1-7
Six months	1.2-7
Nine months	1.5-8
Twelve months	2.8-7
Fifteen months	2.2-8.5
Eighteen months	3-7

The shape was mainly polyhedral with sharp angles and edges and surfaces were smooth with no surface pores, and the round-shaped granules were also present. This observation was the same in all the observed stages of plant growth. These types of granules can be seen in the tuber and root starches [184, 175]. Starch granules can grow bigger with the age of the plant [154, 155]. Growth environment can also influence starch granule size distribution [53].

6.3 The effects of microwave heating and moist heating on starch granular structure

The mode of heating affects the starch granules. This effect will different for each starch extracted from various botanical sources. The microwave heating and moist heating are common steps in the

food industry.   Granular changes during different types of heat treatment at different temperature were observed and compared with corn starch (Figure 6.8 to 6.12).
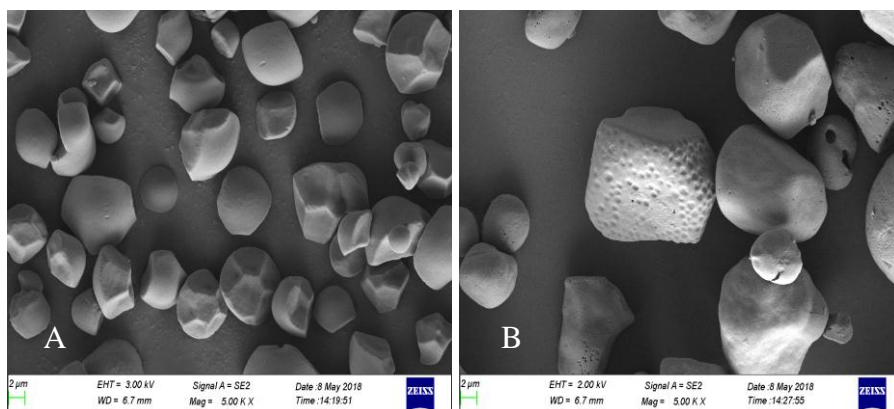
Figure 6.8: Stem (A) and corn (B) starch granules after heating at 40 °C
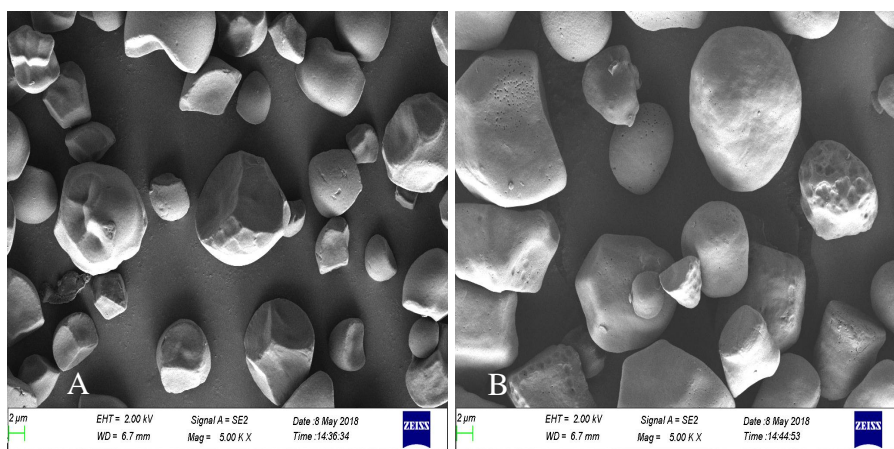
Figure 6.9: Stem (A) and corn (B) starch granules after heating at 60 °C
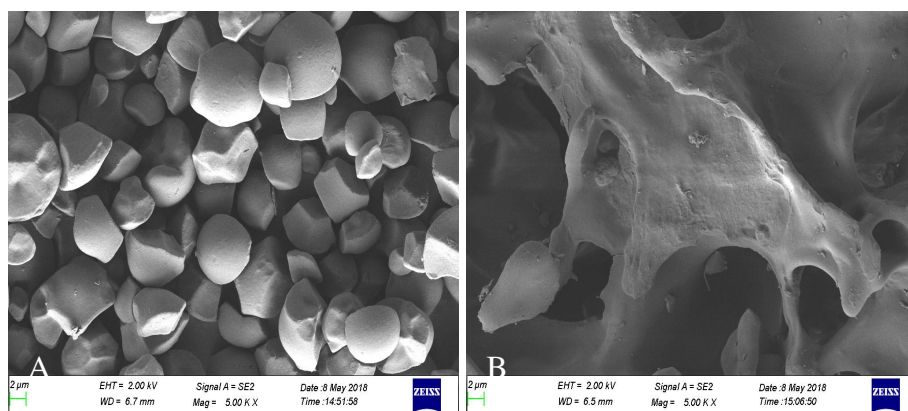
Figure 6.10: Stem (A) and corn (B) starch granules after heating at 80 °C
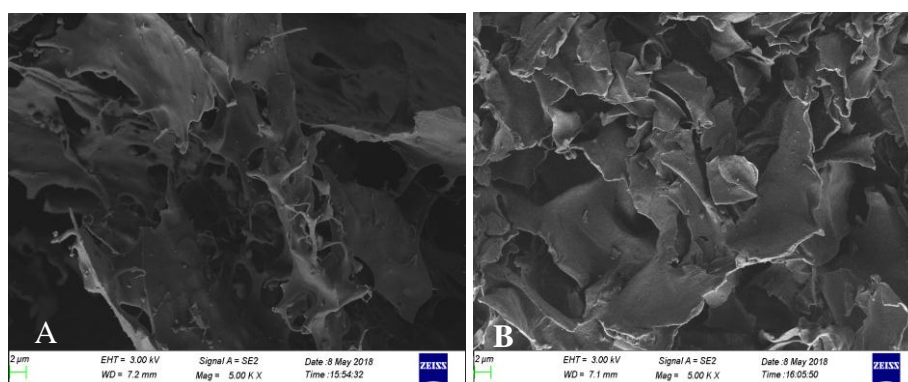
Figure 6.11: Stem (A) and corn (B) starch granules after heating at 100 °C
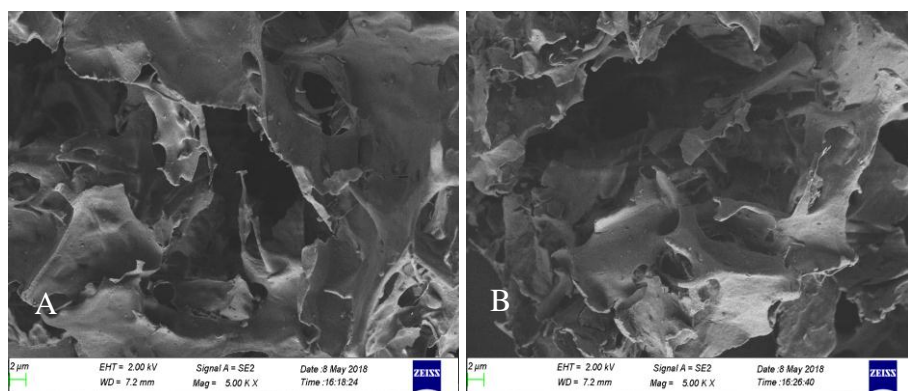
Figure 6.12 Stem (A) and corn (B) starch granules after microwave
heating

Figure 6.11 showed that above 80 °C stem starch granules wholly disrupted. In the case of corn starch, complete disruption occurred below 80 °C (Figure 6.10). It was the indication of higher gelatinization temperature, thus the higher stability of stem starch granules than the corn starch granules. Microwave heating completely disrupted both starch granules.

Starch granules had a layered structure when they heated to their gelatinization temperature, and above that temperature, the layers disappeared. Both types of heating completely disrupted the granules, but the swelling and heating patterns were different for both type of heat [107]. Palav and Seetharaman, 2007 reported that the pattern of starch gelatinization will be different in both types of heating. As the heating increases rapidly in a microwave oven, the structural changes occur earlier than the normal moist heating and so, there will be a lack of granular swelling of starches [111].

6.4 Dispersion of starch in an ionic liquid-'1-butyl-3-methylimidazolium chloride'

Ionic liquids are the salts with weakly coordinated ions [185]. Due to the biodegradable nature of the ionic liquids, there is an increasing trend of using these liquids in industries as solvents. 1-butyl-3-methylimidazolium chloride is one among them. They are preferred due to their ability to solvation at room temperature, biodegradability and are excellent green solvents. These types of ionic liquids could replace many hazardous solvents and thus helps environmental friendly processes [50, 186]. Here the pineapple stem starch

dispersed in an ionic liquid-1-butyl-3 methylimidazolium chloride and observed the granular changes using SEM. All the data were compared with that of commercial corn starch.
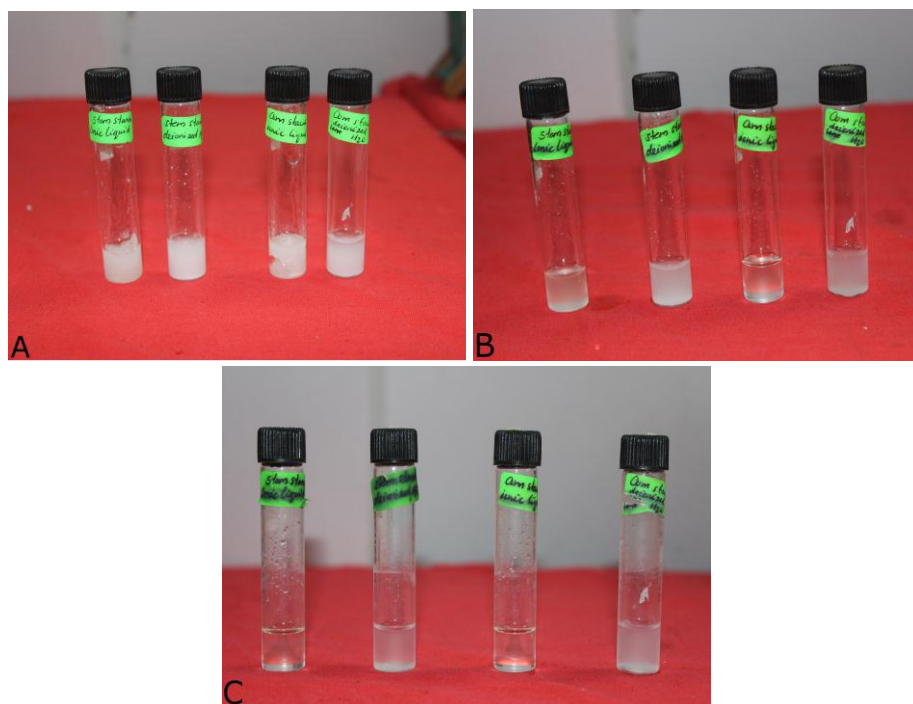
Figure 6:13 A: Stem starch (first and second tube from left) and corn starch (third and fourth from left) in ionic liquid and in deionized water before heating, B: Stem starch and corn starch after heating for 15 minutes from 25 to 80 ºC and C: Stem starch and corn starch after heating for 1 h at 100 ºC.

Before heating, both starch suspension was opaque (Figure 6.13 A). After heating for 15 minutes from 25 to 80 ºC, both starch suspensions were translucent to opaque for both deionized water and ionic liquid (Figure 6.13 B). But corn starch in the ionic liquid was a

somewhat clear solution (Figure 6.13 B-third from left). Both starches dispersed in deionized water was translucent even after heating at 100 ºC for 1 h (Figure 6.13 C-second and fourth from left).

In contrast, dispersion in the ionic liquid of both starches became transparent solutions after heating at 100 ºC for 1 h (Figure 6.13 C-first and third from left). Therefore it can be concluded that the ionic liquid, 1-butyl-3-methylimidazolium chloride was more effective in dispersing starch than deionized water. The result was in agreement with the effect reported by Stevenson et al. 2007 [50].

In scanning electron microscopy, it was observed that both deionized water and ionic liquid disrupted the granular structure of starch compared to the native starch structure. Deionized water treated stem starch showed cracks and rough surface (Figure 6.14 A and B). In corn starch, there were no cracks; the surface was smooth and had no pores (Figure 6.14 C and D). Ionic liquid treated stem starch showed small granular shape (Figure 6.14 E and F). Granules were fused to form large aggregates and were not distinguishable in all other cases.
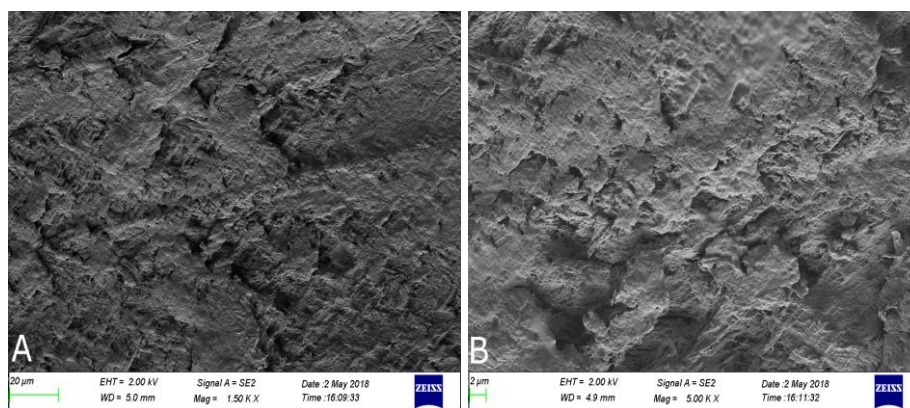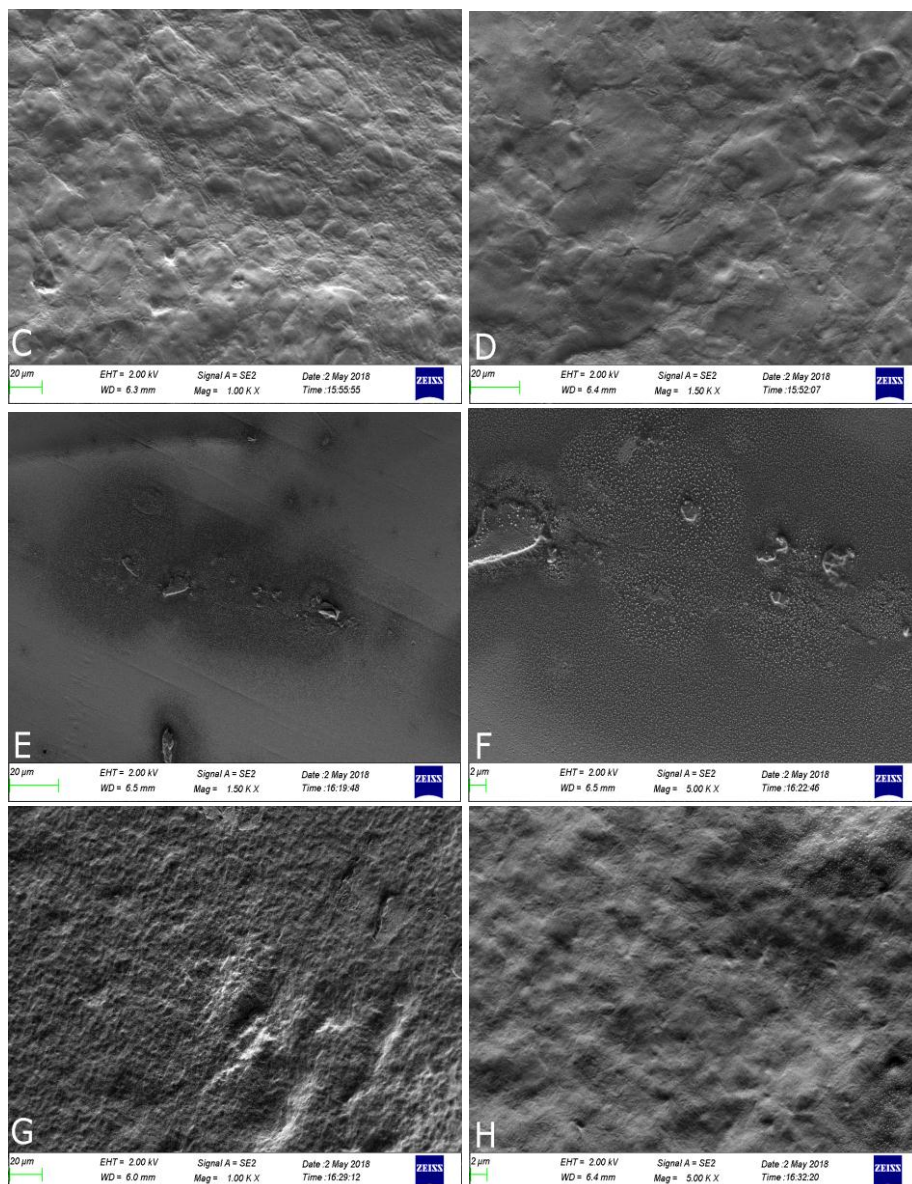
Figure 6.14: Morphological changes in starch granules after dispersed in ionic liquid and in deionized water: Stem starch treated with deionized water (A and B), corn starch treated with deionized water (C and D), stem starch treated with ionic liquid (E and F) and corn starch treated with ionic liquid (G and H)

From the results obtained from the granular studies of stem starch using SEM, it can be concluded that the stem starch granules had no uniform distribution in granule morphology. The shape was mainly polyhedral with sharp angles and edges and surfaces were smooth with no surface pores and smaller size than the corn starch. Small granules are gaining interest in food applications and they can be used as a fat substituent, stabilizers in baking powder, stiffening agent in laundry, and in the manufacture of biodegradable plastics.

Shape of the stem starch granules was the same in all the observed stages of plant growth with an increasing trend was observed in granule size with age.

Both types of heating (moist and microwave) completely disrupted the stem starch granules. In moist heating, the stem starch granules wholly disrupted only after the temperature exceeded 80 °C. In the case of corn starch, complete disruption occurs below 80 °C. It was the indication of higher gelatinization temperature, thus the higher stability of stem starch granules than the corn starch granules. These types of granules are more preferred in applications where stable crystals are needed (e.g. in the preparation of gum candies. Results from the ionic liquid (1-butyl-3-methylimidazolium chloride) treatment revealed that both deionized water and ionic liquid disrupted the granular structure of starch compared to the native starch structure. Ionic liquid treated stem starch showed a small granular shape in their structure, and this was absent in the case of corn starch. It can also be concluded that 1-butyl-3-methylimidazolium chloride is a useful solvent for starches that will also promote green chemistry.

Chapter 7

THERMAL, RHEOLOGICAL AND PESTICIDE RESIDUE ANALYSIS OF PINEAPPLE STEM STARCH

7. THERMAL, RHEOLOGICAL AND PESTICIDE RESIDUE ANALYSIS OF PINEAPPLE STEM STARCH

New food materials produced using starch as raw material, was the result of several characterization studies on starch [56]. Among these, thermal and rheological studies have much importance. Improving or controlling the texture of foodstuffs is the basis of the designing and production of new food products. This was mainly achieved by the viscoelasticity and the gelatinization properties of starch. The dynamic rheological method is the best technique and can be used for viscoelasticity measurements [32]. Differential scanning colorimetry (DSC) is the most commonly used method for studying starch gelatinization.

7.1 Rheological properties of pineapple stem starch

Rheological studies can be used to describe the consistency of different food products using starch which is done by measuring viscosity and elasticity of starch. They are also related to the size, shape, and molecular weight of the starch molecules [187]. Starches from different plant sources show variation in their rheological properties [188]. They possess unique rheological properties with a change of temperature, the concentration of starch and the frequency applied. This can be effectively studied by a dynamic rheometer [56]. Here, we have used the dynamic rheometer, which allowed the continuous assessment of storage modulus, loss modulus, complex viscosity, and phase angle with frequency change of the starch suspensions. The rheological properties of pineapple plant stem starch was compared to

that of commercial corn starch. The study also examined the region-wise (cortex region, middle region and the core region of the stem) difference in the rheological properties of the stem starch (Figure 7.1).
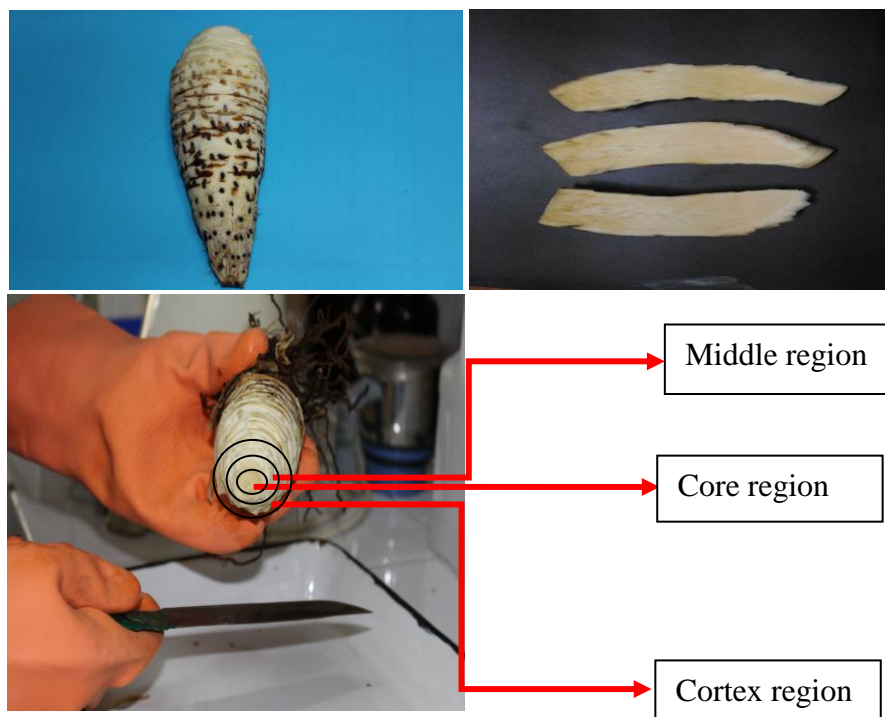
Figure 7.1: Pineapple plant stem and their different regions

It was reported that the rheological properties of a starch mainly depends on its granule size, shape, rigidity, swelling pattern and de-formability, amount and type of amylose/amylopectin which has leached from the granules, surface of starch granule, granule-amylose/amylopectin interactions and granule-granule contact [32, 189, 190].
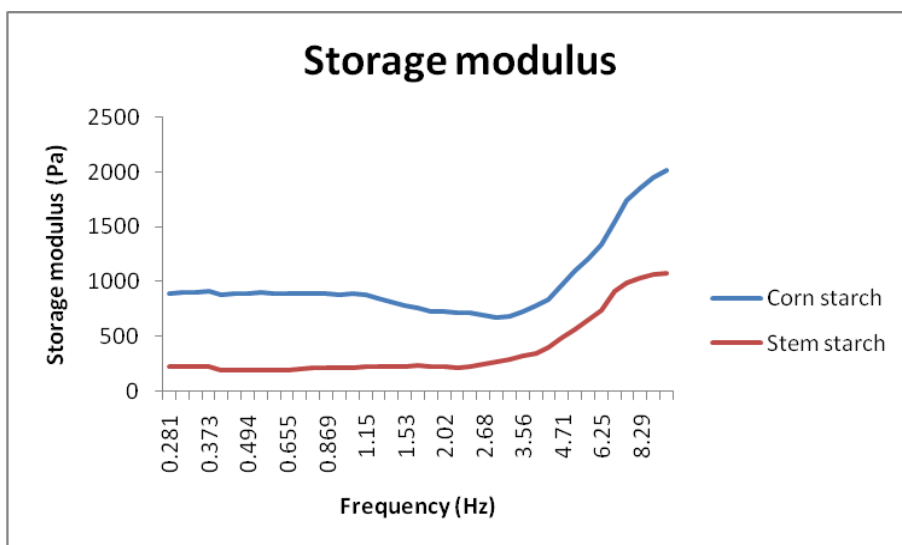
Figure 7.2 Storage modulus of stem starch and corn starch

Rheological parameter-the storage modulus (G') is the measure of energy stored in a material, which describes the elastic properties of that material [32]. The storage moduli of both starches were increasing. Corn starch showed higher values than that of stem starch (Figure 7.2). It was reported that the storage modulus depends on the amylose content and granule structure of starch molecules. Starches with lower amylose content showed a lower value for G' [99, 191]. High amylose content gave high rigidity for starch granule structure [32, 60]. During frequency variation, storage modulus (G') of both the sample showed almost a steady state until 3.56 Hz, and thereafter increased markedly. It was reported that the plateau-like graph of the storage modulus was the indication of the presence of network structures in the starch gels [192]. There was a significant difference in storage modulus for both starches in all the observed frequencies (0.1-10 Hz).
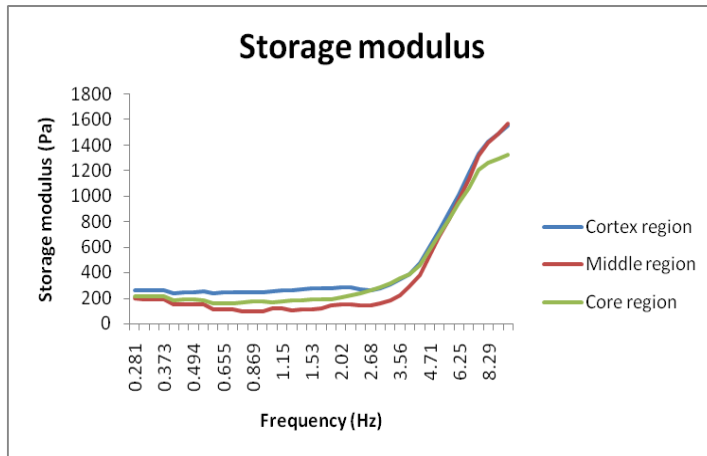
Figure 7.3: Storage modulus of starch from different regions of the plant stem

Figure 7.3 shows the storage modulus of starch extracted from different regions of the pineapple plant stem and there was no significant difference observed at 6.25 Hz.
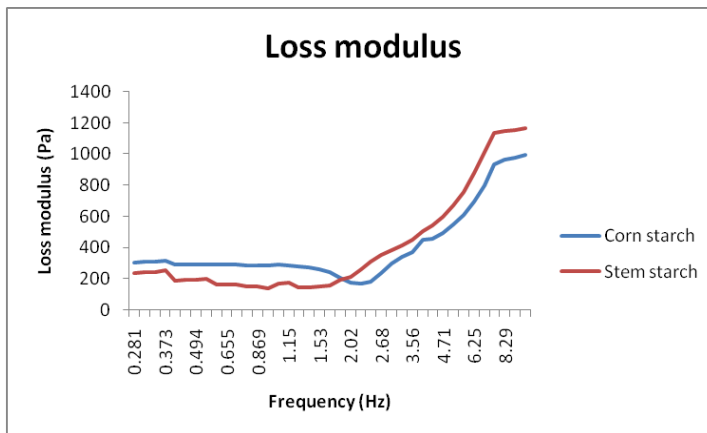
Figure 7.4: Loss modulus of stem starch and corn starch

Loss modulus (G") describes the viscous properties of a material. Stem starch and corn starch showed significant difference in

loss modulus at all the observed frequencies (Figure 7.4). There was no statistical difference in loss modulus of starch from different regions of the stem at the frequencies, 2.02, 2.22 and 2.44 Hz (Figure 7.5).
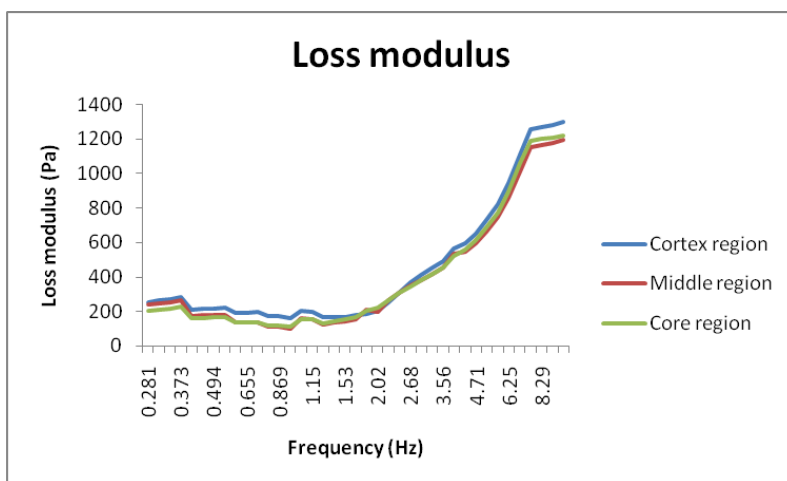
Figure 7.5: Loss modulus of starch from different regions of the plant stem
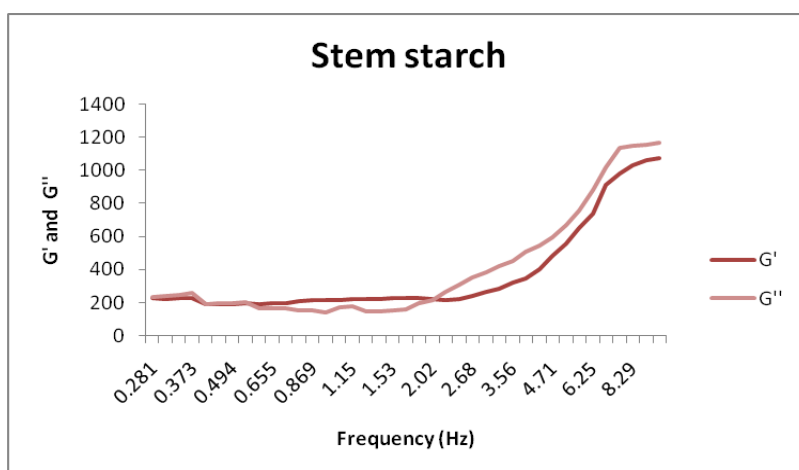
Figure 7.6: Comparison of storage modulus and loss modulus of stem starch
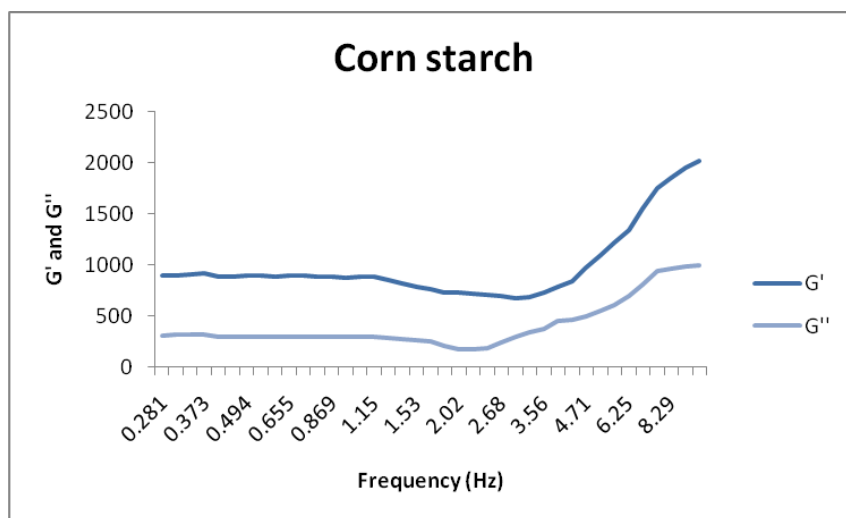
Figure 7.7: Comparison of storage modulus and loss modulus of corn starch

In the case of stem starch, there was no predominant difference in G' and G" values, compared to corn starch. At low frequency, the storage modulus (G') was slightly higher than the loss modulus (G"). This behaviour indicated solid-like behaviour at low frequencies. As frequency increases, G" crosses the G', this response of the material beyond this cross-over frequency was said to be liquid-like behaviour. Stem starch showed a slightly higher value for G" than G' at higher frequencies (after 2.22 Hz) (Figure 7.6). But in the case of corn starch, the G' was found to be consistently higher than G" in the studied frequency range, which indicated a predominant elastic behaviour (solid-like) of corn starch (Figure 7.7). Higher G' was the indication of high stiffness of starch gel [193]. From this, it can be concluded that the stem starch was more viscous (more liquid-like) and its gel was less stiff than corn starch.

Measuring of viscosity during shear stress contribute a major role among the rheological properties as starch is mainly used as a thickening agent in many industries [193]. The dynamic frequency has a significant effect on the complex viscosity of starches [190].
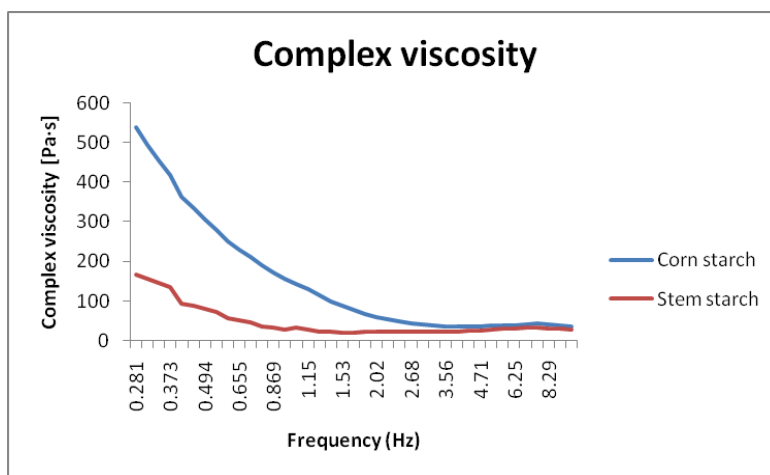
Figure 7.8: Complex viscosity of stem starch and corn starch
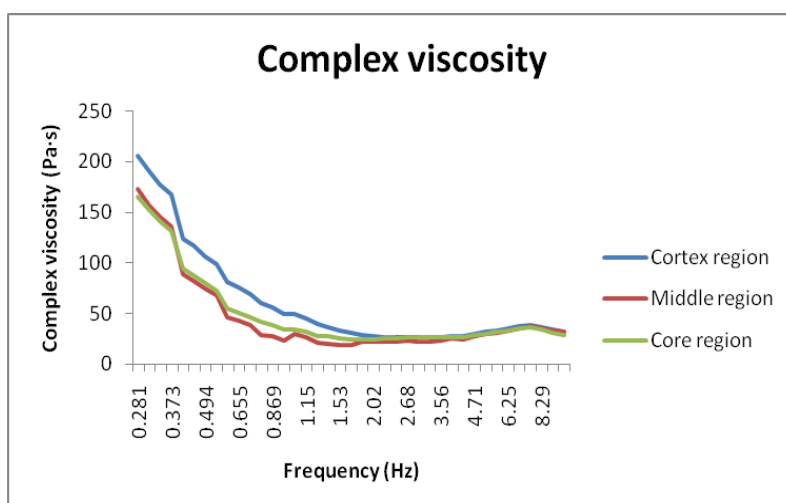
Figure 7.9: Complex viscosity of starch from different regions of the plant stem

As shown in the figure (Figure 7.8), the complex viscosity (η^*) of both starches decreased markedly (shear thinning behaviour) as frequency changed from 0.1 to 10 Hz. A similar result has earlier been reported for lentil starch by Ahmed and Auras in 2011 [60]. The observed viscosity decrease at lower frequencies resulted in a decrease of energetic interactions [192]. During the same frequency change, the native stem starch showed low complex viscosity values compared to corn starch. It was reported that higher the amylose content, the higher will be the shear viscosity and more will be the shear thinning behaviour [188, 194]. So the lower viscosity value of stem starch resulted from the low amylose content of stem starch than the corn starch. However, the two starches followed the almost same pattern of decrease. The complex viscosity of stem starch decreased sharply until around 1.39 Hz and then showed a steady pattern. Corn starch showed a notable decrease until 2.68 Hz and then followed a steady pattern. There was a significant difference observed for complex viscosity of stem starch and corn starch. Starch from different regions of the plant stem also showed significant variation at a 5 % level of significance.

When an input signal (stress or strain) is applied to a sample, it will respond according to the applied stress or strain. There will be a phase displacement between the deformation and the response. The phase angle (δ) or phase shift is the angle formed between the input signal and response. If the phase angle is zero, then the material is purely elastic (solid-like behaviour). If the phase angle is 90°, it means the material is purely a viscous (liquid-like behaviour). Visco-elastic substances have the phase angle between these two limiting values. If

the G' > G", phase angle less than 45°, then the material is solid-like. If the G" > G', phase angle higher than 45°, the material will be liquid-like [192].
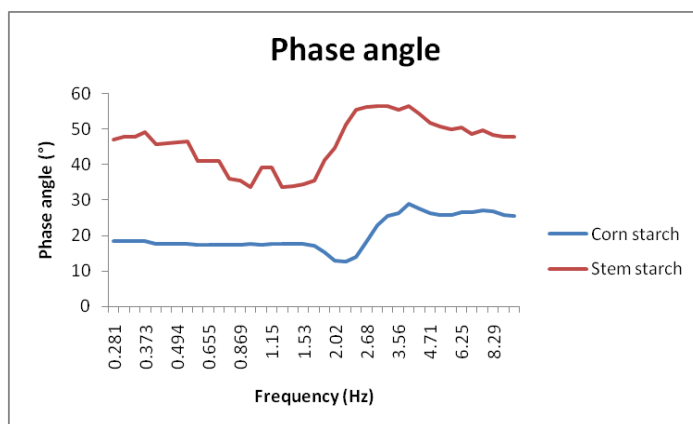
Figure 7.10: Phase angle of stem starch and corn starch
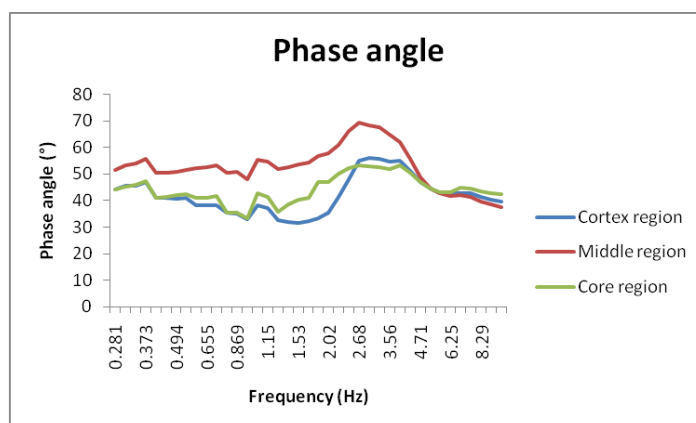
Figure 7.11: Phase angle of starch from different regions of the plant stem

In this study, the corn starch showed lower value of δ than the stem starch for all the observed frequencies. Lower value of δ was the indication of solid-like behaviour [60, 195]. It was reported that as the

volume of swollen granules increases, δ will decrease [196]. In the case of stem starch, at lower frequencies there was a decrease of δ until 1.68 Hz, then the value increased. The maximum value (56.37°) obtained at 2.68 Hz. For corn starch, maximum value observed was 28.91° at 3.91 Hz (Figure 7.10). This was the indication of liquid-like behaviour of stem starch compared to corn starch. There was significant difference observed at all the frequencies (0.1-10 Hz) for both starches. In the case of starch extracted from different regions of the stem, there was no significant difference observed at 4.71, 5.18, 5.69 and 6.25Hz (Figure 7.11).

The rheological properties of corn starch, stem starch, and starch from different regions of the stem at a frequency of 10 Hz is given in the table 7.1

Table 7.1: Rheological properties of corn starch, stem starch, and starch from different regions of the stem at a frequency of 10 Hz

Starch type	Storage Modulus (Pa)	Loss Modulus (Pa)	Complex Viscosity (Pa.S)	Phase angle (°)
Corn starch	2016.50±67	991.33±37	36.06±1.31	25.44±1.05
Pineapple plant stem starch	1074.33±42	1165.67±47	27.69±1.17	47.82±1.99
Starch extracted from the cortex region of the stem	1549.67±65	1300.00±55	32.57±1.42	39.30±1.63
Starch extracted from the middle region of the stem	1565.00±66	1195.67±47	31.05±1.38	37.47±1.52
Starch extracted from the core region of the stem	1321.00±52	1220.00±51	28.41±1.20	42.47±1.76

7.2 Differential scanning colorimetric (DSC) analysis

Differential scanning colorimetry is widely used to study starch gelatinization. Gelatinization is an irreversible structural change (mainly the loss of crystalline structure) that occurs in starch granules in the presence of water and heat [197]. When a starch solution heated in the presence of water, granular swelling occurs and it increases with increasing the heat, which is then followed by structural changes [198]. In a gelatinization process, granule swelling starts in the amorphous region, crystallinity loss and granule breakdown occurs in the later stage of the process [199]. These thermal properties are unique for each starches depending upon organization, length, and number of amylose and amylopectin chains [156].

The gelatinization temperatures-the onset temperature (To), peak temperature (Tp), conclusion temperature (Tc), transition interval-ΔT (Tc-To) and the enthalpy change of gelatinization (ΔH) were calculated and used to compare the thermal properties of the stem starch and the corn starch.
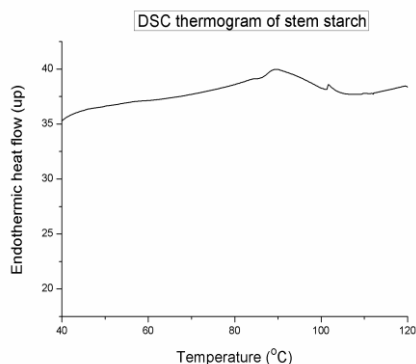
Figure 7.12: DSC thermogram of stem starch
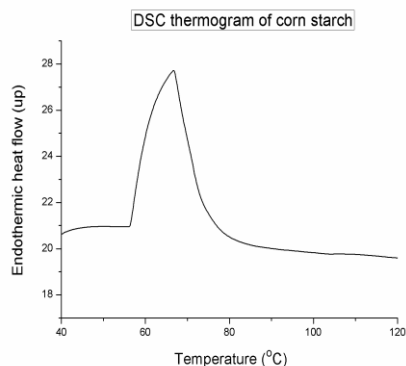
Figure 7.13: DSC thermogram of corn starch

Table 7.2: Gelatinization parameters of stem starch and corn starch

Sample	To	Tp	Tc	ΔT	ΔH (J/g)
Stem starch	84±2.05	89.45±0.41	99.51±2.83	15.61±3.59	15.45±0.43
Corn starch	55.96±1.42	66.73±1.36	74.08±1.69	18.11±1.92	36.55±1.15

Stem starch showed a higher gelatinization temperature than corn starch (Figure 7.12 and 7.13). Higher gelatinization temperature indicates starch crystal stability [156]. Tester and Morrison, 1990 reported that starch with a high gelatinization temperature is more crystalline and have longer chains than those with low gelatinization temperature [200]. According to Vamadevan and Bertoft, 2015 the gelatinization endothermic peak shifts to a higher temperature with decreasing moisture content [156]. It was also reported that A-type crystals have higher thermal transition temperatures [30]. Starches with high gelatinization temperatures have comparatively stable starch gels

and are resistant to acid and enzymatic hydrolysis and can be used in the preparation of gum candies [201, 30].

The gelatinization enthalpy (ΔH) related to the amount of starch in the amorphous phase [202]. Fuentes et al. in 2019 reported that the enthalpy of gelatinization (ΔH) increases with the increase in starch granule size. In this study the stem starch showed lower ΔH than the corn starch (Table 7.2), indicating small granule size of stem starch than the corn starch [203]. The gelatinization transition interval (ΔT) of stem starch was 15.61 ºC and that of corn starch was 18.11 ºC. ΔT is dependent on the heating rate used, and low ΔT is the indication of high homogeneity and purity of the extracted material [156, 52].

7.3 Pesticide residue analysis of the extracted stem starch

Pesticides are the chemicals used to control the pests that damage the crops [204]. They are produced to increase agricultural production and should be used safely. Otherwise, it will harmfully affect the environment [118]. Widely used pesticides include organophosphate and organochlorine pesticides. Organophosphate pesticides are the main components of herbicides and insecticides and can exert adverse effects like impairment of memory, speech loss, nausea, vomiting and weakness [205]. Organochlorine pesticides are the widely used chlorinated pesticides having high persistence in the environment [119].

Here the analysis of pesticides-parathion-methyl, malathion, chlorpyrifos, dichlorvos, ethoprophos, and heptachlor has been done to assess the purity of the extracted starch from pineapple stem, which

was collected from the pineapple farms. The results showed that there was no pesticide content in the extracted starch, which was a positive indication for using this starch in food preparations (Table 7.3).

Table 7.3 Pesticide residue analysis of the extracted starch from the stem of pineapple plant

Sl No.	Pesticides analysed	Results
1	Parathion Methyl	Not detected
2	Malathion	Not detected
3	Chlorpyrifos	Not detected
4	Dichlorvos	Not detected
5	Ethoprophos	Not detected
6	Heptachlor	Not detected

In Kerala, the pineapple crops are not affecting by severe pests and diseases. Leaf spot diseases and mealybugs are seen in the farms. It needs only fungicides like Bordeaux mixture, Zineb or Mancozeb (for leaf spots disease) and 0.05 % of quinalphos (for mealy bugs). An aqueous solution of 25 ppm ethephon containing 2 % urea and 0.04 % calcium carbonate is also used for the uniform flowering of the crop. The pesticide contamination by the pineapple farms has never been reported in Kerala [206]. The result obtained agreed with this statement.

This chapter focused on the rheological properties of pineapple plant stem starch (extracted from the crude stem, and its different regions) and compared the values with corn starch. From the results obtained, it can be concluded that stem starch had a significant

difference in rheological properties with corn starch. Stem starch was more viscous (liquid-like behaviour), had lower amylose content and its gel was less stiff than corn starch. Starches isolated from different plant sources are different in their rheological properties. This rheological data will help to choose the specific application of the starch from a new source (pineapple plant stem), in many industries where viscous starch is preferred. Starch extracted from different regions of the plant stem did not show a significant difference in rheological properties at certain frequencies. In other frequencies, the difference may be due to the region-wise developmental changes in the granular structure and properties.

Higher gelatinization temperature of stem starch indicated higher crystalline nature, longer chains, and more crystal stability. Stable crystals are resistant to acid and enzymatic hydrolysis which are suitable for the products like gum candies, where stable and enzyme resistant starches are required.

Absence of harmful pesticide residues in the extracted stem starch indicated its usefulness in food and feed industries.

Chapter 8

PROXIMATE AND ANTI-NUTRIENT ANALYSIS OF PINEAPPLE STEM AND LEAF

8. PROXIMATE AND ANTI-NUTRIENT ANALYSIS OF PINEAPPLE STEM AND LEAF

8.1 Proximate analysis

The proximate analysis can be defined as the partitioning of compounds present in a feed material into six categories (moisture, ash, crude protein, crude fat, crude fibre, and total carbohydrates) based on their particular chemical properties [207]. For using the pineapple plant stems and leaves as feed material, it is beneficial to find out their proximate contents. For this, moisture, ash, crude fibre, crude protein, crude fat, and the total carbohydrates were calculated on a dry weight basis.

Among these, the moisture content is an essential factor to be measured as it highly depends on the transportation and storage of feedstuffs [208]. It also determines the microbial growth and, thus, the shelf life of feed material. The moisture content of the feed material has a significant influence on the quality of storage and can reduce the post-harvest loss by measuring it [209]. The amount of moisture can vary with several environmental factors like humidity, temperature, time of harvest, climate, and storage conditions [123]. So their determination is crucial for food and feed manufacturers. There is a limit of moisture assigned for all the food products [210]. In the case of feed material, a maximum permissible limit of moisture is 11 % [211]. The pineapple plant stem contained high moisture content (4.79±0.18 %) compared to the pineapple leaf (3.07±0.08 %) and was significantly different ($p < 0.05$). Both values were under the permissible limit.

Ash is the inorganic residue that left after the removal of water and organic materials from a substance using heat [210]. It is the measure of mineral content that a sample possesses because minerals cannot be destroyed by the heating process [123]. Dietary ash helps to maintain the acid-alkaline balance in the blood and also has a role in controlling the hyperglycaemic effect [122]. It was also reported that the ash content could contribute to the pharmacological effect of the plants [212]. Pineapple leaf had significantly higher value for ash (8.87±2.0 %) than its stem (3.69±0.28 %), indicating more top mineral element in leaf than the stem. The ash content can vary with the properties of soil, climate and plant variety [213]. Ash content of some feedstuffs is as follows.

Rice husk: 15-22 %, seaweed meal (*Sargassum spp.*)*:* 23-44.62 %, sugarcane bagasse (fine variety): 2.60-3.49 %, sugarcane bagasse (coarse variety): 1.81-2.58 % and the citrus by-product: 1.7-7 % [214]. Pineapple stem and leaf contained intermediate ash content when compared to these feedstuffs.

The crude fibre is the indigestible parts of feeds, which include cellulose, lignin, pentosan, chitin, etc. Crude fibre value has great importance in feed analysis, because the higher the crude fibre content, lower will be the nutritional value of that feed [215]. The crude fibre content of pineapple leaves was found to be significantly higher (22.43±1.06 %) than its stem (6.30±0.14 %) ($p<0.05$). Crude fibre can aid the active absorption of trace elements from the gut and also helps for proper digestion and effective elimination of waste [216, 123]. It was also reported that due to the very high fibre content, nutrient utilization

is impaired, and the energy values will decrease [217], which will affect the proper growth and reproduction [218]. There is a tendency to refuse the use of rice husk in the animal feed industry because of its high fibre content (39-42 %), roughness, low nutritive value, and digestive tract irritation. However, it can be used after the pre-treatments like supplementation with high-quality protein and the use of microbial enzymes [214].

There is type I, II, and III types of cattle feed for high yielding, medium yielding, and low yielding animals. The maximum permissible limit of crude fibre is 10, 12, and 15 % for type I, II, and III cattle feed respectively [211]. In the case of a pineapple stem, the value is under the limit. But the leaves have high fibre content, and the level can reduce after pre-treatments like in the case of rice husk. It was reported that the fermentation using microorganisms from bamboo sprouts could effectively reduce the fibre content of pineapple waste [28]. The crude fibre content of the pineapple stem and leaf were found to be higher than that of the feedstuff-cottonseed meal (*Gossypium arboreum*) (2-2.7 %) and lower than that of the feed stuff-rice husk (39-42 %). Level of crude fibre in the food materials-millet (2-9 %), green vegetables (1.4-5.3 %), dried fruits and nuts (5-18.3 %) and the feedstuff- palm kernel meal (*Elaeis guineensis*) (8 %) are comparable to that of pineapple stem[214,219].

The total carbohydrates are considered as the primary source of energy in the diet of the animals [212]. They provide energy for maintaining the body temperature and for performing other normal body functions of animals [218]. The stem had 79.85±0.40 % of total

carbohydrates, and leaf had 77.15±1.99 %. There is no significant difference observed for both the samples. It was reported that the leaves of a medicinal plant-*sphaeranthus hirtus* have 78.42±0.05 %, and fruits of another medicinal plant-*amomum subulatum* have 76.20±0.02 % of total carbohydrates [220], a result comparable to that of pineapple stems and leaves.

The fat content of pineapple stem (3.86±0.19 %) and leaf (3.57±0.10 %) was observed to be significantly different ($p<0.05$). They are essential for the proper structural and biological functioning of animal cells [135]. Fat is also a good source of energy for cattle and also helps for the absorption and transportation of fat-soluble vitamins. Fat content increases the feed acceptability by animals as they increase softness and palatability of the feeds [221]. An already used source of animal feed-the citrus by-products contains only 1.2-2.1 % of the crude fat [214]. The minimum requirement of crude fat in cattle feed is reported to be 4, 2.5, and 2 % for type I, II, and III cattle feed, respectively [211]. The crude fat content of both stem and leaf were close to that required for the feed of high yielding cattle (type I cattle feed). Result of total carbohydrate and crude fat contents made the pineapple plant parts as a good source of energy.

Proteins are the building blocks of animal tissues and are essential for proper cell growth and regeneration of tissues [222]. The intake of adequate quantities of protein is essential for growing and breeding animals. Breeding animals require a protein-rich diet [218]. So the determination of crude protein is necessary while preparing the cattle feed formulations. The crude protein content of the stem was

found to be 7.79±0.09 %, and that of leaf 7.33±0.18 %. The crude protein content of pineapple stem and leaf was higher than that of the feedstuffs-citrus by-products (2.2-4.2 %) [214] and corn stover (6.3±0.36 %) [223]. The crude protein content can vary with soil fertility [213]. The pineapple stem and leaf were found to be significantly different for crude protein content at $p<0.05$. The minimum requirement of crude protein in cattle feed is reported to be 22, 20, and 18 % for type I, II, and III cattle feed, respectively [211]. The protein content of both stem and leaf were lower than the required value. From this, it can be suggested that these plant parts are more suitable as a cattle feed ingredient than the cattle feed itself.

The proximate content of pineapple stem and leaf are summarised in Table 8.1

Table 8.1: The proximate content of pineapple stem and leaf (per 100 g dry weight)

Sl No.	Constituents (%)	Stem	Leaf
1	Moisture	4.79±0.18	3.07±0.08
2	Ash	3.69±0.28	8.87±2.0
3	Crude fibre	6.30±0.14	22.43±1.06
4	Crude protein	7.79±0.09	7.33±0.18
5	Crude fat	3.86±0.19	3.57±0.10
6	Total carbohydrates	79.85±0.40	77.15±1.99

8.2 Anti-nutrient analysis

Anti-nutrient compounds are the compounds naturally present in plants which harm the proper growth of the organism that consume

the plants. They also reduce the adequate absorption of other nutrients; thus, there will be inadequate nutrient intake occurs. But in low quantities they are beneficial as they can reduce the level of blood glucose, cholesterol, etc. So the concentration of anti-nutrient factors is crucial while assessing the quality of a plant that we consume [129]. As a part of anti-nutritional analysis, phytate phosphorus, cyanogenic glycosides, oxalic acid, tannins, saponins, trypsin inhibitor activity and lectin/haemagglutinating activity were screened out.

8.2.1　Phytate Phosphorus

Chemically phytic acid is a myoinositol (1,2,3,4,5,6) hexakisphosphoric acid. Inositol penta IP5, tetra IP4, and triphosphates are also known as phytates with molecular formula of $C_6H_{18}O_{24}P_6$ and molecular mass of 660.04 g/mol [224]. The unique structure of phytic acid enables them to chelate with cations like calcium, magnesium, zinc, copper, iron and potassium, form their insoluble salts and impairs their absorption and digestion. Deficiency of Fe, Zn, Ca can have adverse effect in the growth, health and cognitive development in children [225]. It is reported that these phytates can also complex with proteins and thus can alter their structure and function [224].

Phytic acid or phytate (when it is in salt form) is the main storage form of phosphorous in many plant tissues like cereals, legumes and oleaginous seeds [225]. Phosphorous in this form cannot be utilized by human beings, agastric animals, pigs, dogs, birds, etc. because of the lack of the enzyme, phytase [224, 226]. In feed industries,

microbial phytase will add to the animal feed for enhancing the nutritional quality and is widely used in the Netherlands and in the United States [226].

The pineapple stem contained 0.09±0.01 % of phytate P, and the result was very low when compared to the phytate P content of maize and wheat [227]. The pineapple leaf contained 0.24±0.01 % of phytate P and were significantly different from that of the stem. The phytate P content of leaf was lower than maize gluten feed (0.47±0.06 %), wheat feed flour (0.39±0.16 %) and maize feed flour (0.27±0.07 %) [227]. From this, it is clear that the pineapple stem and leaf contain a minimal amount of phytate P when compared to these plant parts which are used as food and feedstuffs.

8.2.2   Cyanogenic glycosides

Cyanogenic glycosides are a group of nitrile-containing plant secondary metabolites. They can produce cyanide by their enzymatic breakdown. Simple chewing or digestion leads to the hydrolysis of the substances, causing the release of cyanide.   Almost all of the cyanogenic glycosides are believed to be derived from only six different amino acids. They are L-valine, L-isoleucine, L-leucine, L-phenylalanine or L-tyrosine and cyclopentenyl-glycine (a nonprotein amino acid) [228]. Production of cyanogenic glycosides in plants varies with their age, variety and also with environmental factors [229, 230]. Toxicity includes inhibition of several enzymes, interference with certain essential amino acids and utilization of associated nutrients and

neuropathy [63]. Symptoms include vomiting, stomach ache, diarrhoea, convulsion, and in severe cases, death may occur [231].

Hydrogen cyanide content of the pineapple stem was found to be 0.995±0.17 mg/100 g, and that of the leaf was 0.994±0.05 mg/100 g. The value was comparable with the HCN level of cocoyam tuber (1.713±0.005 mg/100 g) [126] and lower than that of the feedstuffs-bamboo (*Bambusa arundinacea*)-young short (10-800 mg/100g), sorghum (*Sorghum vulgare*)-leaves (75-79 mg/100 g), giant taro (*Alocasia macrorrhizos*)-leaves (2-3 mg/100 g), bitter almond (*Prunus dulcis*) (470 mg/100 g), lima beans (*Phaseolus innatus*) (200-300 mg/100 g) and flax (*Linum usitatissimum*)-seed meal (36-39 mg/100 g) [232].

8.2.3 Oxalic acid

Oxalic acid is a dicarboxylic acid and will be poisonous when consumes in large amounts. It acts as an anti-nutritional factor by binding with calcium and thus inhibits its absorption [233]. Oxalates exert both direct and indirect toxic effect. Soluble oxalates will directly absorb and accumulate in the kidney and create a toxic effect. Insoluble oxalates are those which bind with calcium and magnesium and cause deficiency of those elements (indirect effect). A most common effect is the kidney damage as the insoluble calcium oxalate crystals eventually accumulate in kidneys. Another affecting system is the nervous system. Insoluble calcium oxalate crystals can accumulate in plasma and cause hypocalcemia leading to muscle twitching and cramps and finally leading to tetany and convulsions [234, 235].

The level of oxalic acid in pineapple stem and leaf were found to be 4.94±0.05 % and 13.55±3.4 % respectively and were significantly different at the p-value of <0.05. Rahman et al. 2017 reported that dietary intake of 4.5g oxalic acid/animal/day is safe and will be dangerous if the level reaches 6.75 g oxalic acid/animal/day or more [235]. The oxalic acid level in the pineapple plant can be reduced by 30 % in stem and 84 % in leaf by simple heat treatment (heating at 80 °C for 1 h) (Table 8.2).

Table 8.2: Reduction of oxalic acid content in pineapple stem and leaf by heating

Sample	Before treatment	Heating at 80 °C for 30 minutes	Heating at 80 °C for 1 h
Stem	4.94±0.05	4.65±0.28	3.45±0.10
Leaf	13.55±3.4	8.84±0.55	2.19±0.17

8.2.4 Tannins

Tannins are high molecular weight polyphenolic compounds considered as an anti-nutrient factor because of their tendency to precipitate proteins. Tannins usually bind with proteins and alter their rate of digestion [236]. These tannin-protein complexes are powerful and cannot be easily broken down. Apart from proteins, they can also complex with divalent metals, cellulose, hemicelluloses, pectin and other carbohydrates [124]. Tannins are classified into two categories- condensed tannins and hydrolysable tannins. Condensed tannins are

mostly the polymers of flavan-3-ol s while hydrolysable tannins are mainly the mixtures of different gallic acid esters of glucose [237].

The tannin content of stem was observed to be 0.88±0.02 %, and that of the leaf was 1.24±0.04 %, and they were significantly different at p<0.05. Atanassova and Christova-Bagdassarian, 2009 reported the tannin content of the edible dried fruits-sweet cherry (1.25 %), Morella cherry (1.11 %), and Aronia (2 %) [238]. Tannin level of the medicinal plant-Borage was 1.80±0.03 % [239]. Some Nigerian medicinal plants like *S. dulcis, S. acuta, S.cayennensis* and *T.procumbens* contained 6.23±0.20 %, 6.08±0.23 %, 9.98±0.32 % and 7.45±0.22 % of tannin respectively [240]. Compared to these data, it was clear that the pineapple plant parts are safe from the adverse effects of tannins.

8.2.5 Saponins

Saponins are also called as natural detergents and they are chemically glycosides in which sugars are linked to triterpene or steroidal aglycone moiety [241]. Most common sugars linked are galactose, arabinose, xylose and glucose [242]. Saponins are found in common plants like soya, peas, beans, potato, yams, asparagus, oats, sugar beet, tea and many medicinal herbs. They are bitter in taste and have the ability to form foam. Their adverse effects include hemolysis of RBC, weight loss in animals, hypocholesterolaemia in humans (by binding with cholesterol and thus impairs their absorption) [242, 63].

The result indicated that the saponin content of pineapple stem was 3.40±0.11 %, and that of the leaf was 7.76±0.26 % and were

significantly different (p<0.05). The edible plants -Soybeans *(Glycine max)* contains 5.6 % and Chickpea *(Cicer arietinum L.)* contains 3.6 % of saponin in dry weight [242]. The saponin content of the medicinal plants-*S. cayennensis* (3.10±0.10 %), *P. bransilensis* (3.92±0.11 %) [240], *Polygala* spp. (8–10 %), *Primula* spp. (5-10 %) and *Quillaja saponaria* (9-10 %) [243] are comparable to that of pineapple stem and leaf.

8.2.6 Trypsin Inhibitor Activity

Protease inhibitors are a diverse group of proteins, widely distributed in plants and can act as defence and regulatory proteins [141]. Trypsin inhibitors-an important anti-nutritional factor come under this category. They are seen in common edible plants like soybeans, beans and seeds, potatoes and legumes. Their adverse effects include the inhibition of trypsin and chymotrypsin, stimulate their secretion, cause hypertrophy and hyperplasia of the pancreas. This effect on pancreas may lead to adenomas and carcinomas of the exocrine pancreas [244]. They can inhibit intestinal protein digestion which will cause decreased growth rate in animals [245]. It was reported that the trypsin inhibitors will be degraded in the rumen of ruminants and thus cannot exert their adverse effect on ruminants [63].

The pineapple stem and leaf had the trypsin inhibitor activity of 1.55±0.36 TIA and 10.75±1.57 TIA, respectively and were significantly different at p<0.05. The value was very low while considering the trypsin inhibitor activity of many edible plants like Soybean-*Glycine max* (14.61±1.41 TIA), Bitter gourd-*Momordica charantia* (58.45±1.85 TIA), Potato-*Solanum tuberosum* (22.01±2.66

TIA), Ginger-*Zingiber officinale* (18.34±1.17 TIA) and Horse gram-*Macrotyloma uniflorum* (97.15±3.05 TIA) [130]. The edible *Oryza sativa* (Asian rice) contains specific trypsin inhibitory activity of 4.26 TIU/mg of protein [141].

8.2.7 Lectin/Haemaglutinating activity

Lectins or agglutinins or phytohemagglutinins are a complex and heterogeneous group of proteins. They are found in most organisms, ranging from viruses and bacteria to plants and animals. Cereals, potatoes and beans contains this protein. Their particular structure includes at least one non-catalytic domain. This domain helps them to selectively recognize and reversibly bind with specific monosaccharides or oligosaccharides present on glycoproteins and glycolipids, without altering the structure and properties of carbohydrate [246, 247]. They contain at least two carbohydrate-combining sites. While reacting with erythrocytes, they combine with the sugars on their surface. They also cause cross-linking of the cells and their subsequent precipitation. This process is known as haemagglutination [248]. Lectins are often found at high concentrations in certain plant tissues like seeds, bark and bulbs [249]. They are essential defence molecules against phytopathogenic microorganisms, phytophagous insects and plant-eating animals [250].

Lectins can exert many adverse effects. They are highly anti-nutritional and are highly resistant to proteolysis in the gastrointestinal tract of monogastric, ruminants and insects. They can bind with the epithelial cells, lining in the small intestine and interfere with intestinal

and systemic metabolism [251]. They can even cause epithelial lesions within the intestine. Excess intake will cause severe intestinal damage, disruption of digestion, nutrient deficiencies, food allergies and immune responses. Haemagglutination mentioned above will cause anaemia [129] and can be used to detect the presence of lectins.

Both the pineapple stem and leaf samples did not show any haemagglutination (Figure 8.1A and 8.1B) and were the indication of the absence of lectins in the samples.
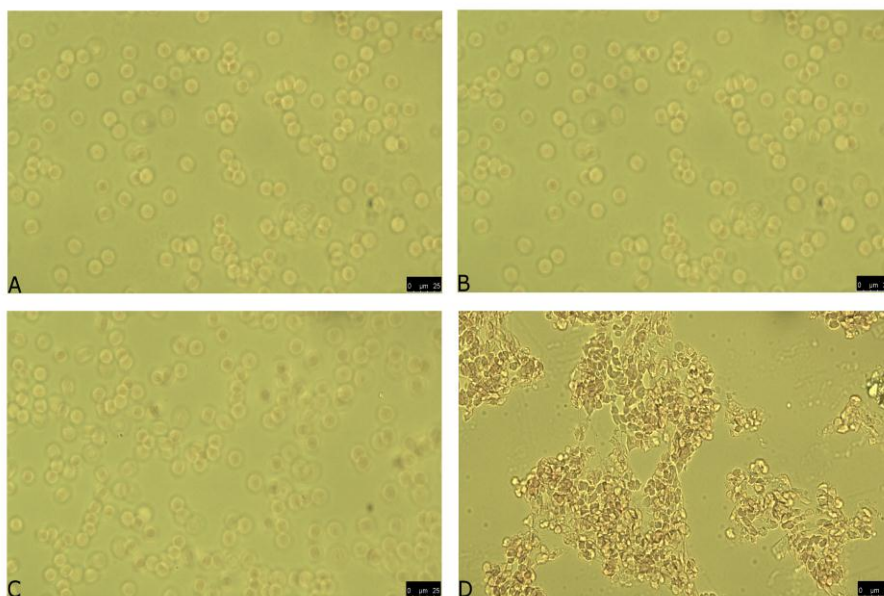
Figure 8.1: Absence of hemagglutinating activity observed by fluorescent microscope (A: Pineapple stem B: Pineapple leaf C: Negative control D: Positive control)

Anti-nutrient factors present in pineapple stem and leaf are summarised in the following table (Table 8.3).

Table 8.3 Anti-nutritional factors present in the pineapple plant stem and leaf

Sl No.	Anti-nutritional factor	Stem	Leaf
1	Phytate P (%)	0.09±0.01	0.24±0.01
2	HCN (mg/100 g)	0.99±0.17	0.99±0.05
3	Oxalic acid (%)	4.94±0.05	13.55±3.4
4	Tannin (%)	0.88±0.02	1.24±0.04
5	Saponin (%)	3.40±0.11	7.76±0.26
6	Trypsin inhibitor activity (TIA)	1.55±0.36	10.75±1.57
7	Lectin/Haemaglutinating activity	Absent	Absent

Results revealed that the pineapple plant stem and leaf contained a considerable amount of nutrients. Level of carbohydrate content and fat indicated that these plant parts were with high levels of energy and the moisture content was within the allowed limit. Fibre content of the stem was also within the permitted range for cattle feed formulations. Ash content revealed that the leaves had higher mineral content compared to the stem. The crude protein content of pineapple stem and leaf was found to be higher than that of the citrus by-products and the corn stover, which were already used as feed ingredients. The anti-nutritional factors except oxalate were within the permissible limit, further we could reduce the oxalate content by applying moderate heating of the raw materials. For the massive production of cattle feed using these ingredients requires further detailed studies.

Chapter 9

SUMMARY AND CONCLUSIONS

9. SUMMARY AND CONCLUSIONS

This research was designed to investigate the production of value-added products like starch and cattle feed from the pineapple agro-waste (pineapple stem and leaf) and thus to provide scientific inputs aiming their effective utilization.

- The starch yield from the pineapple stem was 11.08 ± 0.77 % on a wet basis. In leaf, it was 0.57 ± 0.01 %. As the starch content in the leaf was very low, excluded the leaf starch from further characterization studies.

- Characterization studies of stem starch revealed that some of the characters were comparable to that of corn starch like FTIR and XRD data. FTIR results indicated that the moist and microwave heating did not change the chemical groups already present in the stem starch molecule and did not produce any new chemical groups. However, narrowing of band and change in intensity were observed. This observation was due to the ordering of structure and changes in the specific conformation of starch molecules.

- NMR data obtained was characteristic of other native starches and can be used for further modification studies.

- XRD data revealed that both stem and corn starches had A-type granules, which is a characteristic feature of cereal starches.

- In addition to this, stem starch possesses some unique characters also. There was no uniform granule morphology observed for stem starch. Size of the granule was smaller than the corn starch granules. Comparatively small sized granules of pineapple stem starch were advantageous in the production of a fat substituent, stabilizers in baking powder, a stiffening agent in laundry and the manufacture of biodegradable plastics.

- An increasing trend was observed in granule size with the growth of the pineapple plant. The granule shape was mainly polyhedral with sharp angles and edges and surfaces were smooth with no surface pores, and the round-shaped granules were also present. This observation was the same in all the studied stages of plant growth. These results could be used for identifying starch from the stem at a particular growth stage to choose in the specific starch industry.

- Both types of heating (moist and microwave) completely disrupted the stem and corn starch granules. In moist heating, the disruption of stem starch granules occurred only when the temperature exceeded 80 °C while the corn starch granules were disrupted below 80 °C. It was the indication of higher gelatinization temperature and the higher stability of stem starch than the corn starch granules. The thermal studies confirmed this observation.

- Results from the ionic liquid (1-butyl-3-methylimidazolium chloride) and deionized water treatments (heating and

precipitation) indicated that both of them disrupted the starch granules compared to the native starch structure. Ionic liquid treated stem starch showed small granular shape in their SEM photograph, and this observation was absent in corn starch. It could also be concluded that the ionic liquid, 1-butyl-3-methylimidazolium chloride was more effective in dispersing starch than deionized water.

- In DSC studies, the higher gelatinization temperature of stem starch indicated higher crystalline nature, longer chains and more crystal stability than the starch from the commercial corn. Stable crystals are resistant to acid and enzymatic hydrolysis which are suitable for the products like gum candies, where stable and enzyme resistant starches are required.

- Rheological studies indicated, stem starch was more viscous, had lower amylose content, and its gel was less stiff than the corn starch. Starch extracted from different regions of the plant stem did not show much difference in their rheological properties. Starches with unique characters are always appreciated as the starch industries require new food formulations.

- Both the stem and leaf contained a considerable amount of nutrients needed for cattle feed production. Carbohydrate and fat content indicated that pineapple stem and leaf were with high levels of energy, and the fat content was closer to the requirement of the high yielding animals. The moisture content

was within the allowed limit, and the ash value was comparable to that of feed materials like sugarcane bagasse and citrus by-products. The crude protein content was found to be higher than that of the feed materials-citrus by-products and the corn stover. The fibre content of the stem was at an intermediate level as required for cattle feed formulation.

- The anti-nutrient factors were in a limited quantity and lower than normally used feed stuffs. It is more beneficial as this plant stem and leaf will not need further processing methods to reduce the anti-nutrient level. Processing methods are necessary for many plant samples like coffee pulp to reduce the level of toxic compounds to a safe level. These results make this plant parts as a good feed ingredient.

- If we develop an economically feasible small scale non-polluting technology for the extraction of starch from this agro-waste, we can produce at least 3 tonnes of starch per hectare of pineapple farms (considering the plant density of 63400 plants/ha.). Studies of this kind will aid the production of value-added products like starch with special properties for specific uses in different applications.

- The average weight of the pineapple agro-waste (both stem and leaf) was found to be 2.3±0.26 kg. From this data, it can be calculated that we may get at least 145 tonnes of agro-waste per hectare of pineapple farms.

- For using the pineapple agro-waste as an ingredient for the production of cattle feed, we recommend the method of drying it in the farm itself and transporting to the feed industry.

- This type of researches promises the maximum utilization of arable land, reduction of agro-waste and sustainable agriculture practices. Further research is necessary to develop techniques for the industrial-scale production of cattle feed or cattle feed ingredient from the pineapple agro-waste to overcome the crisis of nutritionally improved feed materials.

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ADDENDUM

PUBLICATIONS/PRESENTATIONS

I. Publication

1. Rinju R and B S Harikumaran Thampi, "Characterization studies on starch extracted from the stem of pineapple plant (Ananas comosus) at different growth stages", Bioscience Biotechnology Research Communications, 2019, DOI: 10.21786/bbrc/12.3/11, ISSN: 0974-6455 (Print), 2321-4007 (Online), 12 (3), pp 623-630.

II. Presentations

1. **Rinju R** and B S Harikumaran Thampi. "Proximate and anti-nutrients analysis of pineapple plant (*Ananas comosu*s) stem and leaves" in 50th Annual International Conference of ICMR-National Institute of Nutrition, Hyderabad on November 15-17, 2018.

2. **Rinju R** and B S Harikumaran Thampi. "Characterization of starch from an agro-waste-the pineapple plant stem" in 30th Kerala Science Congress at Govt. Brennen College, Thalassery on January 28-30, 2018.

3. **Rinju R** and B S Harikumaran Thampi. "XRD Studies on pineapple plant stem starch at different growth stages" in a national seminar on "Recent Trends in Food Technology-Processing Preservation and Byproduct Utilization" conducted by SAFI Institute of Advanced Study, Vazhayur on January 10-11, 2018.

4. **Rinju R** and B S Harikumaran Thampi. "Thermal and Rheological Studies on Starch Isolated from Pineapple Plant Stem-An Agrowaste from Pineapple Farms" in an International Conference on Recent Trends in Agriculture, Veterinary and Life Sciences-2017" at Carmel College for Women, Nuvem, Goa on December 28-30, 2017.

5. **Rinju R** and B S Harikumaran Thampi. "XRD studies on Starch isolated from different regions of pineapple plant stem" in an international seminar on environment, society and economy-AMBIENTE-2017, conducted by St.Joseph's College for Women, Alappuzha, Kerala on December 18-19, 2017.

6. **Rinju R** and B S Harikumaran Thampi. "Rheological properties of pineapple plant stem starch and their comparison with corn starch" in a national seminar, conducted by Inter University Centre for Genomics & Gene Technology, University of Kerala, Kariavattom on March 1-3, 2017.

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Characterization studies on starch extracted from the stem of pineapple plant (*Ananas comosus*) at different growth stages

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ABSTRACT

Starch is the most common carbohydrate in human diets and is contained in large amounts in potatoes, wheat, corn, rice, and cassava. Starch granules are highly variable in their structure, and each has unique structure depending upon their botanical source. In this study, starch was isolated from the stem of pineapple plant at different growth stages-3 months (before flowering), six months (before flowering), nine months (before flowering), 12 months (after flowering), 15 months (after flowering), 18 months (after fruiting). X-ray diffraction (XRD) studies and scanning electron microscopic (SEM) analysis were carried out on these samples as part of characterization studies. Results indicate that maximum starch yield obtained from plant stem at nine months age. X-ray diffraction data revealed this plant stem possess A-type crystals and is same in all growth stages. Size of the granules slightly increased with growth stages and have irregular polygonal in shape. Characterization studies on starch will help assess their specific use in both food and non-food industries.

KEY WORDS: PINEAPPLE PLANT, SEM, STARCH, STEM, XRD