

**STUDIES ON COMBINED TECHNOLOGIES OF
PULSED ELECTRIC FIELD AND MICROWAVE ASSISTED
PROCESS FOR EXTRACTION OF PECTIN FROM
JACKFRUIT RIND AND CORE**

By

NANDHU LAL A. M.

(2017-18-004)



**DEPARTMENT OF PROCESSING AND FOOD ENGINEERING
KELAPPAJI COLLEGE OF AGRICULTURAL
ENGINEERING AND TECHNOLOGY
TAVANUR - 679 573
KERALA, INDIA
2020**

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

**MASTER OF TECHNOLOGY
IN
AGRICULTURAL ENGINEERING**

(Agricultural Processing and Food Engineering)

**Faculty of Agricultural Engineering and Technology
Kerala Agricultural University**



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KELAPPAJI COLLEGE OF AGRICULTURAL
ENGINEERING AND TECHNOLOGY**

TAVANUR - 679 573

KERALA, INDIA

2020

DECLARATION

I hereby declare that this project report entitled “**Studies on combined technologies of pulsed electric field and microwave assisted process for extraction of pectin from Jack fruit rind and core**” is a bona fide record of research work done by me during the course of research and that the report has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Tavanur

Nandhu Lal A. M.

Date:

2017-18-004

CERTIFICATE

Certified that this thesis, entitled, “**Studies on combined technologies of pulsed electric field and microwave assisted process for extraction of pectin from Jack fruit rind and core**” is a record of research work done by Mr. Nandhu Lal A. M. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, associateship to her.

Place: Tavanur

Date:

Dr. Prince M.V.

(Major Advisor, Advisory Committee)

Professor and Head,

Department of Processing

and Food Engineering

K.C.A.E.T., Tavanur.

CERTIFICATE

We, the undersigned members of the advisory Committee of **Mr. Nandhu Lal A. M. (2017-18-004)**, a candidate for the degree of **Master of Technology in Agricultural Engineering** with major in Agricultural Processing and Food Engineering, agree that the thesis entitled “**Studies on combined technologies of pulsed electric field and microwave assisted process for extraction of pectin from Jack fruit rind and core**” may be submitted by Mr. Nandhu Lal A. M., in partial fulfilment of the requirement for the degree.

Dr. Prince M.V

(Chairman,
Advisory Committee)
Professor and Head,
Department of Processing
and Food Engineering
K.C.A.E.T., Tavanur.

Ms. Sreeja R

(Member,
Advisory Committee)
Assistant Professor
(Biochemistry)
Department of Processing
and Food Engineering
K.C.A.E.T., Tavanur.

Dr. Rajesh G. K.

(Member,
Advisory Committee)
Professor
Department of Processing
and Food Engineering
K.C.A.E.T., Tavanur.

Er. Shivaji K. P.

(Member,
Advisory Committee)
Assistant Professor
Department of Farm Machinery
and Power Engineering
K.C.A.E.T., Tavanur

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Nandhu Lal A. M.

Dedicated to
Farmers,
Teachers,
and Family...

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	percentage
&	:	and
°C	:	Degree Celsius
3D	:	Three dimensional
A	:	Ampere
a*	:	Greenness or redness
AIS	:	alcohol insoluble solids
ANOVA	:	Analysis of variance
AOAC	:	Association of analytical chemist
AUA	:	Anhydrouronic acid
b*	:	Blueness or yellowness
BBD	:	Box Behnken Design
CFU	:	Colony Forming Units
cm	:	centimeter
conc.	:	Concentration
cP	:	centi poise
DE	:	degree of esterification
dia	:	diameter
E	:	electric field strength
eq	:	equation
et al	:	and others
etc	:	et cetra
FAO	:	Food and Agriculture Organization
FESEM	:	Field Emission Scanning Electron Microscope
Fig	:	figure
g	:	gram
GalA	:	Galacturonic acid

GHz	:	Giga Hertz
h	:	hour
H ₂ SO ₄	:	Sulphuric acid
ha	:	hectares
HCl	:	Hydrochloric acid
HELP	:	high electric field pulses
HTST	:	High Temperature Short Time
Hz	:	Hertz
i.e.	:	Which is to say, in other words
ICUC	:	International Center for Underutilized Crops
IU	:	International Unit
Kg	:	Kilo gram
KJ	:	Kilo Joule
kV/cm	:	Kilo Volt
kWh	:	kilowatt hour
L	:	litre
L*	:	Lightness or darkness
LDPE	:	Low Density Polyethylene
LOT	:	Line Output Transformer
MAE	:	Microwave assisted extractions
Max	:	Maximum
mg	:	milli gram
MHz	:	Mega Hertz
min	:	minute
MT	:	Million Tonnes
MW	:	Microwave
N	:	Normality
NaCl	:	Sodium Chloride
NaOH	:	Sodium chloride

No.	:	Number
p	:	probability
Pa	:	Pascal
PEF	:	Pulsed Electric Field
pH	:	percentage of H ⁺ ions
PSE	:	pressurized solvent extraction
PVC	:	Poly Vinyl Chloride
rpm	:	revolution per minute
Rs	:	rupees
RSM	:	Response Surface Modeling
RTD	:	Ready to Drink
sec	:	second
SFE	:	supercritical fluid extraction
USA	:	United States of America
V	:	Volt
Vit	:	Vitamin
W	:	Watt
wb	:	wet basis
WHO	:	World Health Organization
Zp	:	cell disintegration index
ϵ'	:	dielectric constant
ϵ''	:	dielectric loss
μ s	:	microseconds

Introduction

CHAPTER 1

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus*) is a fruit crop widely distributed in India and is fast becoming prevalent among the length and breadth of the world. Kerala produces about 281 million tonnes of Jackfruit in the year 2016-17 (www.ecostat.kerala.gov.in). About 60 % of the whole fruit is inedible consisting of inner perigones (non-edible perianth), central core and outer rind, which are unutilized and considered as waste. The Jackfruit processing industry and vendors generally dispose these, which causes negative environmental effects. By-product recovery from such fruit wastes not only improve overall economy of the processing units but also reduce the problem of environmental pollution.

Reports claimed on National Horticulture Database (2018-19) published by National Horticulture Board of India reports an annual production of 1857 MT of Jackfruit in India, cultivated under an area of approximately 1.87 million hectares. According to Ministry of Food Processing Industries India (2009), about 35 % of the total fruits and vegetable production has been lost. Improper processing practices and lack of storage facilities were cited as main factors accounting for this loss. Since these commodities are highly perishable in nature, improper handling accounts for post-harvest losses. Factors such as pathological breakdown and mechanical injuries also accounts for this loss. This in turn results in textural change as well as loss in nutritional quality of food, which makes the food unpalatable and unsafe for human consumption.

Jackfruit is a seasonal, under exploited organic fruit rich in fat, protein, carbohydrate, ash, fiber, vitamins (Vit A, Vit C, riboflavin, thiamine, niacin) and minerals like calcium, potassium phosphorus, sodium and iron (Manjeshwar *et al.*, 2011). The perishable nature of Jackfruit results in huge post-harvest wastage even though the fruit is a highly nutritious commodity. The major types of Jackfruit present in Kerala are 'Koozha' and 'Varikka'. Koozha is thin and fibrous in nature with mushy edible pulp. They are usually very sweet and emit strong odour. In

contrast 'Varikka' is firm, thick, crisp and has comparatively less fragrant pulp. Varikka has more commercial acceptance than Koozha varieties due to its nature.

Inbaraj and Sulochana (2006) reported that about 59 % of ripe Jackfruit peel generally discarded as waste, which might cause serious environmental problems during the season. In India, about 75 % of Jackfruit gets wasted wherein a whopping loss of 35 crore on Jackfruit accounts annually in Kerala alone. Assuming only Rs. 3/- for one Jackfruit and wastage of 50 % per year, India still accounts for a loss of Rs 214 crore worth of food every year. (www.decanherald.com).

Value addition of products by means of by-product recovery using effective and environment friendly methods might serve for value addition and profit, as well as for environmental protection (Koh *et al.*, 2014). Pectin is such a valuable by product that can be obtained from Jackfruit wastes.

Pectin are a family of complex polysaccharides that contain 1-4 linked α -D galacturonic acid organised in a linear backbone. Pectin polysaccharides are of high molecular weight and closely connected with other polymer components in cell walls, which inhibit their release from the cell matrix (Kratchanova *et al.*, 2004). Therefore, the methods used for extraction should allow selective extraction of pectin and should be able to protect the molecular structure without any degradation.

Pectin is recognised as a secondary food ingredient extensively used as gelling, stabilizing and emulsifying agent in food products. Consumption of this polysaccharides has positive effects on human health as they help in reducing cancer development, lowering blood glucose and blood cholesterol level (Jackson *et al.*, 2007). Texturizing applications of pectin in food and similar systems, includes gelling, colloid stabilizing, and viscosity enhancement are fundamentally related to its in-solution (gels or dispersions) behaviour (Lopes da Silva and Rao, 2006). Food sectors like dairy, bakery, and nutraceutical and functional foods as well as pharmaceutical domains like drug delivery uses pectin (Liu, *et al.*, 2007; Phillips & Williams, 2009; Schmidt *et al.*, 2015).

Pectin is obtained commercially by using heat treatment by direct boiling method (Li *et al.*, 2012). This method is not only time consuming but also results in degradation of extracted pectin, which leads to undesired alteration in physicochemical and functional properties of pectin. Therefore, establishment of new methods are essential, by which pectin could be extracted in a shorter time with better quality.

Pulsed Electric Field is a novel non-thermal food processing technology that comprises application of short duration electric field pulses with high intensity to the food material placed between two electrodes. When the potential difference exceeds a critical value, localized electrical breakdown of the cell membrane ensues which results in pore formation on cell membrane which increases permeability (Zimmerman *et al.*, 1974).

First hypothesis of the research is that pre-treatment of the pectin bearing Jackfruit waste using pulsed electric field could cause a rupture of the parenchymal cell of the plant material resulting in the formation of intercellular spaces, the cell will split and pectin can easily get released. This could increase the pectin yield.

Microwave extraction can be efficiently employed for pectin extraction from different plant material and are found to reduce extraction time and energy consumption (Fishman *et al.*, 2006). Microwave heating is an internal heat generation process and the heating is more rapid and homogeneous. Also, during microwave heating, considerable pressure builds up in the material, which modifies properties of tissue material, breaking down the cell structure and increase tissue porosity thus allowing better penetration of extracting solvents and increasing pectin yield during the microwave assisted processes.

It could be hypothesised from the above statements that a combination of PEF pre-treatment followed by a microwave assisted extraction could result in improved mass transfer of pectin to the extraction medium thus increasing the yield. This also could greatly reduce the time of extraction and reduced exposure to the thermal environment which would improve the quality of pectin compared to the

conventional extraction process. Since the plant material behaves differently to the PEF and microwave, the process variables need to be studied and standardised for each plant material under consideration. Though some studies were found reported on extraction of pectin from Jackfruit waste using microwave, studies on combined use of PEF and microwave assisted process were not found reported. Therefore this research entitled “Studies on combined technologies of pulsed electric field and microwave assisted process for extraction of pectin from Jackfruit rind and core” was undertaken with following objectives:

1. To develop a Pulsed Electric Field (PEF) pre-treatment chamber and a microwave assisted system for extraction of pectin from Jackfruit rind and core.
2. To investigate the influence of the combined system parameters on extraction of pectin leading to the optimization of process variables.
3. Characterization of pectin extracted by combined PEF and microwave assisted process and comparison of the same with that obtained through conventional process.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

This chapter contains reviews of researches related to pulsed field and microwave assisted extraction of pectin from Jackfruit rind and core. This chapter provides general information on Jackfruit, characteristics of Jackfruit, Pectin, and its benefits, extraction methods, and application of pulsed electric field, microwave extraction, etc.

2.1 JACKFRUIT

The Jackfruit (*Artocarpus heterophyllus*), a tree belonging to the mulberry family (Moraceae) is assumed to have originated in south western rain forests of India (Boning 2006). This tree is extensively cultivated in tropical regions of India, Nepal, Bangladesh, Malaysia, Vietnam, Thailand, Indonesia, Sri Lanka, and the Philippines. It is also found across African countries like Uganda, Cameroon, Mauritius, and Tanzania, as well as in Brazil and Caribbean nations like Jamaica. In India, Jackfruit is mainly cultivated in Kerala, Karnataka, Goa, Tamil Nadu, coastal Maharashtra, Bihar, Uttar Pradesh, Tripura, Assam, and foothills of Himalayas. (Priya *et al.*, 2014).

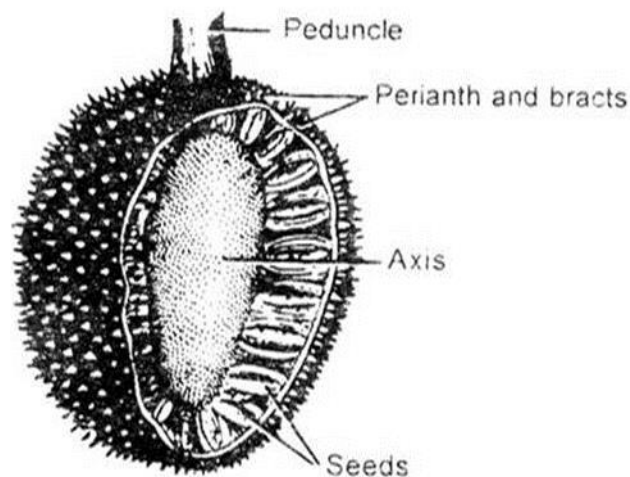


Fig 2.1 Cross section of Jackfruit

2.1.1 Taxonomical classification

Source: USDA National Nutrient data base

Kingdom	:	Plantae-- planta, plants, plants, vegetal
Subkingdom	:	Tracheobionta -- vascular plants
Division	:	Magnoliophyta -- angiosperms, flowering plants, phanérogames
Class	:	Magnoliopsida -- dicots, dicotylédones, dicotyledons
Sub class	:	Hamamelidae
Order	:	Urticales
Family	:	Moraceae -- mulberries
Genus	:	Artocarpus – breadfruit
Species	:	<i>Artocarpus heterophyllus</i> Lam.

India is the second largest producer of jackfruit in the global scenario and is believed to be the motherland of Jackfruit. In India, Jackfruit is cultivated approximately over an area of 1,02,552 ha, of which, approximately 1,00,000 trees are grown as intercrop in back yards along with commercial crops. Kerala owns the largest area under Jackfruit cultivation of about 97,540 ha and produces around 348 million tons of Jackfruit (APAARI. 2012).

Around 60% of the whole fruit contains outer rind, inner perigones (non-edible perianth) and central core, which is inedible and generally considered as wastes (Subburamu *et al.*, 1992). These wastes are undesirable for processing industries as well as for pollution monitoring agencies. Appropriate methods may be used to renovate this unutilized waste into value-added products. By-product recovery might improve the overall economy of processing units and diminish environmental pollution significantly. Pectin is such a valuable by-product that may be obtained from such fruit wastes.

2.1.2 Varieties

Elevitch and Manner (2006) stated that the variation in species depends on tree size, structure, leaf and fruit form, fruit-bearing age, fruit shape, size, colour and texture of edible pulp. Depending on the consistency of the fruit and its pulp, there are two types of morphotypes. One has fruits with small, soft, fibrous and soft flakes with good aroma and sweet carpels. The other is crunchy with crisp carpels, though not as sweet (Odoemelum, 2005; Shyamamma *et al.*, 2008).

In Kerala, based on the quality of pulp, Jackfruit may be classified into 2 major types; *Koozha* and *Varikka*. The fruits are thin, soft, fibrous and musky ranging from sour to very sweet and emits strong aroma when ripe in *Koozha* variety (Elevitch and Manner, 2006). *Varikka* has thick edible pulp, crisp, firm and less odorous. *Varikka* variety is more commercially accepted. Thamara chakka, Vakathanam varikka, Thenga varikka Nadavalam varikka, Thaen varikka, Muttom varikka, Aathimathuram koozha, Ceylon varikka, Rudrakshi, are the main Jackfruit varieties in Kerala. Konkan Prolific, Hybrid Jack, Singapore (or) Ceylon Jack, Burliar-1, PPI-1, PLR-1 are few important varieties introduced by and released from various organizations (Priya *et al.*, 2014).

2.1.3 Nutritional Quality

Table 2.1 Nutrient composition of fresh Jackfruit (per 100 g)

(Narasimham, 1990; Azad, 2000; Haq, 2006)

Composition	Mature fruit	Ripened fruit	Seed
Water (g)	76.2-85.2	72-94	51-64.5
Protein (g)	2.0-2.6	1.2-1.9	6.6-7.04
Fat (g)	0.1-0.6	0.1-0.4	0.4-0.43
Carbohydrates (g)	9.4-11.5	16-25.4	25.8-38.4
Fiber (g)	2.6-3.6	1.0-1.5	1-1.5
Total sugar (g)	-	20.6	-

Minerals			
Total minerals(g)	0.90	0.87-0.90	0.90-1.20
Calcium (mg)	30.00-73.20	20.00-37.00	50
Magnesium (mg)	-	27	54
Phosphorus (mg)	20.00-57.20	38.00-41.00	38.00-97.00
Potassium (mg)	287-323	191-407	246
Sodium (mg)	3.00-35.00	2.00-41.0	63.20
Iron (mg)	0.40-1.90	0.50-1.10	1.50
Vitamins			
Vitamin A (IU)	30.00	175-540	10.00-17.00
Thiamine (mg)	0.05-0.15	0.03-0.09	0.25
Riboflavin (mg)	0.05-0.20	0.05-0.4	0.11-0.30
Vitamin C (mg)	12.00-14.00	7.00-10.00	11.00
Energy (KJ)	50-210	88-410	133-139

Priya *et al.* (2014) reported that Jackfruits have high nutritional and therapeutic values. It can strengthen immune system, protect from cancer, maintains healthy eye and skin, and helps in healthy digestion, prevent anaemia, boosts energy, and lowers high blood pressure, controls asthma, strengthen bones and maintain a healthy thyroid.

Flakes of ripe fruits are rich in nutritive value; every 100 g of ripe flakes contains 30.0-73.2 mg calcium, 287-323 mg potassium and 11-19 g carbohydrates (Elevitch and Manner, 2006).

Jackfruit is “an underutilized crop” in tropical-to-subtropical climate where ignorance, gaps within supply chain systems and lack of post-harvest technology causes wastage of fruits. Jackfruit contains more protein, iron, calcium, vitamins along with essential nutrients compared to common fruits (Prem *et al.*, 2015).

International Centre for Underutilized Crops (ICUC) has been promoting Jackfruit and its use via technology development for its better husbandry and marketing. Jackfruit is broadly valued in tropical Asia where it was instigated as a

cultigen. Though it is broadly used, Jackfruit remains unutilized because of minimal scientific researches (Williams and Haq, 2002).

2.1.4 Post Harvest Utility

Jackfruit can be stored in ambient temperature (25-35 °C) for about 4-5 days and are consumed or marketed soon after harvesting. Under cold storage conditions, its storage life can be extended up to six weeks at a temperature of 11.1°C-12.8°C and humidity of 85-90 % (Bose *et al.*, 2003).

Roy and Joshi (1995) observed that in India, Jackfruit seeds are consumed as such or eaten as a dessert by boiling in sugar. Dried seeds are used to produce flour for producing value-added products. The unfertilized fruits can be used with fruit pulps or to replace it. Pureed Jackfruit is used for the preparation of baby food, jam, jellies, juice, and base for cordials. Fruit-roll, marmalades, candies, ice cream, etc. are made from Jackfruits.

Narasimham (1990) reported the usage of mature Jackfruits as vegetables for cooking, either for salad or curries. Ripe fruits can be eaten raw, or as a dessert by cooking in creamy coconut milk, prepared as candied Jackfruit or Jackfruit leather. Advances in processing technologies such as canning have pushed towards the production of more new products.

Ripe bulbs can be fermented and distilled to produce liquor. A number of products from Jackfruit such as squash, toffee, candy, nectar, pulp, jam, jelly, etc. have been developed and produced on a marketable scale. Preservation methods such as pickle, dehydrated leather, thin papad etc can be made from Jackfruit. (Bhatia *et al.*, 1956a; Bhatia *et al.*, 1956b; Teatota and Awasthi, 1968).

Jackfruits for exporting purpose are generally in “whole-fruit” forms. The high shipping cost of bulk fruits wherein only 40 % of fruits are edible are cost-ineffective for local farmers. Hence, innovative developments in Jackfruit processing are required to ensure a suitable shelf life for export purposes.

2.2 PECTIN

Globally, agro-industrial by-products such as fruit wastes have been recognized useful for extraction of valuable ingredients. Pectin is such a naturally occurring bio-polymer which has been extensively recognized in the food industries as well as in the field of bio-technology. (Khan *et al.*, 2017).

Pectin is a family of complex polysaccharides having 1, 4 - linked α -D galactosyluronic residues. Homogalacturonan, rhamnogalacturonan-I and substituted galacturonans are some of the pectic polysaccharides isolated from the primary plant cell walls. Homogalacturonan (HG) is a linear chain of 1, 4-linked α -D-galactosyluronic residues, with some carboxyl groups having esterified methyl group. (Sharma *et al.*, 2006).

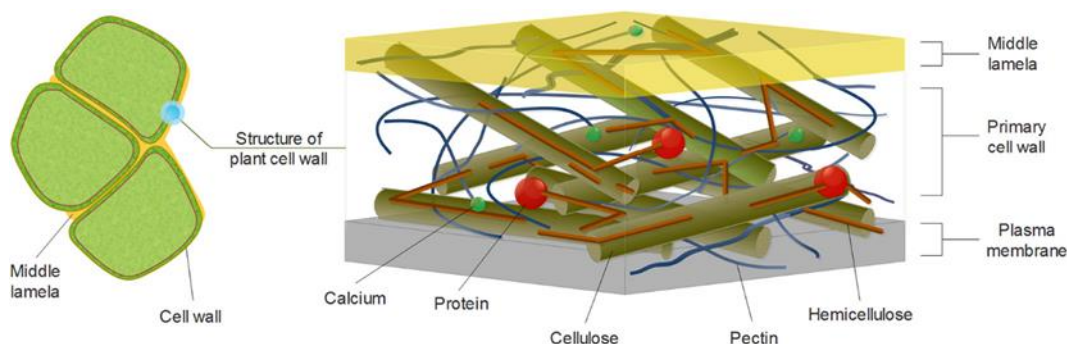


Fig 2.2 Structure of primary plant cell wall (McCann & Roberts (1992))

Pectin is a polysaccharide having hydro colloidal, stabilizing and gelling characteristics. It is widely used in the manufacture of food products, cosmetics, pharmaceuticals, and other products. (Guerrero *et al.*, 2017). Pectin is a component present in primary cell wall of plant cells as well as in the middle lamella of plants tissues. At present, pectin stays as an essential raw material, especially in the food industry as well as in pharmaceutical industries. (Quoc *et al.*, 2015).

Pectin and pectin substances are present in all plants. Pectin substances have the ability to form insoluble complexes enabling the body to get rid of toxins, heavy metals, radionuclide, etc. (Sobol, 2016). Pectin is extensively used as a component

in edible films due to its positive properties such as availability, low cost, and easy processing. (Bykov *et al.*, 2017)

Hosseni *et al.*, (2016) stated that the higher yield, acceptable physico-chemical properties and good degree of purity of obtained pectin substantiate the use of this agricultural waste as a promising source for the pectin production.

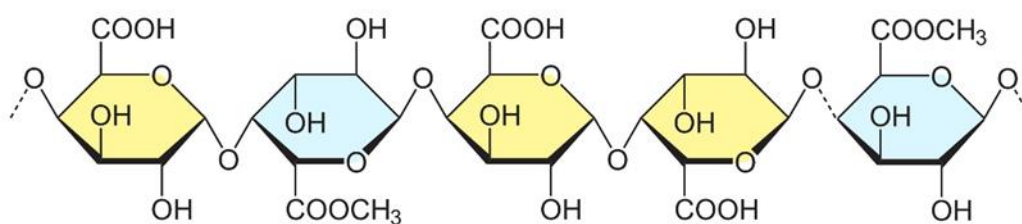


Fig 2.3 Structure of Pectin

The primary monomeric unit of the complex pectin molecule structure is D-galacturonic acid (Gal A), a sugar acid which is an oxidized form of D-galactose. These Gal A units are linked together with 1/4 galacturonosyl linkages which gets interrupted by side-chains bearing L-rhamnose units, disrupting the linear conformation of poly-(Gal A) chain. Specific carboxyl groups among these poly-(Gal A) chain gets esterified by methyl groups which cause deviation in the degree of methyl esterification (DE or DM). Depending on the percentage of DE (greater or less than 50%), pectin is classified as high methoxyl pectins (HMP) or low methoxyl pectins (LMP). Homogalacturonans (HG), rhamnogalacturonan-I (RG-I), and substituted galacturonans (SG or RG-II) are the three general basic classifications of pectins (Ridley *et al.*, 2001).

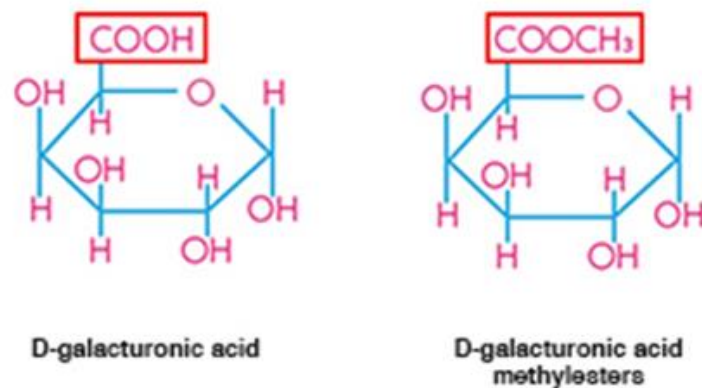


Fig 2.4 Basic structural formula of Galacturonic acid

Extraction practices are generally administered by mass transfer principles. Pectin isolation depends on three factors (Minkov *et al.*, 1996; Pinelo *et al.*, 2006);

- (a) Hydrolysis of proto-pectin (i.e. pectic polysaccharides attached to different cell wall components such as cellulose) from plant material;
- (b) solubilisation of obtained pectin;
- (c) The extraction solvent achieves equilibrium/saturation state.

The pectin extraction follows two processes, hydrolysis and solubilization. The latter comprises diffusion of dissolved pectin into the solvent (Cho & Hwang, 2000; Minkov *et al.*, 1996).

El-Nawawi and Shehata (1987) stated that, the major problems faced in obtaining qualitative and quantitative extract, during pectin extraction may be classified as:

- (i) Technology associated issues (such as optimum operating conditions like temperature, pressure etc.);
- (ii) Intrinsic factors: based on pre-treatment condition (moisture content, particle size etc.);
- (iii) Extrinsic factors: based on solvent (volatility, polarity, solid-liquid ratio, molecular weight, toxicity of solvent etc.).

2.2.1 Pectin Extraction

Srivastava and Malviya (2011) stated that direct boiling and microwave extraction are the most common methods for pectin extraction. The conventional method for extraction is direct boiling, that requires at least 2 hours to obtain a good yield. The microwave extraction requires approximately 15 min for extraction of pectin and is comparatively better in terms of output and quality.

Khalil *et al.* (2013) studied the morphological properties, including relative viscosity for the aqueous and ethanolic extracts of sunflower pectin on citrus pectin and reported that aqueous extraction of pectin from sun flower bases yielded better viscosity, while ethanolic extraction yielded best morphological properties, which indicates the advancement of sunflower pectin in comparison with citrus pectin on physical properties.

Lizardo *et al.* (2015) assessed the practicability of pectin of saba banana peel for usage in food processing. Pectin from saba banana peel was extracted under two different extraction conditions. Results revealed higher ash content (11.15 and 13.83 % respectively) than commercial citrus pectin (1.76%) whereas moisture content of ripe and unripe saba banana peel pectin was analogous. The equivalent weight obtained from ripe peels (953.89) was comparable to commercial citrus pectin (893.00). Anhydrouronic acid (AUA) and methoxyl content values were significantly lower than commercial citrus pectin, while the degree of esterification (DE) values for both stage of ripeness were comparable to commercial citrus pectin. Strawberry jam prepared using the obtained pectin was used to assess its gelling ability and sensory evaluation (Triangle test) was conducted for comparison studies.

Leong *et al.* (2016) studied the consequence of citric acid, nitric acid and sulfuric acid based on yield and physico-chemical properties of pectin obtained from Jackfruit and chempedak rind. Yield and physicochemical properties such as color, uronic acid content, degree of acetylation and degree of esterification were found and compared. Pectin yield was lowest for nitric acid extraction (with a yield of 14.81 ± 1.02 % and 17.62 ± 0.69 %, respectively). The uronic acid content

obtained was more than 65 % for all pectins. High methoxyl pectin, having a degree of esterification ranging from 72-75 % for Jackfruit rind pectin and 66-69 % for chempedak rind pectin were obtained in this study. The degree of acetylation was lower than 1%. Citric acid-extraction resulted in darker pectin and is least preferred. Sulfuric acid proved to be the best extractant among the acids studied, giving high yield of pectin. Brighter pectin solution were obtained.

Gazala *et al.* (2017) stated that physic-chemical characteristics of pectin extracted varied significantly. Citric acid extraction resulted in pectin with higher anhydrouronic acid (65%), higher methoxyl content (10.39 %) and degree of esterification (49.35%). The pectin extracted from both sources showed a lower degree of esterification (50%) that discloses its low methoxyl content.

Guerrero *et al.* (2017) informed that, pectin yield increased proportionally to time and temperature. However, there was no observable variation in methoxyl percentage ($2.65\% \pm 0.16$ and $2.90\% \pm 0.03$). The best extraction parameters using citric acid solution of pH 3, at a temperature of 70 °C for 95 min. Under this condition, 8.82 g pectin/100 g pectin yield from cocoa husks was attained with an esterification degree $71.88\% \pm 0.78$, and galacturonic acid percentage of $26.86\% \pm 0.86$. These results show that the husks from cocoa industry can be considered as a substitute source to extract pectin.

Khan *et al.* (2017) reported 22.55 % maximum extraction of pectin from grapefruit peel at a temperature of 120 °C, at pH 1.5. Besides, adding the obtained pectin for jam preparation showed a substantial effect on the texture of final product. The results stated that pectin holds great potential for application in fruit industries for best quality products and value addition.

2.3 PULSED ELECTRIC FIELD

Electronics plays imperative role in the development of various automatic machinery equipment in the arena of Agricultural Engineering. Mechatronics is the combination of mechanical systems with electronics. There are numerous

applications of electronics in the machinery used in the various branches of Agricultural engineering. Applications of electronic-based equipment such as Atomic absorption spectrometer, LASER leveler, and non-thermal electro-technology like Pulse Electric Field are some of promising applications of electronics in the area of Agricultural Engineering (Aware, 2009). Pulsed electric field (PEF) is a non-thermal method used for disintegration of plant tissues that can increase juice yield and enhance extraction of bio-active compounds.

The goal of novel technologies is to preserve bio-active components with a marginal impact on its sensory qualities, and to increase safety and quality control alongside the food chain. Also other important objectives of novel technologies comprise the producing shelf stable, semi fresh products, reduction in food wastage by increasing shelf life, reducing water and energy usage, the producing food ingredients using various byproducts. To achieve these goals new technologies such as sensor technology, Nano and micro technology, non-thermal pasteurization, and sterilization, the utilization of by-products, sustainable packaging, low water application and low energy technology along with enhanced knowledge transfer should be applied. Non thermal methods such as pulsed electric field, high hydrostatic pressure, and cold plasma facilitate enzyme and microbial inactivation with a negligible impact on the sensory properties. (Hribar *et al.*, 2016). Pulsed electric field (PEF) can be considered as a novel emerging technology that can substitute conventional thermal (HTST) pasteurization.

Pulsed electric field (PEF) is a promising and innovative method for non-thermal food processing. It is a noble substitute for conventional cell membrane permeabilization methods like thermal treatments, and chemicals and enzymes addition. Electroporation is the prime effect occurring due to pulsed electric field interaction with biological cells. In this method, the creation of pores on membrane cells occurs, resulting in an increase in permeability.

Disadvantages in food products caused by thermal processing and the rising demand for safe, healthier and high-quality foods by consumers lead to the development of novel food preservation technologies. The pulsed electric field

technology is a non-thermal novel method which can break down the cell membrane of microbes as well as in food. This characteristics enables pulsed electric field to enable operations such as drying, extraction and facilitates microbial inactivation in the food industry. Use of pulsed electrical field the food industry has been limited due to its high investment and operating costs as well as uncertainty on process parameters for various foods (Güven, 2017).

Pulsed electric field uses short bursts of electricity for microbial inactivation and causes marginal or less unfavorable effect on food quality features. PEF technology promises safety as well as marketability of food products. PEF technology has been used for cold-pasteurization and less number of products are commercially available in the market. PEF can be considered as a continuous processing method, for semi-liquid and liquid food products. Application of PEF technology has been efficaciously established for the pasteurization of products such as milk, yogurt, juices, liquid eggs, and soups. PEF is also useful to increase extraction of sugars and other cellular contents like sugar beets from plant cells. PEF is also used in reducing the sludge from waste water. (Giri and Mangaraj, 2013).

Protein is one of the most important components present in food, and its functional properties determine the quality of food. Protein modification methods mainly include physical, chemical, enzyme, and genetic engineering, etc. Physical modification has lots of advantages such as short treatment time, low cost, safety, and smaller effects on nutrition in comparison with other methods. Moreover, novel physical modification methods have advantages, for instance, low treatment temperature, low energy cost, low consumption in protein functionality and high product quality. But novel physical modification methods are of limited application in the food industry and is still in the laboratory stage. There is limited knowledge about the mechanism of some novel modification methods and the materials used in such methods. So extended knowledge is required to provide new ideas for protein modification processing (Guo *et al.*, 2017).

Pulsed electric field (PEF) has received exceptional attention among non-thermal treatments due to its possible use in liquid foods processing and its viable applications in continuous flow processing. PEF is a non-thermal preservation method primarily used in liquid foods for inactivation of micro-organisms and enzymes and retaining volatile components and nutrients in food. Improving milk quality and other dairy products with PEF processing might seek pertinent attention to products development research. Pulsed electric field (PEF) processing has already been established as an alternative that can be applied to convey safe shelf-stable products. (Swami, 2010).

Maillard reaction which occur mostly in heated and stored foods is one of the main non-enzymatic browning reactions amongst amino groups in proteins and reducing sugars. Maillard reaction could cause desired or undesired effects on quality attributes of food, affecting its taste, color, nutritional value. Currently, studies are conducted on non-thermal processes with the aim of reducing these negative changes resulting from these complex reaction and favorable outcomes are achieved. The application of such non-thermal methods in the food industry may increase shelf-life of products and in producing more reliable foods that have better quality related to its nutritional and sensory characteristics. Thus, non-thermal processes have proven to be alternate to existing processing methods, comprising thermal processing methods. (Topdas, 2012)

The influence of high electric field pulses (HELP) as a non-thermal, novel procedure on cell disintegration and oil extraction of palm fruit mesocarp was examined by Paoplook *et al.* (2013). The process parameters (pulse number 5 to 30 pulses, Field strength 1 to 5 kV/cm, and the capacity of capacitors 0.49 to 1.98 μF) showed a thorough effect on cell disintegration. Increasing field strength increased cell disintegration. About 97% of cell disintegration was achieved after HELP treatment at 5 kV/cm and 30 pulses. In addition, increasing pulse number from 5 to 30 pulses (at a constant field strength of 4 kV/cm) increased the cell disintegration. Likewise, increasing the capacity of capacitors up to 1.98 μF exhibited a positive effect on cell disintegration of palm fruit mesocarp. The energy consumption and

processing time in the course of cell disintegration using HELP were distinctly lower (about 12 kJ/kg, less than 30 sec.) in comparison to thermal treatment (at 80°C, 2h, about 210 kJ/kg). Pressings of mesocarp indicated that the remaining pulp after pressing for HELP pretreated samples (at 4 and 6 kV/cm) was noticeably lower (about 20 and 18.6 % respectively) compared to thermally treated samples (21.5 %). The measurement of oil yield exhibited a progressive effect of HELP pretreatment. Increasing field strength increased oil yield. The peroxide value of crude oil pretreated with HELP was marginally higher related to untreated or thermally treated samples.

Kumar *et al.* (2015) in the study on Pulsed electric field and combination processing of mango nectar and its effect on volatile compounds and HMF formation reported that PEF treatment reduced the negative effect on retention of volatile compounds. Also, the HMF concentration obtained was lower compared to other treated samples and was found insignificantly ($p > 0.05$) different from the untreated sample, imparting the fresh-like character of the product.

Lamanauskas *et al.* (2015) in his work, treated frozen/thawed European blueberry (*Vaccinium myrtillus* L.) fruits with pulsed electric field to enhance the cell membrane permeabilization and to increase the quality of blueberry juice at the time of pressing process. The pulsed electric field is an effective method for cell membrane permeabilization of food tissues on fresh plant cells. Freeze/thawing is also well known for its capability of cell membrane permeabilization. The Blueberries tissues were exposed to 20 μ s monopolar square wave pulses with different electric field strength ($E=1-3-5$ kV cm⁻¹) and total specific energy input ($WT=1-5-10$ kJ kg⁻¹), with their permeabilization being characterized by electrical impedance measurements and cell disintegration index (Z_p). The juice obtained after pressing (1.32 bar), was analyzed for anthocyanin content, total polyphenols and antioxidant activity. The cell disintegration index ($p < 0.05$) increased significantly from 0.2 to 0.6 with increasing pulsed electric field treatment intensity (E and WT). Consequently, in comparison with control, PEF treatment prompted a slightly higher release of polyphenols (up to +8.0 %) and anthocyanins (up to

+8.3%), thus enhancing the antioxidant activity of blueberry juice (up to +16.7 %). In brief, frozen/thawed blueberries could be treated using pulsed electric field to further increase juice quality.

Kumar *et al.* (2015) subjected Ready to Drink (RTD) mango nectar to PEF treatment. The effect of process parameters including pulse width (15-24 μ s) and pulse frequency (70-120 Hz) on carotenes, coliforms, total plate count, molds and yeast, and overall acceptability were studied using response surface methodology. Mango nectar showed high retention of carotene content at higher frequencies and pulse widths in comparison to lower levels. Conversely, severe PEF treatments reduced the carotene content. Maximum inactivation of native microflora (4.1 log CFU/ml), retention of carotene content (2100 μ g/100 ml) and overall acceptability (8.3) scores were obtained at 38.0 kV/cm for 24 μ s using bipolar pulses at 120 Hz.

Xiong *et al.* (2015) studied the extraction effect of total flavones from rape pollen, by using a PEF wall-breaking method on the basis of 3 single factors - pulses number, electric field intensity, and backflow temperature considering total flavones extraction rate as response value, the best extraction method for total flavones were optimized using Box-Behnken method. The results indicated that electric field intensity had a substantial effect on rape pollen cell wall breaking. Under the optimal extraction conditions, i.e. electric field intensity of 28 kV/cm, pulses number of 508 and backflow temperature of 82 °C, total flavones extraction rate could extent to 4.508 %.

Ferrari *et al.* (2016) reported that PEF pre-treatment of red raspberries was found to be a promising technique improving the efficiency of industrial processing of raspberries.

Liu *et al.* (2016) characterized the extraction effect of corn bran polysaccharide using high voltage pulsed electric field (PEF) assisted extraction and proved PEF can increase the extraction rate of corn bran polysaccharides, and stated PEF assisted enzymes were better in comparison.

Teusdea *et al.* (2017) studied the effect of various pulsed electric field treatments on producing high quality red wines. Different Pulsed Electric Field (PEF) treatments were applied in the pre-maceration stage of mash derived from 'Pinot Noir' and 'Merlot' grapes which were harvested from Crişana-Santimreu vineyard, Romania, in 2016, with the aim of increasing the content of total phenols, flavonoids, monomeric anthocyanin pigment and colour intensity of 'Pinot Noir' and 'Merlot' wines. All PEF treatments applied in the pre-maceration stage lead to an increase in bioactive compounds and color intensification. PEF technology thus proved to be suitable for extracting phenols from grapes and can be applied in the food industry to attain wines rich in bioactive compounds with antioxidant capacity.

Bellebna *et al.* (2017) analyzed the effectiveness of PEF pre-treatment of beet juice extraction. Response Surface Modeling (RSM) was used for ascertaining the set point for betanin extraction process. The number of pulses and influence of PEF strength on betanin extraction were investigated in this work. The results revealed that investigated parameters of PEF pre-treatment shown significant effect in juice yield as well as enriched betanin concentration of final product.

2.4 MICROWAVE ASSISTED EXTRACTION

Mason *et al.* (1999) stated that the primary step in obtaining crude extract from plants is extraction. The extract thus obtained is then subjected to analysis and identification of active components. Conventional extraction methods include Soxhlet extraction, maceration, soaking, water percolation, etc. These techniques requires long extraction time which results in risk of thermal degradation of thermo labile active components.

Microwave assisted extractions (MAE), pressurized solvent extraction (PSE) and supercritical fluid extraction (SFE) are some of the novel techniques available for extraction. Among these, Microwave-assisted extraction has attained significant research attention in several fields, particularly in medicinal plant research, because of its moderate capital cost, special heating mechanism and its good performance

in atmospheric conditions (Howard, B., 1995; Eskilsson and Björklund., 2000; Li *et al.*, 2003)

Electromagnetic waves with frequencies ranging from 300 MHz - 300 GHz are called Microwaves. (Basak *et al.*, 2013). The microwave field comprises alternating magnetic field, in which, polarity of molecules changes from the original random thermal motion in line with the direction of orientation of the electric field (2.45 billion times per second) (Menendez *et al.*, 2010). The capability of a food material to convert microwave energy into heat is determined by its dielectric properties (Curet *et al.*, 2014; Franco *et al.*, 2015).

Srinivasa and Malviya (2011) reviewed on pectin sources, extraction and its applications on pharmaceutical industry cited that microwaves penetrate into food materials and interacts with polar molecules to generate heat. This energy from heating of microwave acts directly on polar molecules as a result of ionic conduction and dipole rotation. Thus selective and targeted constituents can be heated relating to their dielectric constant. During microwave heating, high pressure builds up inside material which modifies the physical properties of plant tissues. This pressure breaks down cell structure and improves capillary porous structure of plant tissues. This increases penetration rate of extracting solvent into tissues, increasing the extraction rate of pectin.

The efficiency of microwave heating relies on the dissipation factor of the material, $\tan \gamma$, which measures the ability of the sample to absorb microwave energy and dissipate heat to the surrounding molecules, given by Eq. (1) (Mandal *et al.*, 2007)

$$\tan \gamma = \epsilon''/\epsilon' \quad (1)$$

Where, ϵ'' is the dielectric loss indicating the efficiency of converting microwave energy into heat. ϵ' is the dielectric constant which measures the ability of the material to absorb microwave energy.

The rate of conversion of electrical energy into thermal energy in the material is defined by Eq. (2) (Chen *et al.*, 1993)

$$P = K \cdot f \epsilon'' E^2 \tan \gamma \quad (2)$$

where P is the microwave power dissipation per unit volume, K is a constant, f is the applied frequency, ϵ'' is the material's absolute dielectric constant, E is the electric field strength and $\tan \delta$ is the dielectric loss tangent.

Microwave heating gained popularity in food processing because of its ability to attain high heating rates, more uniform heating, reduction in cooking time, ease of operation, safe handling and low maintenance (Salazar-Gonzalez *et al.*, 2011, Zhang *et al.*, 2006). Moreover, microwave heating reduces changes in flavour and nutritional quality of food contrasted with conventional heating during cooking or reheating process (Vadivambal and Jayas, 2010).

Drying, tempering, sterilization, thawing, pasteurization, baking of food materials etc. are some of the applications of microwave heating in food processing (Metaxas and Meredith, 1983, Gupta and Wong, 2007).

Letellier & Budzinski (1999) stated that the two basic principles of extraction using microwave energy, are

- (i) Direct heating of microwave-absorbing matrix, which leads to the release of target compound into the cool solvent;
- (ii) Heating polar solvent up to boiling point, which results in extraction of the compound.

Increased microwave power raises temperature within the system which leads to increase in pectin yield (Maran *et al.*, 2013; Wang *et al.*, 2007). But after an observable threshold, further increase in MW power reduces pectin yield depending on the thermolabile nature of pectin. This latter effect was also reported for MAE of flavonoids (Routray & Orsat, 2012). This initial rise in extraction efficiency maybe due to lower viscosity and surface tension, which accelerates hydrolysis and increases solubility of proto-pectin and pectin. It is, therefore, imperative to select an optimal MW power such that time required to attain the fixed temperature is minimized.

Li *et al.* (2013) conducted a research to compare the higher extraction rate between alcohol deposition with acid hydrolysis and microwave-assistant extraction technique of pectin from orange peels. The experimental results showed that the former method produced a better extraction effect. For alcohol deposition

with acid hydrolysis method, the technique parameters were optimized using L9 orthogonal experiment based on the result of single-factor experiments, after investigating the influence of pH, solid to liquid ratio, extraction time and temperature on pectin extraction rate. On the basis of these ideal technical parameters, the microwave-assisted technique was used for pectin extraction. The selected parameter was acquired using L16 orthogonal experiment after studying the effect of pH, solid to liquid ratio, microwave treatment period, extraction temperature and microwave power on pectin extraction yield. Repetitive analyses of two methods were carried out separately, pectin extraction rate using the first technique was 14.57 % and that with the second technique was 11.62 %. The characteristics of pectin sample obtained using alcohol deposition with acid hydrolysis were investigated further. The research validated that mass percent of pectin was 87.23 %. The degree of esterification of pectin was 75.66 %, and color of the pectin sample ranged from creamy white to pale yellow.

Koh *et al.* (2014) in his study compared the efficiency of microwave-assisted extraction of pectin from Jackfruit rind against conventional extraction method. This study also investigated the effect of power level on quality and yield of pectin extracted from Jackfruit rinds. For conventional extraction, water-based extraction method was conducted with an extraction duration of 1 h and 10 min for MAE, respectively. The temperature of conventional extraction was fixed at 90 °C and the power levels of MAE were fixed as 450 W, 600 W and 800 W. High yields of pectin were achieved from conventional extraction (14.59%), and MAE (16.72-17.63%). Except for moisture and ash content, all quality characteristics determined were found to be insignificant for pectin extracted from both MAE and conventional extraction. Increase in microwave power has an insignificant effect on yield and quality characteristics of pectin. The results showed that MAE requires less time than conventional extraction for the extraction of comparable amount and quality of pectin from Jackfruit rind. Microwave-assisted extraction at a power of 450 W was the most economic and effective extraction condition amongst the different tested power levels. Microwave-assisted extraction (MAE) was thus found to be more efficient than the conventional method in extracting pectin.

Rahmati *et al.* (2015) reported that the maximum expected yield of pectin extraction was 18.53 % by application of microwave-assisted extraction and stated that microwave-assisted extraction can produce high-quality dragon fruit peel pectin.

Zarei *et al.* (2017) studied the effect of microwave-assisted extraction on yield and quality of apple pomace and lemon peel pectin and showed that Microwave-assisted extraction resulted in higher pectin yields of 10.07% and 8.83% in pretreated samples using the microwave and 9.4% and 8.0% in the extraction of dried samples after microwave treatment in lemon peel and apple pomace, respectively. Lemon peel pectin in pre-treated samples using microwave and extraction of dried samples after microwave treatment exhibited a higher degree of esterification 71.8 % and 70 %, respectively, while 68 % and 65.4 % were obtained from apple pomace in same treatments. Moreover, lemon peel pectin displayed the highest galacturonic content of 74.5 % in the extraction of pre-treated samples using the microwave, while apple pomace pectin showed higher galacturonic acid content of 70.5 % and 70 % in both extractions of dried after microwave treatment and in the extraction of dried samples. Texture analysis of jellies prepared using various extracted pectin showed the highest fracturability in the microwave assisted drying treatment of 32.5 N and 33 N for lemon peel and apple pomace pectin, respectively.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

This chapter outlines the design and development of a Pulsed Electric Field (PEF) and Microwave assisted extraction system and methodologies adopted for studying the effect of PEF assisted microwave treatment for extraction of pectin from Jackfruit rind and core. The materials used for fabrication of the PEF system and the instrumentation employed for measurements of parameters were explained. The process of evaluation of the developed system and optimization of process parameters for PEF assisted microwave extraction of pectin from Jackfruit rind and core and the methods for determining various quality parameters are also explained.

3.1 DEVELOPMENT OF A PULSED ELECTRIC FIELD PRE TREATMENT SYSTEM FOR PECTIN EXTRACTION

In this study, a Pulsed Electric Field (PEF) pre-treatment system was conceptualised and developed for pectin extraction. When pulsed electric field is applied to the material, an extra membrane potential difference develops across the plant cells. When this potential difference exceeds a critical value, localized electrical breakdown of cell membrane occurs resulting in an increase in cell permeability and pore formation. This in turn increases mass transfer rate of extraction process which in turn increases the extraction efficiency in the subsequent extraction process.

A lab scale PEF unit was conceptualized, designed and fabricated based on thorough review of research conducted on principles of PEF system. The developed PEF system as shown in Fig 3.1 and Plate 3.1 consists of following parts.

1. Outer Protective Chamber
2. Inlet System
3. Pulse Generating System
4. Treatment Chamber
5. Display Unit
6. Cooling System
7. Treated Sample Outlet

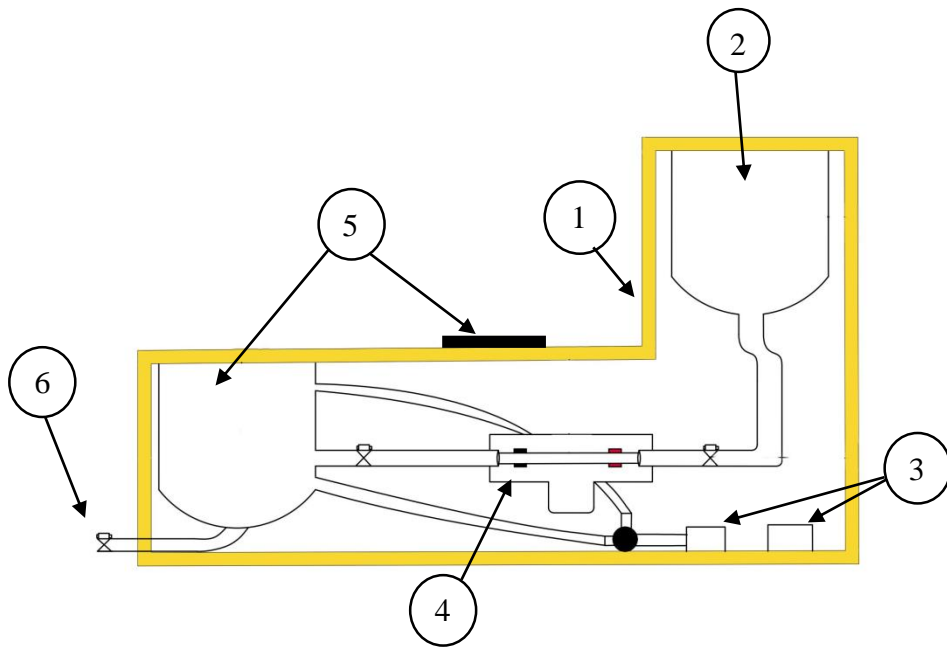


Figure 3.1 Schematic diagram of PEF pre-treatment chamber

1. Outer Protective Chamber
2. Inlet System
3. Pulse Generating System
4. Treatment Chamber
5. Cooling System
6. Treated Sample Outlet



Plate 3.1 Front view of the pulsed electric field system

3.1.1 Outer Protective Chamber

The main frame and supporting structures of PEF treatment system was made using 1 inch mild steel angles. Aluminium sheets of 10 gauges thick were used on frame casing. All display and controls for monitoring and adjusting parameters like electric field strength, pulse frequency and power switch are provided on this outer casing. Special insulation is provided to isolate outer frame work from the internal circuits for the safety of operator.

3.1.2 Inlet System

It consists of a stainless steel feed vessel of 5 L capacity connected to a 2 cm diameter stainless steel pipe. The alcoholic insoluble residue from the feed vessel flows to the treatment chamber via the steel pipe (Plate 3.2). A flow control valve connects the inlet system to treatment chamber, which regulates the feed rate of solution into the treatment chamber.



Plate 3.2 Inlet system

3.1.3 Pulse Generating System

The pulse generating system constitutes the key component of PEF system. This high boost circuit was designed to generate pulses of voltage ranging from 5 to 20kV. The input supply requirements are 230 V, 12 W power and 5A current.

In this system, pulse generation is achieved by a LOT (Line Output Transformer) and a controlling unit with micro controller. The primary function of LOT is to step up input voltage (230 V) to a required voltage (up to 20 kV) at an input current of 5 A. Controlling unit with micro controller adjusts the frequency and amplitude of pulses. Embedded C was the language used to set up micro controller.

Initially, Line Output Transformer is extensively used in switched mode power supply for supplying high voltage. A wide range of low and high voltages can be generated using this transformer having less number of windings through rectification. The primary winding of LOT transformer is subjugated by a switch from power supply. When power supply is switched ON, the primary inductance builds up current, which quickly raises the voltage in the output winding within a second until limited by load conditions. The resultant output current is in pulsed wave form that repeats at horizontal frequency of the display.

A filtering unit is attached along with the LOT to check the properties of generated pulses. Filter unit filters the input current and prevents component damage from high voltage fluctuations. Filter unit and LOT constitute circuits fabricated with different diodes, resistors, capacitors and transformers. Plate 3.4, Fig 3.2 and Fig 3.3 shows LOT and filter circuits respectively. Since the LOT circuit produces pulse with varying voltages, an isolated feedback circuit is installed between the electrode and LOT to ensure the transmission of pulses of required voltage into the treatment chamber. Circuit diagram of feedback circuit is represented in Fig 3.4.

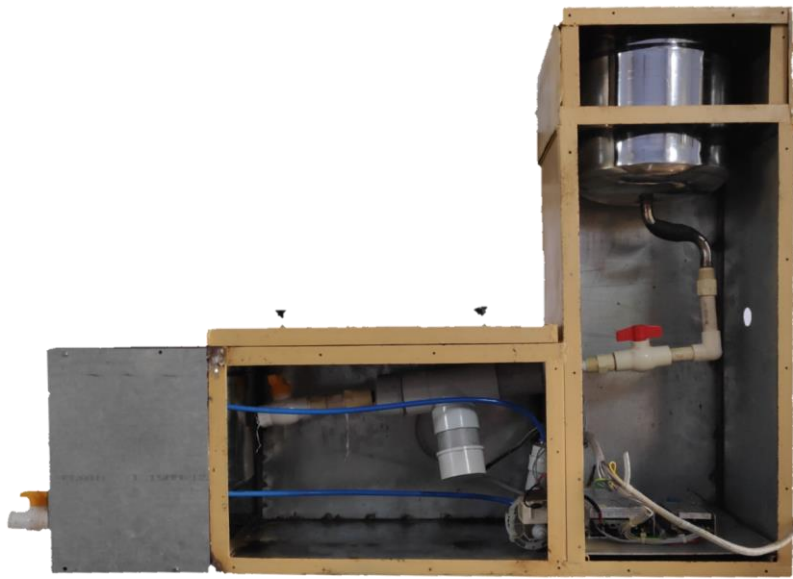


Plate 3.3 PEF treatment chamber

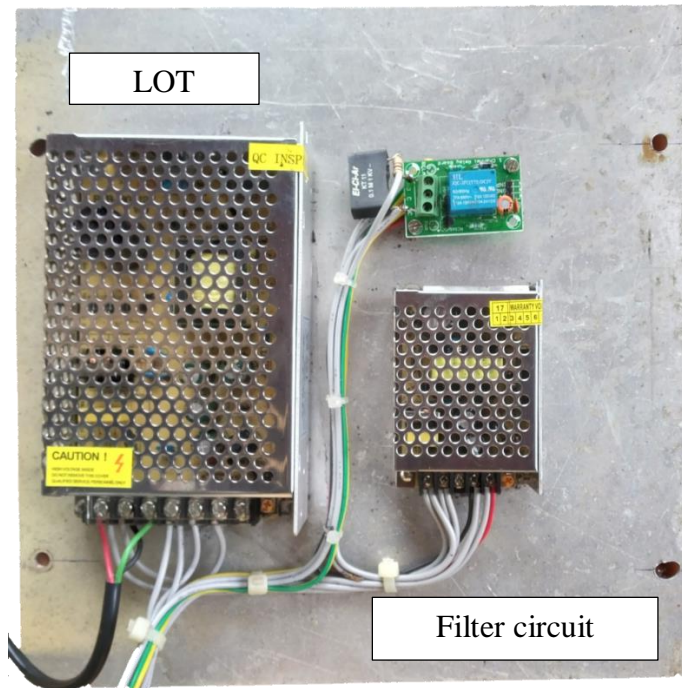


Plate 3.4 LOT and filter unit

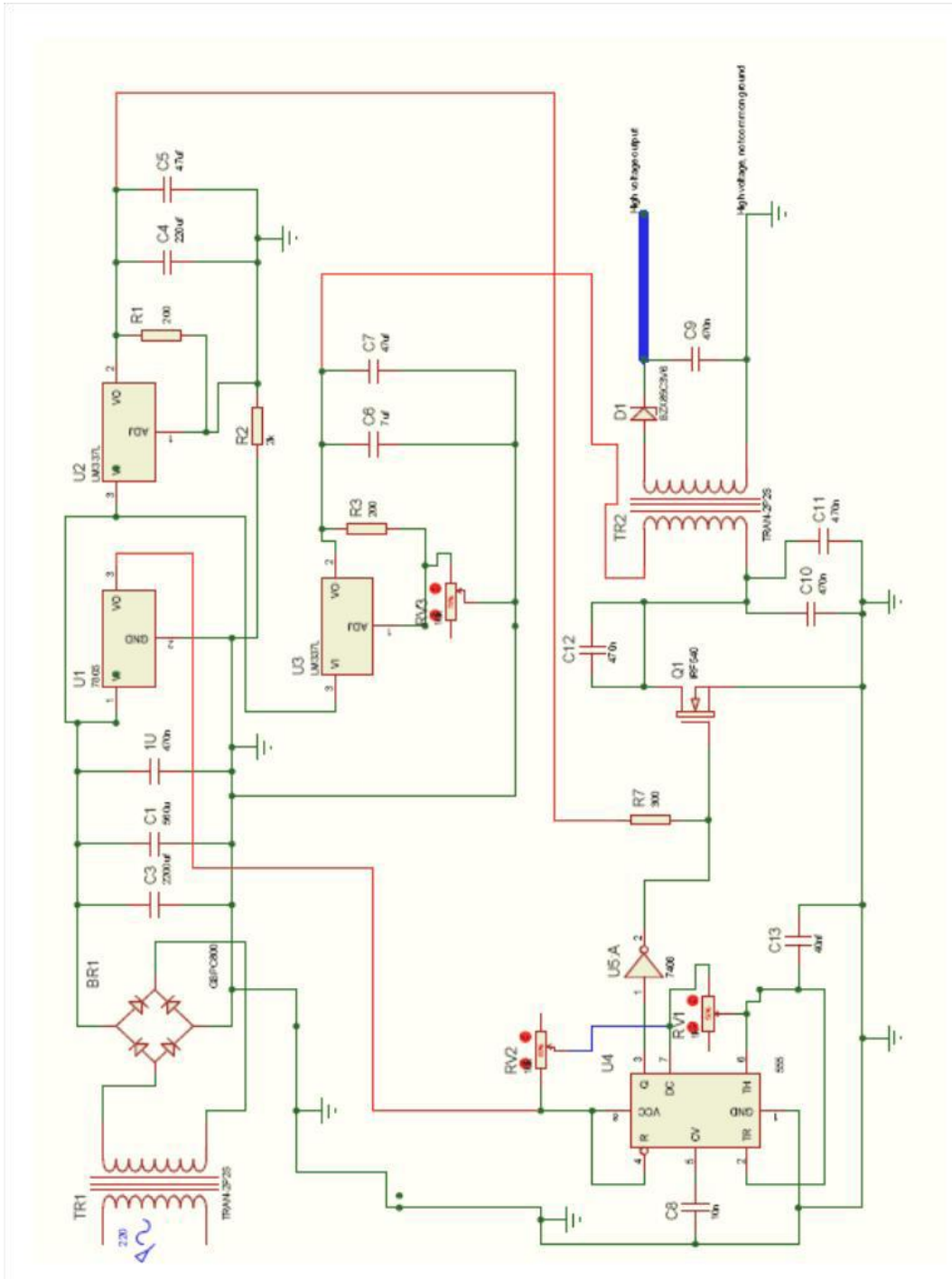


Figure 3.2 Circuit diagram of LOT

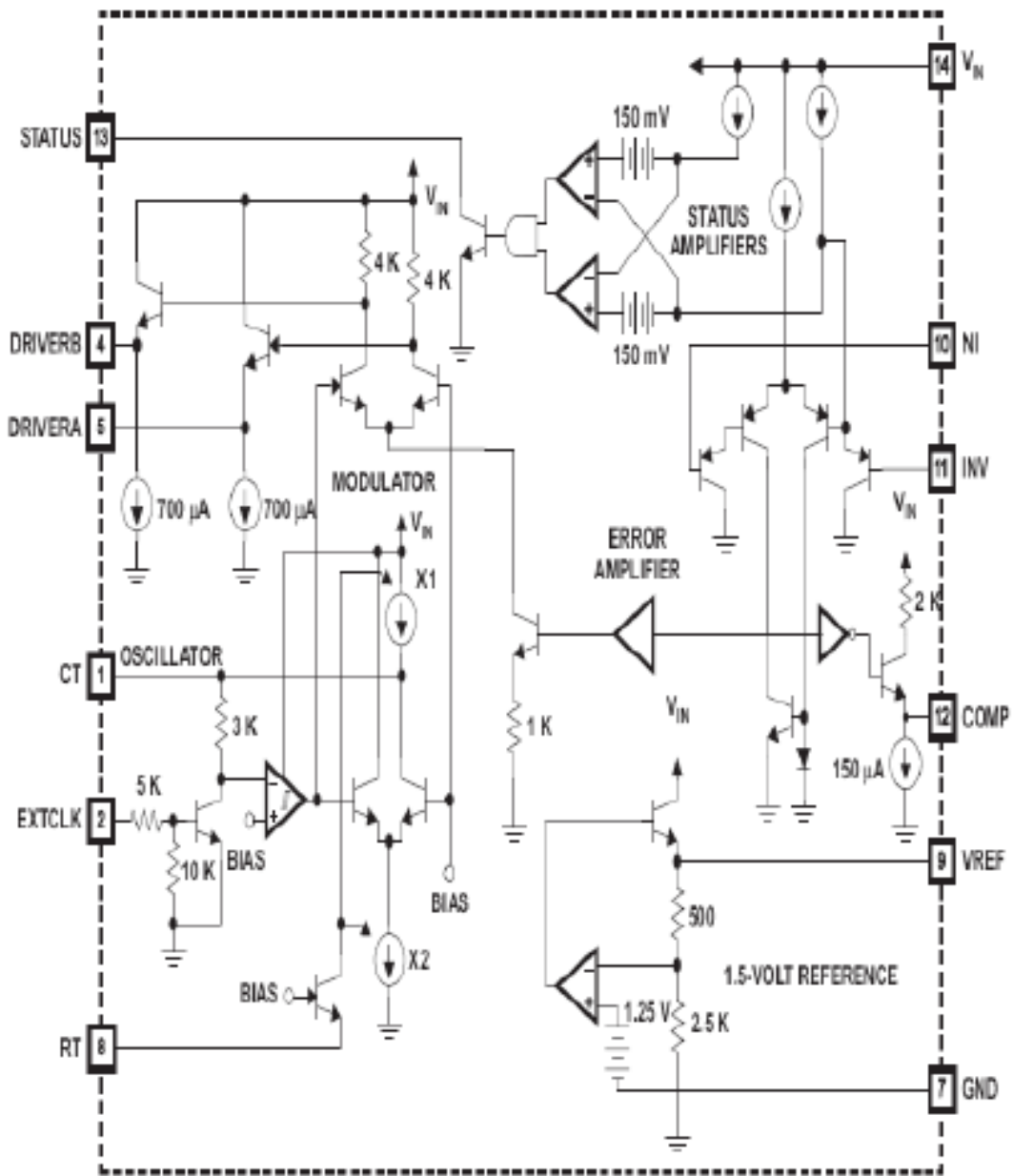


Figure 3.3 Circuit diagram of isolated feedback circuit

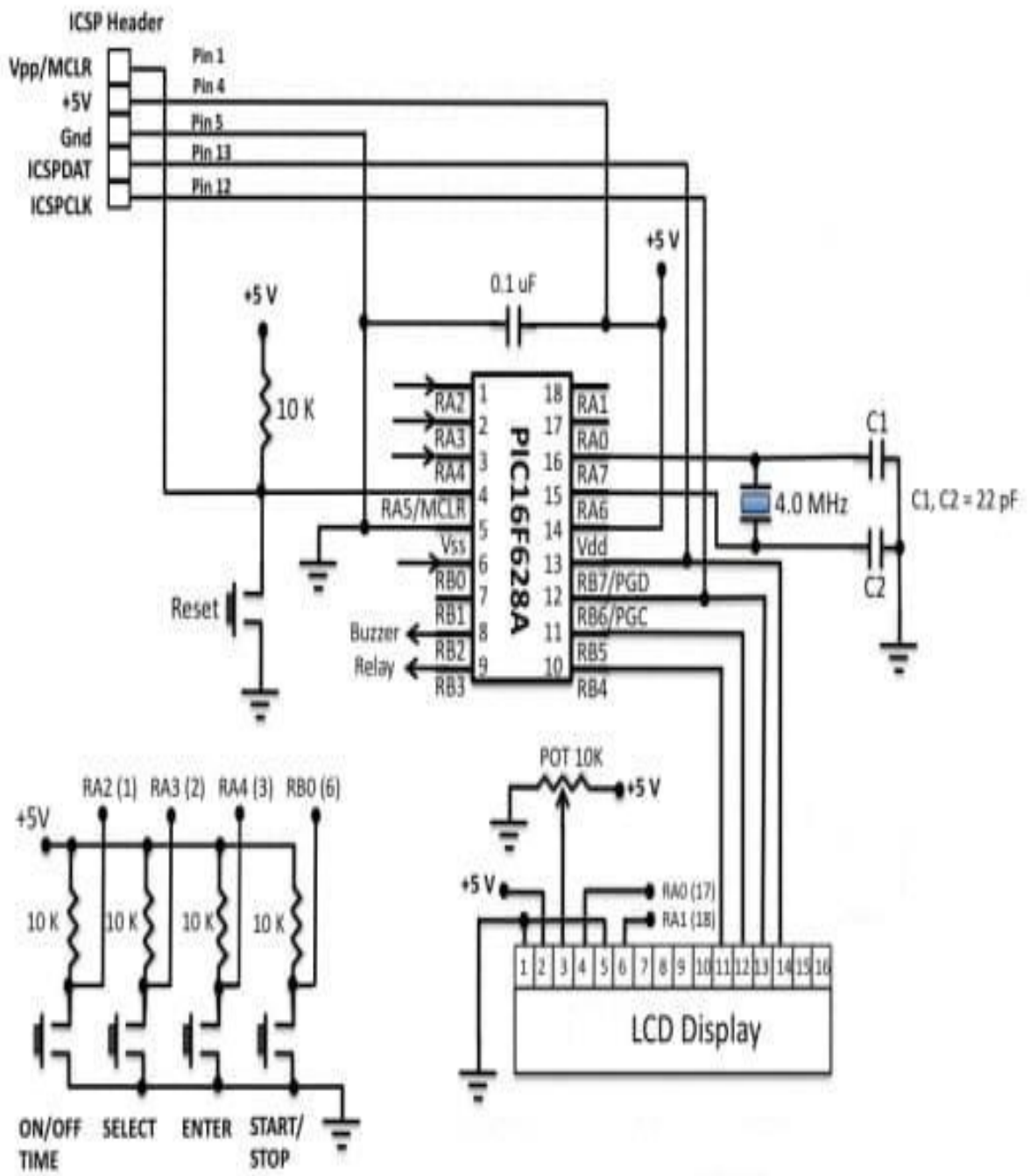


Figure 3.4 Circuit diagram of filter unit

3.1.4 Treatment Chamber

The treatment chamber (Plate 3.5) comprises of two stainless steel electrodes installed at a gap of 5 cm encased in a T-shaped PVC chamber. To prevent electric shocks and to prevent transmission of pulses to exterior, the treatment chamber is provided with an inside coating of Teflon. The chamber includes a sensor connected to the electrodes for measuring the field strength and number of pulses. The high voltage pulses from pulse generator are transmitted to the electrodes in the treatment chamber according to required modes. These pulses transmit through the solution via electrodes resulting in electroporation of cells.

Two flow control valves made of PVC are placed on both ends to regulate the flow. Valve 1 is opened and valve 2 is closed while the solution is fed into the treatment chamber. Both valves are closed during PEF treatment. After treatment, valve 2 is opened and the solution flows out of the treatment chamber.



Plate 3.5 Treatment chamber

Table 3.1.Specifications of treatment chamber

Dimension of electrodes	200 x 50 mm
Gap between the electrodes	20 mm
Thickness of Teflon coat	10 mm
Length of PVC pipe	220 mm

3.1.5 Display Unit

The display unit displays frequency and electric field strength of generated pulses. This unit consists of 5x8 dots display along with a cursor and is programmed using Embedded P language. The frequency and field strength can be set using ‘up’ and ‘down’ switches. Plate 3.6 and Fig 3.5 depicts Display unit and block diagram of display unit respectively.



Plate 3.6 Display unit

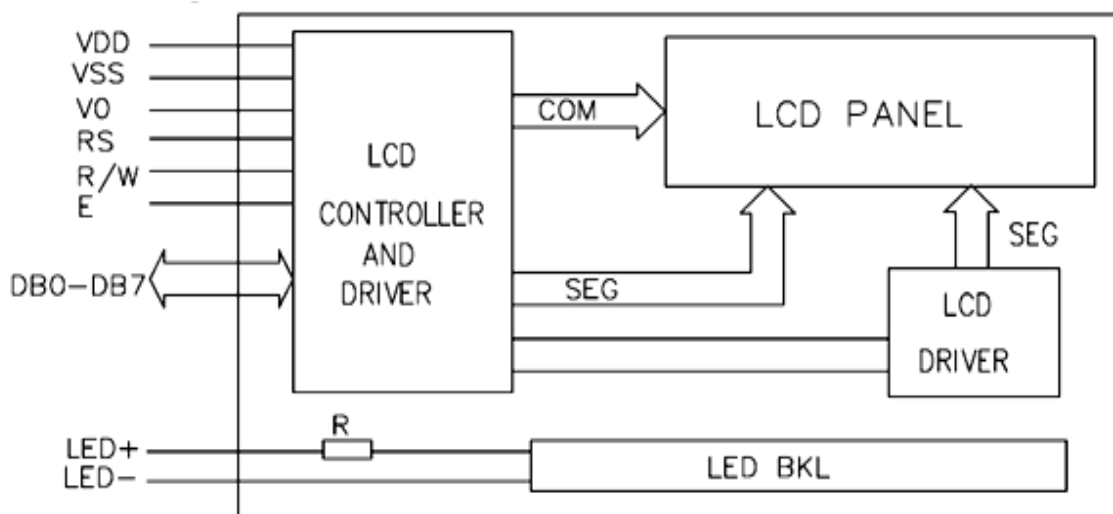


Figure 3.5 Block diagram of display unit

3.1.6 Cooling System

Cooling of treated pectin sample helps to prevent degradation from sudden exposure to high temperature. Two cooling units are provided in the system. A cooling fan (Plate 3.7) is provided on the top of outer frame. This fan is activated automatically by a microcontroller, which makes the fan ON during the high voltage firing of pulses and stops when the firing halts.

A cooling bath of 5 L capacity made of stainless steel is installed at the outlet side to cool the treated mix following exposure to pulsed electric field. The vessel is filled by water and treated sample flows through the stainless steel pipe of 2 cm diameter, immersed in the water bath so that the same gets cooled. Water at ambient temperature is circulated by a pump for efficient cooling. The cooling bath is presented in Plate 3.8.



Plate 3.7 Micro controller activated cooling fan



Plate 3.8 Cooling bath system

3.1.7 Treated Sample Outlet

The outlet system (Plate 3.9) consists of a PVC control valve connected to the outlet pipe which regulates the outflow of PEF treated sample.



Plate 3.9 Treated sample outlet

3.2 MICROWAVE EXTRACTION SYSTEM

The pulsed electric field pre-treated samples were subjected to microwave treatment for extraction of pectin. The microwave extraction results in shorter extraction time and subtly low energy consumption. Microwaves heat the material throughout its volume whereas conventional heating is from the outside and requires heat conduction from the outside in contact with a hot surface. In this process, microwave irradiation heats in-situ water of plant cells, which leads to immediate internal change. This in turn leads to rise of pressure inside plant cells, which further disrupt the cell walls and causes the release of molecules to be extracted (Vinatoru *et al.*, 2017).

For subjecting the pulsed electric filed pre-treated samples to microwaves, requires a microwave reactor. Towards this end, a commercial domestic microwave oven (Model: Whirlpool; Magicook™ MW 20 BC); with following specifications is employed.

Table 3.2 Specifications of microwave extraction unit

Power consumption	230 V/50 Hz, 1200 W (Microwave Output) 1100 W (Grill power) 2000 W (Convection power, Max)
Microwave frequency	2450 MHz
Outside dimensions	262 mm x 452 mm x 325 mm
Oven cavity dimensions	195 mm x 315 mm x 325 mm
Oven capacity	20 litres



Plate 3.10 Microwave source

The microwave power density and the time of exposure to microwaves are the major process variables that influence the extraction process and therefore needed to be optimised. The microwave reactor as specified above has facilities for varying power levels from 110 to 1100 W and to the required time of exposure.

3.3 PRELIMINARY STUDIES ON EXTRACTION OF PECTIN FROM JACKFRUIT RIND AND CORE

As available literature on combined PEF and microwave extraction of pectin is limited, preliminary studies on the pulsed electric field pre-treatment and microwave assisted extraction were carried out to determine the best combinations of solid-solvent ratio, pH of the solvent, range of PEF treatment time, microwave power, exposure time of both treatments.

Based on a thorough review of literature and the preliminary studies conducted, the following parameters were fixed for further analysis and optimisation.

Sl no.	Parameter	Value/Range
1	Solid-Solvent ratio	1:20
2	pH of citric acid	2
3	PEF field strength	5, 10, 15 kV/cm
4	PEF exposure time	2,4,6 min
5	Microwave power density	450, 550, 650 W/g
6	Microwave exposure time	5, 10, 15 min

Citric acid among other acids was chosen as solvent for acidic extraction of pectin as it is food grade.

3.4 EXPERIMENTAL DESIGN

The process parameters which influence the pectin yield and energy consumption for extraction were selected as independent variables based on detailed review of literature and preliminary studies conducted. Yield of pectin and energy consumed during extraction were taken as dependent variables.

Independent variables

I. Pulse Field strength (kV/cm)

- a) V_1 : 5
- b) V_2 : 10
- c) V_3 : 15

II. PEF treatment time (min)

- a) T_1 : 2
- b) T_2 : 4
- c) T_3 : 6

III. Microwave power density (W/g)

- a) D_1 : 450
- b) D_2 : 550
- c) D_3 : 650

IV. Time of exposure (min)

- a) t_1 : 5
- b) t_2 : 10
- c) t_3 : 15

Dependent variables

- a) Pectin yield
- b) Energy consumption

3.5 EXPERIMENTAL PROCEDURE

The experiments were conducted at Food Engineering lab, KCAET, Tavanur to evaluate the effect of PEF assisted microwave treatment to determine the yield of pectin from Jackfruit rind and core and to optimize the process parameters, according to the design of experiments stated in section 3.6.

Freshly harvested raw Jackfruit (*Varikka* variety) purchased from farm of KCAET, Tavanur were used for this study. The jackfruit was then cut using a stainless steel knife and its core (axis) and inner perigones were separated manually. These were then dried in a cabinet drier at 60 °C for 6 hours. The dried plant material is then powdered in a lab under pulveriser mixer and stored in LDPE pouches at ambient temperature.

The alcohol insoluble solids (AIS) from Jackfruit rind and core were prepared by treating the Jackfruit powder with 80 % ethanol for 45 minutes at 1:4 (Jackfruit powder : ethanol) ratio. This procedure is repeated again and unwanted pigments, free sugars and alcohol soluble impurities from jackfruit powder were removed. The treated powder is then dried in an oven at 55 °C until constant weight is achieved. Ten gram of this alcoholic insoluble residue is mixed with 200 ml of 1 % citric acid solution until pH 2.0 is reached, mixed well and is used for each treatment.

3.5.1 Pulsed Electric Field Treatment

The power supply of pulsed electric field generator is switched on. The parameters such as electric field strength and pulse frequency were configured on the configuring unit on the display panel. The alcohol insoluble residue from Jackfruit rind and core is fed into the PEF treatment chamber through the inlet vessel through the flow control valve (Valve 1) at the inlet section (Plate 3.2), while all other valves are closed. Once the treatment chamber is filled with the alcoholic insoluble residue of Jackfruit rind and core, the inlet valve is closed. Then the pulse generator is switched on and the sample is treated with the electric pulses of predetermined electric field strength and pulse frequency for required treatment time. Meanwhile, the microcontroller activated cooling fan gets started during the treatment and go off once the treatment is completed. After the treatment, the pulse generator is stopped and the outlet valve (Valve 2) of treatment chamber (Plate 3.5) is opened and the sample is let into the cooling system of the unit. The treated sample is then collected by opening the treated sample outlet (Valve 3) (Plate 3.9). The treated sample is then subjected to microwave assisted extraction of pectin.

3.5.2 Microwave Extraction of Pectin

The PEF treated samples at different pulse field strength and treatment times were then subjected to microwave assisted extraction. The PEF pre-treated samples obtained after each set of combinations of process variables were kept in the microwave reaction chamber at different power densities and exposure time as per the experimental design (Section 3.4). After microwave treatment, the samples were allowed to cool for 5 minutes and used for further analysis.

3.5.3 Alcoholic Precipitation of Pectin

Alcoholic precipitation of pectin was conducted using method by Kratchanova *et al.*, (2004) with slight modifications. The cooled treated sample was added with equal amount of 95 % ethanol. The mixture was stirred for 30 min using magnetic stirrer at room temperature and then kept under refrigeration at 5 °C for 1.5 h. The coagulated pectin was then separated by filtration. This extracted pectin was washed again with 80 % ethanol for two times until all impurities are removed. The sample is then dried in an oven at 70 °C for 6 hours and then ground to powder and stored.

3.6 OPTIMIZATION OF PROCESS PARAMETERS FOR PEF AND MICROWAVE ASSISTED EXTRACTION OF PECTIN

The process parameters leading to maximum pectin yield with minimum energy consumption were then optimized. The pulsed field strength, PEF treatment time, microwave power density and time of exposure to microwave were the process variables that were to be optimized. The analysis are conducted as per the experimental design as explained in section 3.4.

Response surface methodology (RSM) was selected for design of experimental combinations to optimize process variables considering its emphasize on modelling and analysis of problem wherein the responses are vastly influenced (Montgomery, 2001). The method of RSM is attributed to multivariate non-linear

model which was an extensively used technique for optimisation process. It is expedient in analysing the interactions of various parameters that affects the process. The major advantage of RSM is that it reduces the total number of experimental runs required for providing adequate information for statistically acceptable results (Montgomery, 2001).

Box-Behnken designs for four variables of three different levels at three centre point combinations for each were employed for best experiment combination (Gopika and Ghuman, 2014). Pulse Field strength(X1), PEF treatment time (X2), Microwave power density (X3) and time of exposure (X4) were taken as independent variables. Process variables for statistical process calculations were coded as -1, 0 and +1. The values of corresponding independent variables at three levels were shown in Table 3.3.

3.6.1 Pectin Yield

The pectin concentrate obtained after the microwave assisted extraction is dried in a hot air oven at 70 °C for 6 hours. The yield of powder so obtained is calculated using the following equation;

$$\text{Yield of pectin} = \frac{\text{weight of pectin obtained}}{\text{weight of alcoholic insoluble solid}} \times 100 \quad \text{..... (1)}$$

3.6.2 Energy Consumption

A three phase AC static watt hour energy meter (Plate 3.11) was connected to pulsed electric field generator and microwave oven for measuring the energy expended during PEF pretreatment and microwave extraction process. The electrical energy consumed for the pulsed electric field pretreatment and for the microwave extraction for all the treatments as per the experimental design was found. These were then added to find out the total energy requirement for each set of experiments. The energy requirements for conventional extraction were also found out.



Plate 3.11 Energy meter

Table 3.3 Values of independent variables at three levels of Box-Behnken design

Independent variable	Symbol		Level	
	Coded	Uncoded	Coded	Uncoded
Pulsed field strength (kV/cm)	X1	A	-1	5
			0	10
			+1	15
PEF treatment time (min)	X2	B	-1	2
			0	4
			+1	6
Microwave power density (W/g)	X3	C	-1	450
			0	550
			1	650
Time of exposure (min)	X4	D	-1	5
			0	10
			+1	15

According to BBD, total numbers of experiments to be accomplished were twenty nine for four independent variables as shown in Table 3.4.

Table 3.4 Experimental design used for combined PEF and microwave assisted extraction of pectin

Std	Run	Block	Coded variables				Un coded variables			
			Field strength	PEF time	MW power density	MW time	Field strength	PEF time	MW power density	MW time
1	10	Block 1	-1	-1	0	10	5	2	550	10
2	16	Block 1	1	-1	0	10	15	2	550	10
3	24	Block 1	-1	1	0	10	5	6	550	10
4	26	Block 1	1	1	0	10	15	6	550	10
5	4	Block 1	0	0	-1	5	10	4	450	5
6	25	Block 1	0	0	1	5	10	4	650	5
7	11	Block 1	0	0	-1	15	10	4	450	15
8	7	Block 1	0	0	1	15	10	4	650	15
9	14	Block 1	-1	0	0	5	5	4	550	5
10	18	Block 1	1	0	0	5	15	4	550	5
11	8	Block 1	-1	0	0	15	5	4	550	15
12	27	Block 1	1	0	0	15	15	4	550	15
13	13	Block 1	0	-1	-1	10	10	2	450	10

14	1	Block 1	0	1	-1	10	10	6	450	10
15	2	Block 1	0	-1	1	10	10	2	650	10
16	21	Block 1	0	1	1	10	10	6	650	10
17	6	Block 1	-1	0	-1	10	5	4	450	10
18	29	Block 1	1	0	-1	10	15	4	450	10
19	20	Block 1	-1	0	1	10	5	4	650	10
20	3	Block 1	15	0	1	10	15	4	650	10
21	15	Block 1	0	-1	0	5	10	2	550	5
22	19	Block 1	0	1	0	5	10	6	550	5
23	28	Block 1	0	-1	0	15	10	2	550	15
24	23	Block 1	0	1	0	15	10	6	550	15
25	17	Block 1	0	0	0	10	10	4	550	10
26	5	Block 1	0	0	0	10	10	4	550	10
27	12	Block 1	0	0	0	10	10	4	550	10
28	22	Block 1	0	0	0	10	10	4	550	10
29	9	Block 1	0	0	0	10	10	4	550	10

To optimise independent variables and to check the adequacy of experimental design, a second order non-linear regression equation was fitted between independent variables and dependant variables (Lee *et al.*, 2006).

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 + b_{12}X_1 X_2 + b_{13}X_1 X_3 + b_{14}X_1 X_4 + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4$$

Where,

Y = response value

b_0, b_1, b_2, b_3, b_4 = regression coefficients of linear terms

$b_{11}, b_{22}, b_{33}, b_{44}$ = regression coefficients of quadratic terms

$b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34}$ = regression coefficients of cross-product terms

X_1, X_2, X_3, X_4 = coded values of independent variables.

Coefficient of determination (R^2) determines the quality of fit whereas statistical significance of the second order test is determined using F test. P value was determined to analyse the significance of regression coefficient. Analysis of variance (ANOVA) was conducted for finding the final predictive quadratic equation. These experiments were designed using Design Expert Software, (Version 7.7.0), State-Ease, Minneapolis, MN. Statistical analyses of the experimental data were also conducted using the same software. Using Box-Behnken design for four independent factors, twenty nine experiments were to be conducted as depicted in Table 3.4. The response surface equation for response variables and contour plot analysis by fixing one independent variable as central value and changing other two variables were conducted using this software.

3.7 CONVENTIONAL EXTRACTION PROCESS

The alcohol insoluble residue obtained from Jackfruit rind and core is added with 200 ml 1 % citric acid solution of pH 2. The solution is then kept in heating water bath at a temperature of 80 °C for 45 minutes. After 45 min, the sample is allowed to cool for 5 minutes. The sample in solution is then added with equal amount of 95 % ethanol. The solution is then kept under refrigeration for 1.5 hours at 5 °C. The pectin is then separated by filtration, followed by drying in hot air oven at 60 °C for 6 hours. The dried pectin is powdered and stored.

3.8 CHARACTERIZATION OF PECTIN EXTRACTED BY COMBINED PEF AND MICROWAVE ASSISTED PROCESS

3.8.1 Moisture content

Moisture content was determined by the method suggested by AOAC 2000. Three gram of sample was weighed in a petri dish and placed in oven and dried at 105 °C for 3 hours. Weight after drying was taken. The moisture content in the sample was measured using the formula,

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \dots\dots\dots (2)$$

Where; W_1 = weight of sample before drying

W_2 = weight of sample after drying

3.8.2 Solubility

The solubility of dry pectin in cold and hot water are found using method used by Lokhande *et al.*, 2016. The procedure is as follows.

3.8.2.a Solubility of Dry Pectin in Cold and Hot Water

About 0.5 g sample of dried pectin were weighed and added to 10 ml of 95% ethanol in conical flasks. 50 ml distilled water was added to the above solution. The visual appearance of the pectin in cold water was noted. The mixture was then shaken vigorously to form a suspension and then heated at 85-90 °C for 15 min. After heating, the visual appearance of pectin was noted for solubility of pectin in hot water.

3.8.2.b Solubility of Pectin Solution in Cold and Hot Alkali

Two conical flasks containing 10 ml of 0.1 N NaOH solution was taken and added with 5 ml of pectin solution (0.5 g dried pectin sample in 10 ml of 95% ethanol). The visual appearance of the pectin in cold alkali was noted. The second flask was then heated at 85- 90 °C for 10 min. After heating, the visual appearance of pectin was noted for solubility of pectin in hot alkali.

3.8.3 Viscosity

The viscosity of pectin powder was measured using Brookfield DV-E Viscometer (shown in Plate 3.12). This viscometer essentially consists of a spindle which can rotate inside the sample. The spindle inside feed matrix offers a resistance force during its rotation. The instrument measures the force quintessential for overcoming this resistant force.



Plate 3.12 Brookfield DV-E Viscometer

The equipment is calibrated without placing spindle from 0 to 100 rpm before the experiment. The instrument is levelled using the mercury level present at the top of the instrument. Spindle LV-1 (61) was selected for viscosity measurement of aqueous solution of pectin at a speed of 100 rpm. Five hundred ml aqueous solution of pectin prepared by adding 10 g sample into 500 ml water is taken in a beaker. The spindle is immersed carefully inside the solution to a marked level in such a way that it is not touching the side walls or bottom of beaker. The speed in rpm is set on the dial to obtain torque. The viscometer is switched on and allowed to attain a stable value of viscosity. After attaining stability, the viscosity values were noted and expressed in centi poise.

3.8.4 Colour

The colour of the pectin was found using a Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virginia, USA; model: Hunter-Lab's Colour Flex EZ) (Plate 3.13).



Plate 3.13 Hunter-Lab's Colorimeter

The colorimeter comprises an opaque cover, measurement (sample) port and a display unit as its parts. Hunter-lab colour flex meter works based on the principle of focusing light to the sample and quantifying the energy reflected from it across the entire visible spectrum. A mathematical model named Hunter model describes the colour by matching the colour sequence across the visible spectrum using primary lights. The colour of sample is read in terms of L^* , a^* and b^* values where, luminance (L), which indicates whiteness to darkness forms vertical axis. a (-) greenness, a (+) redness, b (-) blueness and b (+) yellowness, defines the chromatic portion of solids.

For colour analysis, pectin powder samples were filled in a transparent glass cup positioned above the port of colorimeter and covered with an opaque cover to exclude the interference of any external light. Before measurements, the instrument was calibrated using definite colours like black and white tiles.

After calibration, the samples were placed above the port and instrument is turned on and 'L*', 'a*' and 'b*' values were recorded.

3.8.5 Ash Content

Ash content was determined by procedure as specified by AOAC 2000. About 5 g of sample was taken in silica dish. The dried sample and dishes were ignited on a Bunsen burner. The samples were then heated in muffle furnace at 550 °C for 4 to 6 hours and cooled overnight. The dishes were then cooled and weighed. The difference in weight gives total ash content of the sample in percentage.

3.8.6 Crude Protein Content

The crude protein content present in the sample was calculated by AOAC, 2005 using micro Kjeltac distillation apparatus. Finely grounded sample (0.8 g) was taken into the digestion tube. Digestion mixture (0.8 g copper sulphate and 7 g of potassium sulphate) was prepared by mixing the above mentioned compounds. Sample along with 0.5 g of this digestion mixture is added to 10 ml of conc. H₂SO₄. This mixture was digested in the digestion unit until the mixture became colourless. The tubes were then allowed to cool. The mixture was then transferred to distillation unit where 40 ml of NaOH (40%) solution was endorsed into the tube. The liberated ammonium was absorbed by (4%) boric acid solution mixed with 10 ml bromocresol green and 7 ml of methyl red indicator. The pink colour of this solution turns to green. This solution was then titrated against 0.1 N HCL until a pink colour appears. The protein percentage was calculated using the formula:

$$\text{Protein (\%)} = \frac{(\text{ml of HCl} - \text{ml of blank}) \times \text{molarity} \times 14.007 \times 100}{\text{mg test protein}} \times 6.25 \dots \dots \dots (3)$$

3.8.7 Equivalent Weight

Equivalent weight of pectin sample was determined as follows. One gram of pectin sample was taken in a conical flask and mixed with 5 ml 95 % ethanol followed by addition of 1 g of NaCl. Hundred ml of distilled water was added to

the mixture and mixed thoroughly. Few drops of phenol red indicator was added to the solution and titrated against 0.1 N NaOH solution until the pink colour appears. The equivalent weight is then found using the formula:

$$\text{Moles of NaOH} = (\text{Titre value}) \times 1/1000 \text{ (ml/L)} \times 0.1104 \text{ mol (NaOH/L)} \dots (4)$$

$$\text{Equivalent weight (g/mol)} = \text{weight} / \text{no. of moles of NaOH} \dots(5)$$

The neutralized solution so obtained as above is referred to as Titration (A) and is used for methoxyl percentage determination.

3.8.8 Methoxyl Percentage

The methoxyl percentage of pectin sample was determined using the neutralized solution (Titration A) obtained from equivalent weight calculation. 25ml of 0.25 N NaOH solution was added to neutralized solution followed by vigorous stirring using shaker and was allowed to stand for 30 min at room temperature. After 30 min, 25 ml of 0.25 N HCl was added to this solution and titrated against 0.1 N NaOH solution until color of indicator turns pink. Methoxyl content is determined by the following formula.

$$\text{Methoxyl content (\%)} = \frac{(\text{ml of alkali} \times \text{Normality of alkali} \times 3.1)}{(\text{Weight of sample})} \dots\dots (6)$$

3.8.9 Anhydrouronic Acid (AUA) content

The anhydrouronic acid content of pectin samples were calculated by applying the values of previously determined equivalent weight and methoxyl percentage to the following equation.

$$\text{AUA \%} = [176/Z] \times 100 \dots\dots (7)$$

Where,

176 = molecular weight of anhydrouronic acid

Z = (weight of sample (mg))/ (m eq of alkali for free acid + m eq of alkali for methoxyl)

3.8.10 Degree of Esterification

The DE (%) of extracted pectin was calculated using the data obtained from methoxyl and anhydrouronic acid content determinations to the following formula.

$$\text{DE \%} = [(176 \times \text{MeO \%}) / (31 \times \text{AUA \%})] \times 10 \quad \dots\dots (8)$$

3.9 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy (SEM) analysis was used to investigate the morphological characteristics of Jackfruit rind and core powder before and after extraction. Scanning electron microscope magnifies images using electrons. Scanning electron microscopy of cross sections of Jackfruit rind and core powder before and after extraction were carried out using Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM) at SEM centre, NIT, Calicut (Plate 3.14). The pectin samples were coated with non-conducting gold using E 1010 ion sputter coating unit (Plate 3.15), followed by examination under SEM. Electron photomicrographs were taken using an electronic gun (Tungsten Schottky emission electron source) at 10 kV to desired magnifications.

The salient features of SU 6600-FESEM were:

- Resolution : 1.2 nm/30 kV, 3.0 nm/1 kV
- Probe current : 1pA~200nA
- Specimen Size : Max 150 mm dia. × 40 mm H
- Magnification : 500,000 x.
- Specimen chamber pressure : 10⁻⁴ Pa (high vacuum)
10-300Pa (low vacuum)



Plate 3.14 Hitachi SU6600 Variable Pressure Field Emission Scanning Electron Microscope (FESEM)



Plate 3.15 Ion sputter coating unit

3.9 COMPARISON OF COMBINED PEF AND MICROWAVE ASSISTED EXTRACTION PROCESS WITH CONVENTIONAL PROCESS

The extraction process parameters and quality attributes of the pectin obtained through conventional extraction method and combined Pulsed Electric Field and Microwave assisted extraction process were then compared through analysis of the results obtained.

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

This chapter outlines the results on the development of pulsed electric field and microwave assisted extraction system and evaluation of developed system towards the extraction of pectin from Jackfruit rind and core. The outcome of the experiments conducted to analyse the influence of the combined system parameters leading to their standardization are discussed in detail. Also, the results of the experiments conducted to determine the quality characteristics of combined PEF and microwave assisted extracted pectin are analysed, discussed and compared with that obtained through conventional extraction processes.

4.1 DEVELOPMENT OF A COMBINED PULSED ELECTRIC FIELD AND MICROWAVE ASSISTED EXTRACTION SYSTEM

A combined pulsed electric field and microwave assisted extraction system for pectin extraction from Jackfruit rind and core was conceptualised and fabricated (Plate 3.1). The essential parts of the pulse electric field generation system comprises of an inlet unit, outer protective chamber, treatment chamber, display unit, pulse generating system, and a cooling system (Figure 3.1).

The main frame of the pulsed electric field system was constructed using mild steel angles. For ensuring operational safety, outer casing was provided with additional insulation to safeguard the operator from getting electric shocks.

The display unit for controlling and monitoring parameters are positioned on the outer covering. The display unit as shown in Plate 3.6 displays the pulse frequency and electric field strength was programmed using Embedded P language. The inlet system constitutes a stainless steel vessel of 5 L capacity for feeding sample and a 2 cm diameter control valve for controlling the feed rate of the solution to the treatment chamber.

The pulse generating system constitutes a high boost circuit that can generate pulses in the range of 5-20 kV voltage with an input supply of 5 A, 12 W and 230 V forms the quintessential component of PEF pre-treatment system. A LOT circuit produces electric pulses inside the system. The filtering unit coupled with the treatment chamber checks the properties of pulses formed. LOT circuit produces pulses with varying voltages. An isolated feedback circuit installed between LOT and the electrode controls the voltage of pulses produced by LOT and regulates the required voltage of pulses to be transmitted to the electrode.

The treatment chamber contains two stainless steel electrodes connected together inside a T shaped PVC chamber. The high voltage pulses generated from pulse generator are transmitted into the electrodes. The pulses get transmitted to the sample inside these electrodes. Two valves of 2 cm diameter are placed on both ends of the chamber to regulate the flow of sample between the electrodes. After pre-treatment, the valve is opened and the sample moves to the cooling unit.

Two cooling systems are provided on the PEF system (Plate 3.7 and Plate 3.8). A cooling bath of 5 L capacity stainless steel vessel filled with water is used for cooling after treatment. A fan positioned on the top of outer frame is also used as cooling system. It is propelled by a microcontroller, which transmits signal to the start the fan when high voltage firing is initiated. The fan turns off when the pulsating field repose.

The outlet system comprises of an outlet and a control valve to regulate the outflow of sample after treatment.

The pulsed electric field pre-treated samples were subjected to microwave treatment for extraction of pectin. The microwave extraction system has a maximum power of 1100 W. The microwave power density and time for extraction was adjusted at the control panel provided on the microwave reactor chamber. The energy consumption for pectin extraction was measured using a three phase energy meter. The PEF pre-treated powder was subjected to microwave heating which is a volumetric heating through kinetic effects. The PEF pre-treatment of Jackfruit

waste powder could cause rupture of the parenchymal cell of the plant material, resulting in the formation of intercellular spaces, the cell will split and pectin could get easily released. During microwave heating of sample, considerable pressure builds up in the material modifying the properties of the tissue material, breaking down cell structure and increase tissue porosity allowing better penetration of extracting solvents, thus increasing pectin yield.

4.2 STANDARDISATION OF THE PROCESS PARAMETERS OF COMBINED PEF AND MICROWAVE ASSISTED EXTRACTION SYSTEM

A series of experiments were performed according to the experimental design for evaluation of combined PEF and microwave assisted extraction system for extraction of pectin from Jackfruit rind and core, and to optimise the process parameters. For the experiments, three levels of Pulse field strength (5, 10 and 15kV/cm), PEF treatment time (2, 4 and 6 min), Microwave power density (450, 550 and 650 W/g) and time of exposure (5, 10 and 15 min) as input variables were employed. The experiments were performed as per the methodology described in Chapter III under the section 3.5. The process parameters of PEF and microwave assisted extraction of pectin from Jackfruit rind and core was then standardised using the results of experiments conducted for extraction by the combined technologies. The mean values of pectin yield and energy consumption obtained during the experiments are tabulated in Table 4.1.

Response surface methodology (RSM) was performed for the optimisation of process variables. Box- Behnken method was used in the design for optimisation of process parameters for the study wherein twenty nine experimental data were analysed in accordance with response surface methodology. The statistical software Design Expert (Trial version 7.0.0, STAT-EASE Inc.) was used for analysis of these experimental data; the same was also used for the analysis of variance (ANOVA), regression coefficient calculation and for graphical analysis/response surfaces of the experimental data.

A second order quadratic model was used to relate the independent process variables. The coefficient of each terms of the second order polynomial equation was determined by multiple regression analysis using Design Expert software by fitting selected models to the experimental data. Analysis of variance (ANOVA) was performed for analysing the statistical significance of terms in regression equation in order to determine the sufficiency of the quadratic model. 'p' values were used to verify the significance of each coefficients and to comprehend the correlation between the test variables. A smaller value of 'p' ($p < 0.05$) indicates significance of the corresponding coefficient. The adequacy of the regression model was checked by R^2 , Adjusted R^2 , Adequate Precision and Fisher's F-test (Montgomery, 2001).

Adjusted R^2 is the measure of variation in mean in relation with the number of terms present in the model. Adjusted R^2 decreases with increase in number of terms, if those additional terms do not add any value to the model. Adequate precision method compares the range of predicted values at design points with average prediction error. The significance of all the terms in quadratic polynomial was conceived statistically by assessing the F-values at probability (p) 0.1 to 0.01. A complete second order quadratic model was designed to fit the data and was checked by performing R^2 , predicted R^2 (measurement of effectiveness of prediction of response value in a model), Adjusted R^2 , and Fischer F-test for verifying the adequacy of the model (Haber and Runyon, 1977). Smaller values of R^2 during explanation of variation in behavior represent the insignificance of dependent variables in the model. Partial differentiations of process parameters were conducted to optimize the quadratic model with respect to each parameters. The resulting function is equated with zero to solve the equation. Statistical calculations using regression coefficients were also performed to generate three-dimensional plots for the regression models.

4.2.1 Effect of Process Parameters on Pectin Yield

The pectin yield extracted from Jackfruit rind and core with respect to various combinations of process parameters are shown in Table 4.1.

Table 4.1. Effect of process parameters towards extraction of pectin

Sl no.	Sample	Pectin yield (%)	Energy consumption (kWh)	Total time of exposure (min)
1	A ₂ B ₃ C ₁ D ₂	16.3	0.194	16
2	A ₂ B ₁ C ₃ D ₂	15.9	0.1916	12
3	A ₃ B ₂ C ₃ D ₂	17.4	0.1932	14
4	A ₂ B ₂ C ₁ D ₁	14.5	0.098	9
5	A ₂ B ₂ C ₂ D ₂	18.3	0.1916	14
6	A ₁ B ₂ C ₁ D ₂	14.6	0.1916	14
7	A ₂ B ₂ C ₃ D ₃	17.6	0.2885	19
8	A ₁ B ₂ C ₂ D ₃	15.3	0.2885	19
9	A ₂ B ₂ C ₂ D ₂	18	0.1932	14
10	A ₁ B ₁ C ₂ D ₂	14.2	0.1916	12
11	A ₂ B ₂ C ₁ D ₃	16.1	0.2885	19
12	A ₂ B ₂ C ₂ D ₂	17.8	0.1932	14
13	A ₂ B ₁ C ₁ D ₂	14.3	0.1916	12
14	A ₁ B ₂ C ₂ D ₁	13.9	0.098	9
15	A ₂ B ₁ C ₂ D ₁	15.7	0.0964	7
16	A ₃ B ₁ C ₂ D ₂	15.2	0.1916	12
17	A ₂ B ₂ C ₂ D ₂	16.9	0.1932	14
18	A ₃ B ₂ C ₂ D ₁	16.2	0.098	9
19	A ₂ B ₃ C ₂ D ₁	17.4	0.0988	11
20	A ₁ B ₂ C ₃ D ₂	16.4	0.1932	14
21	A ₂ B ₃ C ₃ D ₂	17.3	0.194	16
22	A ₂ B ₂ C ₂ D ₂	17.7	0.1932	14

23	A ₂ B ₃ C ₂ D ₃	16.8	0.2893	21
24	A ₁ B ₃ C ₂ D ₂	15.8	0.194	16
25	A ₂ B ₂ C ₃ D ₁	18.1	0.098	9
26	A ₃ B ₃ C ₂ D ₂	17.6	0.194	16
27	A ₃ B ₂ C ₂ D ₃	16.9	0.2885	19
28	A ₂ B ₁ C ₂ D ₃	17	0.2869	17
29	A ₃ B ₂ C ₁ D ₂	15.6	0.1932	14

To analyse the relationship between dependant and independent variables, Response Surface Methodology was used. The ANOVA table for the response “pectin yield” is given in Appendix A (Table 1). Using the experimental values, a second order non-linear regression equation was fitted between independent and dependent variables. The resultant regression model obtained for prediction of pectin yield with combined PEF and microwave assisted extraction of Pectin is as follows;

$$\text{Pectin yield} = 17.74 + 0.72A + 0.74B + 0.94C + 0.33D + 0.20AB + 0.000AC - 0.18AD - 0.15BC - 0.47BD - 0.53CD - 1.32A^2 - 0.77B^2 - 0.70C^2 - 0.52D^2 \dots (4.1)$$

Where,

A- Pulse Field strength (kV/cm)

B- PEF treatment time (min)

C- MW power density (W/g)

D- Time of exposure (min)

It is perceptible from Equation (4.1) that total pectin yield was in positive correlation with Pulsed field strength, PEF treatment time, microwave power density and time of exposure.

The ANOVA table for the response “pectin yield” is shown in Appendix A (Table A.1). From Table A.2, R- Squared (89.37 %), Adj R Squared (78.74 %) and

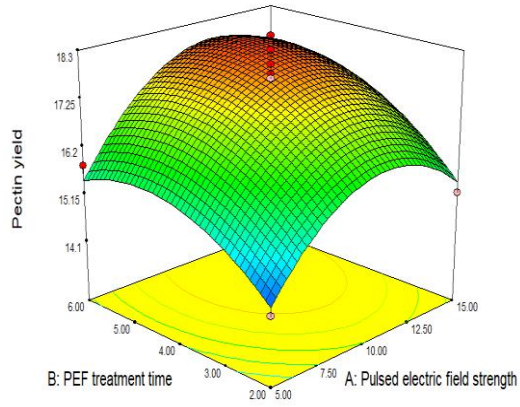
Pred R-Squared (48.66 %) were respectively obtained for total pectin yield. From Table A.1, it can be confirmed that the process parameters had significant effect on total pectin yield at one per cent ($p < 0.001$) level of significance.

In the regression model analysis, the value obtained for coefficient of determination (R^2) was 89.37 % proving 89.37 % variability in data for the model. The Pred R^2 value (48.66 %) and Adj- R^2 value (78.74 %) are reasonably in agreement with each other. Lack of fit was insignificant for the model. F-value for the model shows that the model was significant at one percent and five percent level of significance. The adequate precision value obtained was 8.795 indicating the effectiveness of prediction of model within the design space, as it is greater than the standard value of 4. Consequently, the designed second order model proved to be efficacious for describing the total yield of pectin.

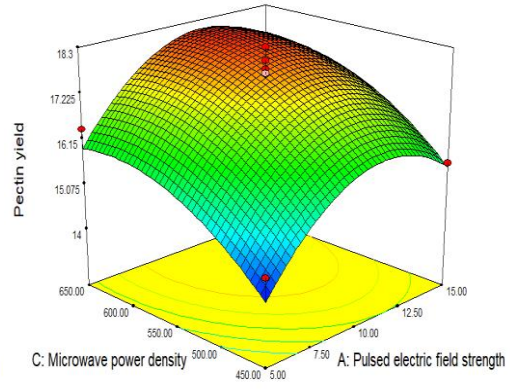
The pectin yield extracted from Jackfruit rind and core powder varied between 13 to 18 %. The maximum pectin yield (18.3 %) was obtained for a sample with pulsed electric field strength of 10 kV/cm treated for 4 min, at microwave power density of 550 W/g for 10 min.

The relationship between pulsed electric field strength, pretreatment time, microwave power density and time of exposure on total pectin yield from jackfruit rind and core is illustrated by plotting 3D graphs representing the response surface generated by the model (Equation. 4.1). The 3D responses were shown in Figure 4.1.

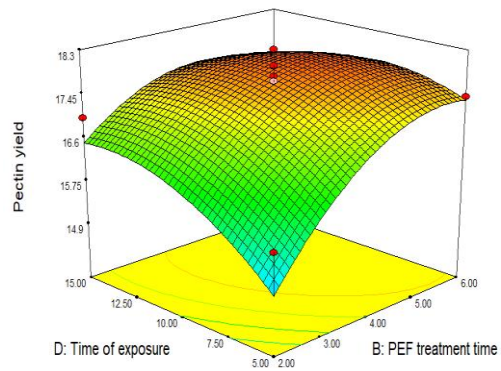
Yeoh *et al.* (2008) reported that yield and other characteristics of pectin depends on raw materials, extraction conditions (pH, time, temperature, type of solvent) etc.



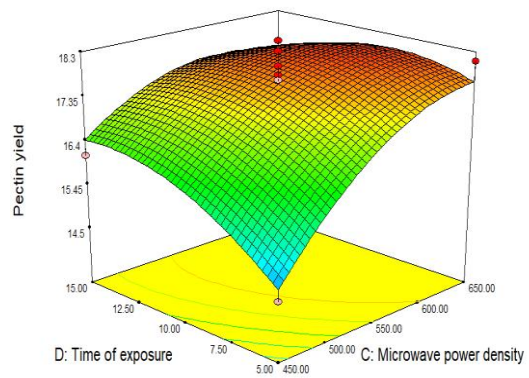
(a)



(b)



(c)



(d)

Figure 4.1. Effect of process parameters on pectin yield

From the graph (Fig 4.1 (a)), it can be seen that pectin yield increases with increase in PEF pre-treatment up to a limit followed by decrease in yield. The increase in yield could be due to the electroporation of cells by pulsed electric field. PEF pre-treatment of the pectin bearing Jackfruit waste could cause a rupture of the parenchymal cell of the plant material resulting in the formation of intercellular spaces, the cell will split and pectin can easily get released. But after certain treatment time and pulsed field strength, these phenomena could not contribute to the yield.

The pectin yield increased with increase in microwave power density as illustrated in Fig 4.1. (d). Since microwave heating is an internal heat generation process in which heating is more rapid and homogeneous, considerable pressure builds up in the material, which modifies properties of tissue material, break down cell structure and increase tissue porosity thus allowing better penetration of extracting solvents and increasing pectin yield.

Guolin *et al.* (2012) reported that direct interaction of microwaves with solvent results in the release of intra-cellular materials to the solvent, which in turn reduces time of pectin extraction along with increment in extraction efficiency. Kratchanova *et al.* (2004) reported that microwave heating inactivates pectinesterase enzyme, which in turn decreases the solubility of pectin, thereby increases extraction efficiency of pectin. Pectin yield increased with increase in time of exposure of microwave treatment up to a limit, followed by limited increase in yield with further increase in time of exposure. This could be due to the fact that maximum extraction would have happened and only limited quantity of pectin is available for further extraction which would take a long time as the diffusion rate of the remaining pectin would slow down since the concentration gradient is reduced.

The combined effect of pulsed electric field and microwave heating on cell wall matrix wherein the skin tissues opens as a result of breakdown of parenchymal cells, enabling solvent to penetrate into skin tissues, which enhances extraction efficiency resulting in increase in pectin yield for pulsed electric field and

microwave assisted extraction, in comparison with conventional extraction. Thus PEF electric field pretreatment followed by microwave extraction can be seen as an efficient method to increase the yield of pectin.

4.2.2 Effect of Process Parameters on Total Energy Consumption

Total energy consumption for combined PEF and microwave assisted extraction of pectin at various combinations of process parameters is shown in Table 4.1. The total energy consumption varied between 0.098 and 0.2885 kWh with minimum energy obtained at a PEF strength of 5 kV/cm for 2 min treatment time at microwave power density of 450 W/g exposed for 5 min.

A second order non-linear regression equation was fitted between independent and dependent variables. The regression model obtained to predict the total energy consumption for PEF and microwave assisted extraction is as follows.

$$\begin{aligned} \text{Total energy consumption} = & 0.19 + (1.333\text{E} - 004)\text{A} + (1.200\text{E} - 003)\text{B} + (1.333\text{E} \\ & - 004)\text{C} + 0.095\text{D} + 0.000\text{ AB} - (4.000\text{E} - 004)\text{ AC} + 0.000\text{ AD} + 0.000\text{ BC} + \\ & 0.000\text{BD} + 0.000\text{CD} + (2.667\text{E} - 005)\text{ A}^2 - (1.733\text{E} - 004)\text{ B}^2 + (2.667\text{E} - 005)\text{ C}^2 + \\ & (2.767\text{E} - 004)\text{ D}^2 \end{aligned} \quad \dots\dots (4.2)$$

Where,

A- Pulse Field strength (kV/cm)

B- PEF treatment time (min)

C- MW power density (W/g)

D- Time of exposure (min)

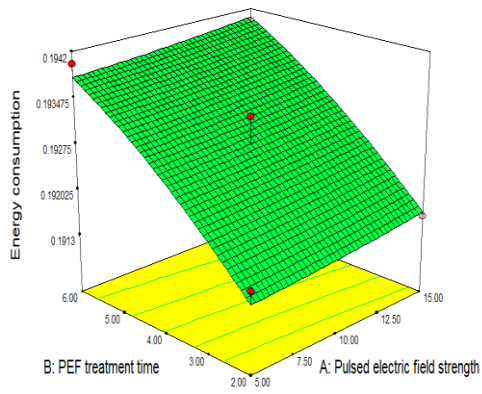
It is perceptible from Equation 4.2 that total energy consumption proved to be in positive correlation with Pulse Field strength, PEF treatment time, microwave power density and time of extraction.

The ANOVA table for the response “energy consumption” is shown in Appendix B (Table B.1). From Table B.2, R- Squared (100 %), Adj R Squared (99.99 %) and Pred R-Squared (99.99 %) were respectively obtained for energy consumption. From Table B.1, it can be confirmed that the process parameters has significant effect on total pectin yield at one per cent ($p < 0.001$) level of significance.

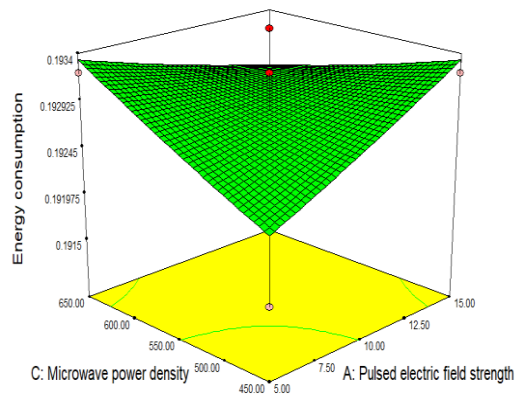
In the regression model analysis, the value obtained for coefficient of determination (R^2) was 100 % proving 100 % variability in data for the model. The Pred R^2 value (99.99 %) and Adj- R^2 value (99.99 %) are reasonably in agreement with each other. Lack of fit was insignificant for the model. F-value for the model shows that the model was significant at one percent and five percent level of significance. The adequate precision value obtained was 5.68648 indicating the effectiveness of prediction of model within the design space. Consequently, the designed second order model proved to be efficacious for describing the total energy consumption for the extraction of pectin.

The relationship between total energy consumption and independent variables are illustrated by plotting 3D graphs representing the response surface generated by the model (Equation. 4.2). The 3D responses were shown in Fig 4.2.

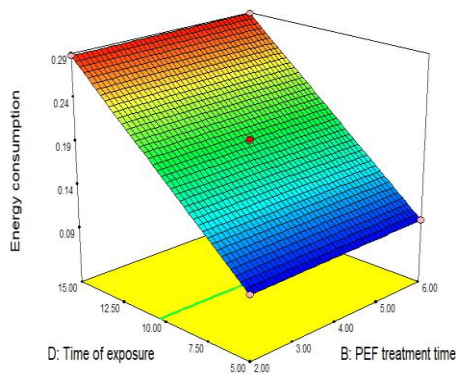
It is evident from Fig 4.2 (a) that the energy consumption increased linearly with increase in PEF treatment time but marginally with pulsed electric field strength. The same trend was observed for microwave treatment also that the increase in energy consumption was linear and high with increments in exposure time and marginal with increase in power density (Fig 4.2 (d)). Fig 4.2 (b) and Fig 4.2 (c) substantiate these findings.



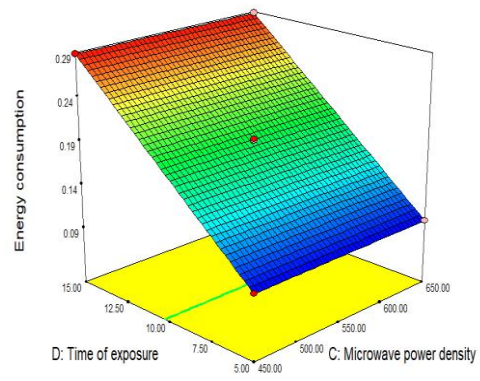
(a)



(b)



(c)



(d)

Figure 4.2. Effect of process parameters on total energy consumption

4.3 DESIRABILITY

Desirability analysis was performed using Design Expert software. The value of desirability ranges from zero to one for any applied response. Desirability analysis combines individual responses into a single value and then analyze for the best overall desirability. A value closer to zero indicates one or more responses falls outside desirable range, whereas desirability value of one represents ideal case. A value closer to one indicates the responses are in desirable range (Maran *et al.*, 2013).

From the desirability analysis, the optimum operating conditions for combined PEF and microwave assisted extraction of pectin from Jackfruit rind and core were found to be as follows:

Pulsed electric field strength : 11.99 kV/cm

PEF treatment time : 5.47 min

Microwave power density : 647.30 W/g

Time of exposure : 5.00 min

The pectin yield obtained from desirability analysis was 18.2426 % and energy consumption was found to be 0.0987488 kWh. The desirability after optimization was found to be 0.987. Since the value of desirability is close to one, the optimized values could be considered as ideal.

Table 4.2. Optimal level obtained from desirability analysis

Sl no.	Response	Units	Desirability	Optimal level	Minimum level	Maximum level
1	Pectin yield	%	Maximize	18.2426	13.9	18.3
2	Energy consumption	kWh	Minimize	0.0987488	0.0964	0.2893

4.4 CHARACTERIZATION OF PECTIN EXTRACTED BY COMBINED PEF AND MICROWAVE ASSISTED PROCESS

The physicochemical and morphological characteristics of the extracted pectin from both combined PEF and microwave assisted process and conventional process were determined as described in section 3.7 and are tabulated in Table 4.3. The pectin obtained after combined PEF and microwave extraction and conventional extraction process are shown in Plate 4.1.

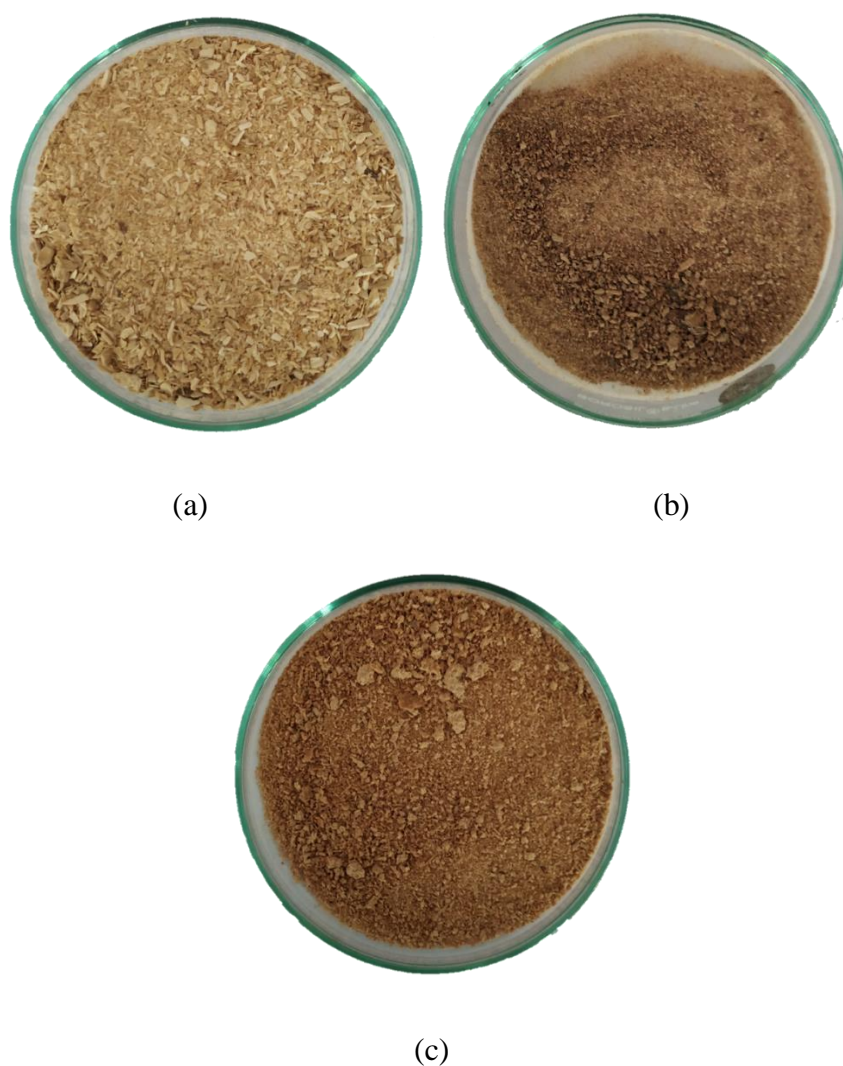


Plate 4.1 (a) Raw Jackfruit powder; (b) Pectin extracted through conventional extraction process; (c) Pectin extracted through combined PEF and microwave assisted extraction process

4.4.1 Moisture Content

The moisture content (wb %) of the samples obtained using various extraction methods are shown in Table 4.3.

Table 4.3 Physico-chemical characteristics of the extracted pectin from Jackfruit rind and core powder

Test	Unit	Conventional extraction	PEF and MW assisted extraction
Moisture content	%	10.04	8.95
Solubility in cold water	-	Insoluble	Insoluble
Solubility in hot water	-	Soluble	Soluble
Solubility in cold alkali	-	Soluble with precipitate	Soluble with precipitate
Solubility in hot alkali	-	Soluble	Soluble
Viscosity	cP	38.14	39.78
L*	-	56.61	73.63
a*	-	8.44	5.88
b*	-	17.06	16.59
Ash	%	7.27	6.78
Protein	%	9.98	3.283

The lowest moisture content of 8.95 % (wb) was noticed in combined PEF and microwave assisted extraction. The results obtained shows that the moisture content of the extracted pectin samples using different extraction techniques are in the acceptable range (below 12 %) for both extraction process, which satisfies the requirements suggested by Food Chemical Codex. Higher moisture content in pectin causes production of pectinase enzyme, which could initiate microbial growth and results in quality deterioration of pectin (Muhamadzadeh *et al.*, 2010; Ismail *et al.*, 2012). The lowest moisture content recorded for combined PEF and microwave assisted indicates its storage stability compared to that produced by conventional extraction process.

4.4.2 Solubility

The solubility of pectin in cold water, hot water, cold alkali and hot alkali were analyzed and presented in Table 4.3. The results showed that all pectin samples were insoluble in cold water, but soluble in hot water. The solubility of pectin in hot water is essential during the preparation of products like jams, where pectin is used as gelling agent.

The solubility of pectin in 0.1 N NaOH alkali solutions, both in cold and hot alkali was analyzed. All samples were partially soluble in cold alkali with the formation of precipitate. The pectin samples obtained from various extraction methods were soluble in hot alkali and the powder gets suspended in the alkali solution. Fishman (1993) reported the solubility test in alkali for pectin as a conformation test for pectin in solution. The pectin obtained from combined extraction method using PEF and microwave satisfies this conformation test.

4.4.3 Viscosity

Begum *et al.* (2007) reported that the typical characteristics of pectin samples in solution is its shear thinning behaviour, wherein the viscosity decreases with

increase in shear rate, due to elongation of pectin's polymer chain in the direction of flow, resulting in reduction in resistance to flow. Rao (1993) stated that measures of molecular weight of pectin such as solubility and viscosity affects the gelling ability of pectin.

The results of viscosity of 2 % aqueous solution of pectin obtained through PEF and microwave assisted process and conventional process are shown in Table 4.3. The results indicates that viscosity of pectin obtained from combined PEF and microwave assisted extraction is higher than that of conventional extraction method. Increase in viscosity might be due to low exposure to heat. Higher viscosity indicates higher molecular weight, which indicates the extracted pectin is of better grade (Rao, 1993).

4.4.4 Colour

Hunterlab colorimeter was used to analyze the colour of pectin samples based on L*, a* and b* values, as represented in Table 4.3. Colour of pectin is an important factor as it affects the colour of final product prepared using pectin. The colour of pectin mainly depends upon the raw material and method of extraction (Mohamed and Hasan, 1995).

The pectin extracted by combined PEF and microwave assisted extraction was in light brown colour. The colour of pectin obtained using conventional extraction method gave darker brown colour. Colour of pectin is a vital factor affecting the colour and appearance of gel formed during food preparation. Lighter colour indicates minimal effect of heat during extraction on the colour of pectin and thus preferred (Koh *et al.*, 2014). The results were comparable with the results obtained by Koh *et al.*, (2014), which reported a brownish orange colour for pectin. Pectin obtained by combined PEF and microwave assisted extraction yielded lighter colour which indicates better quality of pectin comparing to that obtained through conventional process.

4.4.5 Ash Content

The lowest ash content of 6.78 % was observed in combined PEF and microwave assisted extraction, which was lower than pectin obtained through conventional extraction (7.27 %). The results obtained shows that the ash content of the extracted pectin samples using both extraction techniques are in the acceptable range (below 10 %) for both extraction processes, which satisfies the requirements suggested by FAO. Similar results were obtained by microwave assisted extraction of pectin by Koh *et al.*, (2014).

Ash content of pectin indicates the amount of inorganic impurities present in pectin (Suhaila and Zahariah, 1995). Yapo (2019) reported the reduction in ash content in pectin as an indication of purity of pectin contributing to good gelling strength. Lower values of pectin obtained through combined PEF and microwave assisted extraction indicates that the pectin extracted is superior in quality. According to Sonmez and Giray (2011), shorter extraction time results in lower ash content, which is in accordance with the result obtained in this study. The results obtained are shown in Table 4.3.

4.4.6 Crude Protein Content

The crude protein content of the pectin extracted by the combined and conventional extraction methods are given in Table 4.3. The lowest crude protein content of 3.283 % was observed in combined PEF and microwave assisted extraction, which was lower than that of pectin obtained through conventional extraction (9.98 %). The results obtained shows that the crude protein content of the extracted pectin samples using both extraction techniques are lower, indicating better quality of pectin. According to Yapo (2009), lower protein content of pectin results in better gelling properties of products.

4.4.7 Equivalent Weight

Equivalent weight of pectin is the total amount of unesterified free galacturonic acid in the molecular chains of pectin (Ranganna, 1986). The equivalent weight of the pectin samples found out by potentiometric titration method are presented in Table 4.4. The results obtained shows that pectin extracted through combined PEF and microwave extraction has higher equivalent weight than that obtained through conventional extraction. The decline in equivalent weight might be due to depolymerization of pectin into pectic acid. Reports by Meilina and Illah (2012) claimed that longer extraction time results in increase in depolymerization of pectin into pectic acid, resulting in decreased esterification of galacturonic acid groups. Putra (2009) reported that the declination of equivalent weight is due to increase in demethylated pectin methoxyl groups, and causes pectin degradation.

Table 4.4 Quality characteristics of pectin samples

Type of extraction	Unit	Conventional extraction	PEF and MW assisted extraction
Equivalent weight	(g/mol)	466.905	557.473
Methoxyl percentage	(%)	9.376	8.37
Anhydrouronic acid	(%)	67.85	69.44
Degree of esterification	(%)	78.45	68.43

4.4.8 Methoxyl Percentage

Methoxyl percentage is an indication of number of methyl ester groups in pectin (Putra, 2010). The methoxyl content is an important factor determining the gel forming ability of pectin. The methoxyl percentage of pectin obtained through both extraction methods were greater than 7, indicating high methoxyl content Wignyanto *et al.* (2014). From the results, it can be inferred that pectin obtained through PEF and microwave assisted extraction is of desirable purity. On the basis of results, both pectins were classified as high methoxyl pectin.

4.4.9 Anhydrouronic Acid (AUA) Content

The results of the anhydrouronic acid content obtained from combined PEF and microwave assisted extraction of pectin were slightly lesser than the galacturonic acid percentage obtained from commercial sources of pectin such as citrus (72.1 %) and apple (73.5 %) reported by Savary and Nuñez (2003). The results were comparable with the results (69.47-75.34%) obtained by Bagherian *et al.*, (2011).

Galacturonic acid is the major anhydrouronic acid present in pectin. Anhydrouronic acid determination is a major criterion for characterization of pectin (Canteri *et al.*, 2012). Higher galacturonic acid content and lower ash content are major requisite for purity of pectin (Liang *et al.*, 2012). Food and Agricultural Organisation (FAO) suggests a requirement of minimum 65 % of galacturonic acid content for pectin to be pure.

The results obtained as shown in Table 4.4 shows that the pectin obtained through various treatment methods are comparable and the pulsed electric field and microwave treated sample gives higher amount of galacturonic acid content, which can be inferred as increase in purity of pectin with pulsed electric field treatment and microwave extraction.

4.4.10 Degree of Esterification

Degree of Esterification is an important quality characteristic that affect the gel formation capability of pectin. Pectin is categorized into two based on the degree of esterification. Pectin with DE above 50 % are classified as higher ester pectin whereas pectin with DE less than 50 % are classified as lower ester pectin. Decrement in DE % results in poor settling of gel. Gelling ability of pectin increases with increase in percentage of degree of esterification.

The results of the Degree of Esterification as shown in Table 4.4 reveals that pectin obtained through combined PEF and microwave assisted extraction gives higher methoxy pectin which determines the purity and gelling ability of pectin obtained. In this case, both extraction method yielded a DE of higher than 50 % and is graded as higher ester pectin and would result in high gelling power.

4.5 SCANNING ELECTRON MICROSCOPY

The scanning electron microscopy was performed to analyse the morphological changes and surface appearances of the jackfruit rind and core powder before extraction and after extraction. The micrographs were analysed for structural changes after extraction and compared to raw jackfruit rind and core samples. The micrographs obtained on 500 x and 1000 x magnification are shown in Plate 4.2 and 4.3.

From the obtained micrographs shown in Plate 4.2 (a) and 4.3 (a) it may be observed that the jackfruit samples contain relatively smooth surfaces before extraction. The micrographs obtained after conventional extraction (Plate 4.2 (b) and 4.3 (b)) shows pores indicating structural decomposition and rupture of cells in larger size, which might be due to exposure to heat for higher time. The micrographs obtained after combined PEF and microwave assisted extraction (Plate 4.2 (c) and 4.3 (c)) shows higher number of pore formation and cell disruption indicating rapid structural rupture than conventional extraction method.

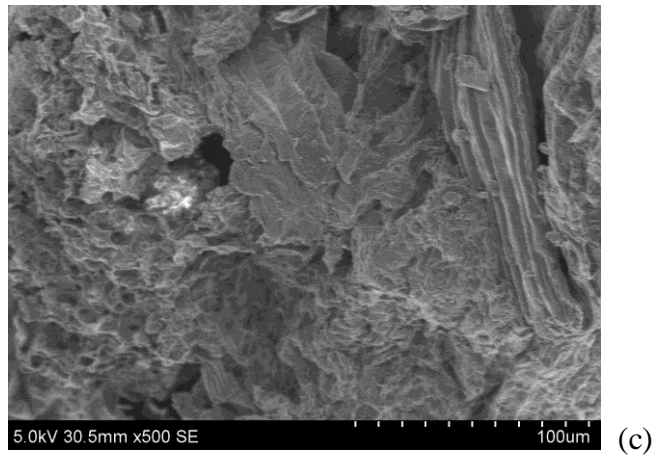
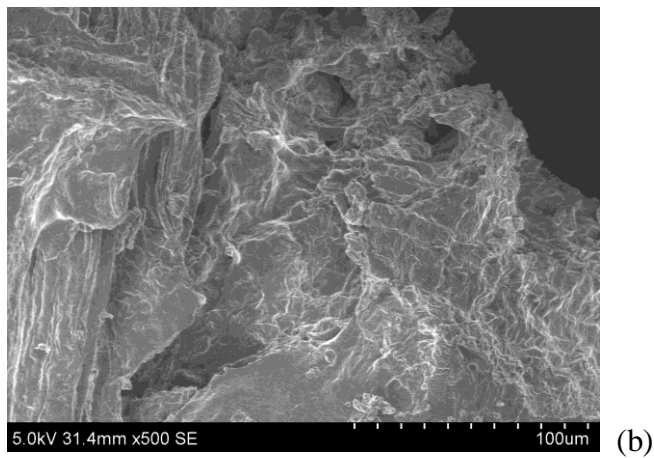
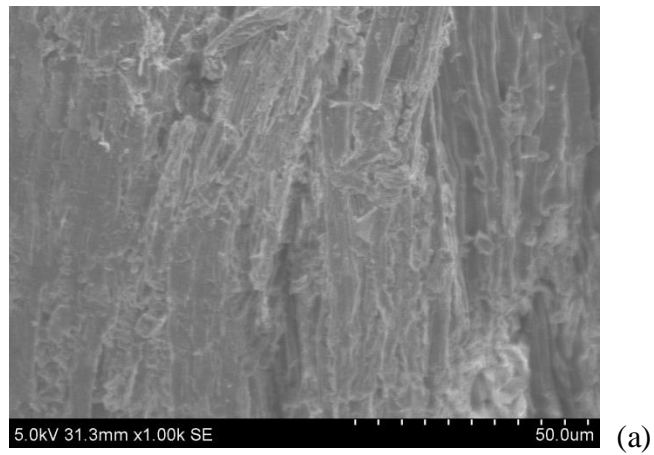
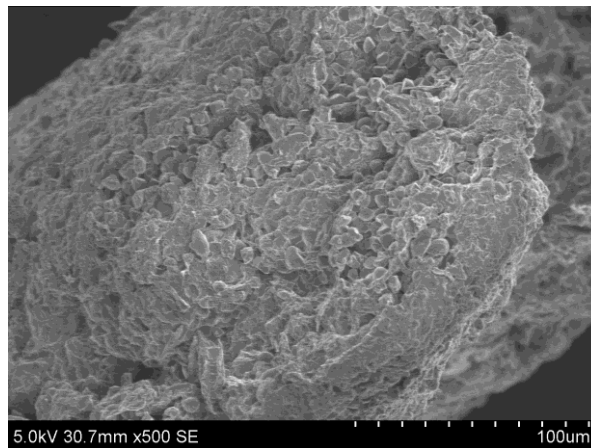
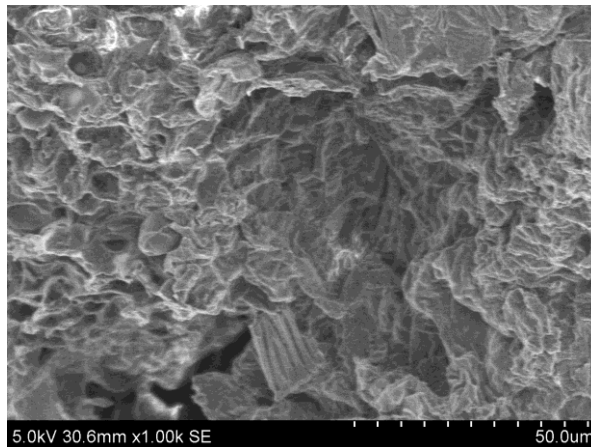


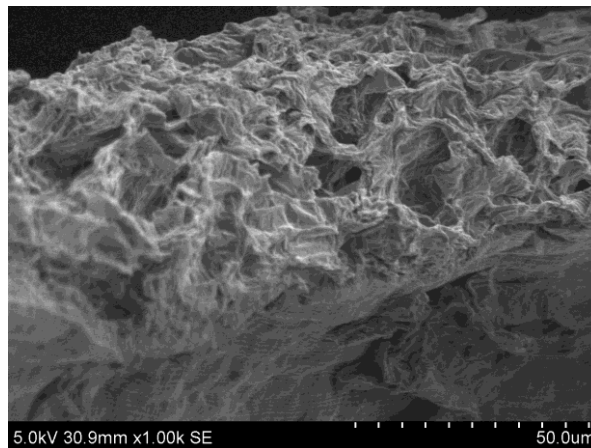
Plate 4.2 SEM micrograph of Jackfruit powder at 500 x magnification (a) before extraction (b) after conventional extraction (c) after combined PEF and microwave assisted extraction



(a)



(b)



(c)

Plate 4.3 SEM micrograph of Jackfruit powder at 1000 x magnification (a) before extraction (b) after conventional extraction (c) after combined PEF and microwave assisted extraction

From the micrographs it could be seen that the structure of Jackfruit rind and core powder changed after extraction process as pores have been visualised in the structure after extraction (Plate 4.2 and Plate 4.3). As a result of intensive vapor formation in the capillary-porous structure of the plant material, large pressure is built up, modifying its physical properties. The capillary-porous characteristics of the fruit tissue improved as a result of the increase in the pressure and temperature in the inside of the tissue. The combined pulsed electric field and microwave assisted heating of Jackfruit rind and core powder led to severing of the parenchymal cells. The damage to the plant tissue increased with the rise in the intensity of the treatment, which was expressed in increase of the intracellular spaces, as visualized on micrographs. More severing of cells are seen on Jackfruit rind and core powder samples after combined PEF and microwave extraction, compared to conventional extraction. This indicates better extraction efficiency.

The pulsed electric field and microwave treated samples (Plate 4.2 (c) and Plate 4.3 (c)) were found to have more cell rupture in comparison with samples that have undergone conventional methods of extraction. This clearly indicates the effect of pulsed electric field and microwave treatment causes increased cell rupture which increases extraction efficiency of pectin from Jackfruit rind and core, proving it an efficient method for pectin extraction with minimal application of heat.

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

Jackfruit (*Artocarpus heterophyllus*) is a fruit crop widely distributed in India having multiple benefits. About 60 % of the fruit, consisting of core, inner perigones and outer rind is inedible and generally discarded as waste. Discarding these waste might lead to negative environmental effects. Value addition through by-product recovery might by using efficient and environment friendly methods might help in profit and solving environmental problems. Pectin is such a valuable by product that can be obtained from jackfruit wastes.

Pectin are a family of complex polysaccharides that contain 1-4 linked α -D galacturonic acid organised in a linear backbone. Pectin, a secondary food ingredient, is used as gelling, stabilizing and emulsifying agent in food products. Consumption of pectin reduces risks for cancer development, lower blood glucose and blood cholesterol level in body. Food sectors like dairy, bakery, and nutraceutical and functional foods as well as pharmaceutical domains like drug delivery use pectin.

Present methodology used for pectin extraction includes direct boiling method using acidified water, which is time consuming and results in degradation of extracted pectin, causing undesired alteration in physicochemical and functional properties of pectin. Novel methods like microwave extraction has been proven efficient than conventional heating.

Pulsed electric field is a novel non-thermal food processing technology in which short duration electric field pulses with high intensity are passed to the food material placed between two electrodes. The potential difference causes breakdown of cell membrane resulting in the increase in permeability due to pore formation. The application of microwave radiation results in internal heat generation which heats up moisture leading to evaporation causing tremendous pressure on cell wall.

This in turn results in swelling and rupture of plant cells resulting in leaching out phyto constituents in cell, leading to extraction.

This study envisages the development of a pulsed electric field pre-treatment system and microwave assisted extraction system for extracting pectin from Jackfruit rind and core. The essential components of the system includes a pulsed electric field system and microwave extraction system. The pulsed electric field system consists of an inlet unit, outer protective chamber, and treatment chamber, pulse generating system, cooling system, display unit and treated sample outlet. The main frame was built using mild steel angles and the outer protective covering containing display unit was made using aluminium sheets. A stainless steel vessel of 5 L capacity forms the inlet system. The inlet system is connected to treatment chamber using steel pipe of 2 cm diameter, which the product flow is regulated by a flow control valve. Pulse generating system comprising of LOT, controlling unit, filter unit and micro controller forms the main component of PEF system. With an input supply of 230 V, 12 W power and 5 A current supply, the high boost circuit generates short pulses of voltage range of 5-20 kV. LOT produces pulses with different voltage levels, wherein the filter circuit regulates the frequency and amplitude of generated pulses. The product is treated in the treatment chamber which consists of two electrodes through which high voltage pulses is transmitted to the product, resulting in electroporation of cells which enables easier extraction of pectin. The cooling fan present at the top of treatment chamber gets automatically switched ON when the machine is started and gets off when pulse generator is stopped. After treatment the valve 2 present at the end of treatment chamber is opened for the product to pass to cooling unit. The cooling chamber consists of a cooler in which water is filled and circulated and the product is allowed to cool. The display unit consists of provisions to set electric field strength and frequency of pulses. The display unit is designed using embedded P language. After cooling, the product passes through control valve present at outlet system which regulates the outflow of product.

For microwave extraction of pectin from jackfruit rind and core, a commercial domestic microwave oven was used as microwave reactor. The sample to be treated with microwave are placed inside the chamber and treated for a pre-determined time. The parameters such as microwave power density and time of exposure are preset on the display panel.

Preliminary studies were conducted in order to determine the best combinations of solid-solvent ratio, pH of the solvent, range of PEF treatment time, microwave power, exposure time of both treatments and suitable values were fixed. For evaluation of efficiency of developed system towards extraction of pectin from Jackfruit rind and core, process parameters which influence the pectin yield and energy consumption for extraction were selected as independent variables based on preliminary studies and review of literature. Pulsed field strength (5, 10 and 15 kV/cm); PEF treatment time (2, 4 and 6 min); microwave power density (450, 550 and 650 W/g) and time of exposure (5, 10 and 15 min) were chosen as independent variables. Pectin yield and Energy consumption were chosen as dependent variables.

The extraction process consists of two parts; pre-treatment using pulsed electric field and extraction using microwave reactor. For pulsed electric field pretreatment, the required parameters like pulse frequency and electric field strength are configured in the display panel. The outlet valve and valve 2 present in the outer end of treatment chamber is closed and the inlet valve is open. The weighed sample is fed through inlet unit to the treatment chamber and the inlet valve is closed. The pulsed generator is switched ON and time is noted in a stopwatch. After the required pretreatment time is achieved, the pulse generator is turned off and the valve 2 is opened to allow the treated pulp to move towards the cooling chamber. After cooling is achieved, the sample is taken out by opening the outlet valve. The sample is then taken for extraction process.

The sample is kept inside the microwave chamber and closed. The parameters such as microwave power density and time of exposure are set in the configuring panel of microwave reactor. The microwave oven is switched on and the sample is

treated for extraction. After the treatment time is achieved, the microwave reactor is switched off and the sample is allowed to cool. The sample is then added with equal amount of 95 % ethanol and refrigerated for 1.5 hours at 5 °C. The coagulated pectin is then separated by filtration and dried at 70 °C for 6 hours and then ground to powder and stored.

The optimization of process parameters was undertaken to verify the sufficiency of experimental design. A second order nonlinear regression equation for dependent and independent variables was fitted and analysis of variance (ANOVA) was performed for the final predictive equation using statistical software. Design Expert (Trial version 7.0.0, STAT-EASE Inc.) software was used wherein Response Surface Methodology (RSM) was adopted. Box-Behnken design of four variables and three levels, each with 3 center point combinations were used and the response surface equation was optimized for these response variables using this software. SEM analysis was performed in samples before and after extraction of pectin to study changes in morphological characteristics during extraction.

The results obtained shows that the pretreatment using pulsed electric field and microwave heating has significant effect on extraction of pectin. Pulsed electric field and microwave assisted extraction yielded pectin in the range of 13.9 to 18.3 % and power consumption of 0.098 to 0.2893 kWh. From the results it was found that the process parameters have impact on the extraction of pectin from Jackfruit rind and core powder. Desirability analysis was conducted to optimize the process parameters. The optimized conditions obtained after desirability analysis were: Pulse field strength (11.98 kV/cm); PEF treatment time (5.46 min); Microwave power density (647.55 W/g) and time of exposure (5 min), and were taken for characterization and comparison studies.

Characterization of pectin was accomplished by analyzing various physicochemical properties and quality attributes of pectin and comparison with conventional extraction process. The optimal level of pectin extracted through

combined PEF and microwave extraction were 18.24 % of pectin at 10.47 min of extraction which was better compared to conventional extraction (17.1 %) at 45 min of extraction. The combined pulsed electric field and microwave assisted extraction of pectin proved to be energy efficient as the energy consumption for the same was 0.0987 kWh which was comparatively lesser than the conventional (1 kWh) extraction method.

The physicochemical parameters of the samples such as moisture content, ash content, protein content, viscosity, solubility and colour of samples were determined and compared with conventional extraction method. The moisture content of PEF assisted extraction was within range of 12 % and lower than conventional extraction. The ash content (6.78 %) and protein content (3.283 %) of the pectin obtained through combined PEF and microwave extraction were within desirable range and found to be better than pectin obtained through conventional extraction. The viscosity of pectin obtained through combined PEF and microwave extraction was higher than pectin obtained through conventional extraction. PEF assisted microwave extraction yielded lighter colored pectin than conventional extraction which is desirable for the product preparations in which pectin is used.

The quality parameters of pectin were analyzed by finding equivalent weight, methoxyl percentage, galacturonic acid percentage and degree of esterification of the pectin obtained through combined PEF and microwave extraction and was compared with conventional extraction process. All the parameters were in the range as suggested for good quality pectin and were yielded better properties than pectin obtained through conventional extraction process. The obtained pectin was classified as high methoxyl pectin since its degree of esterification was above 50 %. The results obtained from Scanning Electron Microscopy depicted the morphological changes in samples with jackfruit rind and core powder before and after extraction and more rupture of cells and severing of parenchymal cells were found in combined PEF and microwave extraction, which indicates better extraction efficiency.

The optimized conditions for PEF assisted microwave extraction from the studies are pulsed electric field strength (11.98 kV/cm), pretreatment time (5.46 min), microwave power density (647.55 W/g) and time of exposure (5 min) respectively. From this study it is concluded that pectin obtained from inedible parts of jackfruit can be utilized as a convenient source for pectin and combined technology of pulsed electric field treatment and microwave assisted extraction could be considered as an efficient extraction technique for pectin extraction.

The following suggestions can be applicable for further research works on PEF assisted microwave extraction processes.

1. A continuous system for pectin extraction may be developed incorporating PEF treatment system and microwave extraction system.
2. Comparison studies with pectin obtained from various indigenous fruit crops maybe conducted to study and cross check the quality of pectin and its characterisation.

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Appendices

APPENDIX A

Table A.1. ANOVA for Pectin yield

Source	Sum of Squares	df	Mean Square	F value	p-value Prob > F	
Model	41.36	14	2.95	8.41	0.0001	significant
A-PEF strength	6.31	1	6.31	17.95	0.0008	
B-PEF time	6.6	1	6.6	18.78	0.0007	
C-MW power density	10.64	1	10.64	30.28	< 0.0001	
D-time of exposure	1.27	1	1.27	3.61	0.0784	
AB	0.16	1	0.16	0.46	0.5108	
AC	0	1	0	0	1	
AD	0.12	1	0.12	0.35	0.5643	
BC	0.09	1	0.09	0.26	0.6207	
BD	0.9	1	0.9	2.57	0.1314	
CD	1.1	1	1.1	3.14	0.0983	
A2	11.3	1	11.3	32.16	< 0.0001	
B2	3.85	1	3.85	10.94	0.0052	
C2	3.13	1	3.13	8.91	0.0098	
D2	1.75	1	1.75	4.99	0.0423	
Residual	4.92	14	0.35			
Lack of Fit	3.83	10	0.38	1.4	0.3984	not significant
Pure Error	1.09	4	0.27			
Cor Total	46.28	28				

Table A.2. Values obtained from ANOVA table for pectin yield

Std. Dev.	0.59
Mean	16.37
C.V. %	3.62
PRESS	23.76
R-Squared	0.8937
Adj R-Squared	0.7874
Pred R-Squared	0.4866
Adeq Precision	8.795

Appendix B

Table B.1. ANOVA for Energy consumption

Source	Sum of Squares	df	Mean Square	F Value	P-value Prob > F	
Model	0.10889	14	0.007778	34960.38	< 0.0001	significant
A-PEF strength	2.13E-07	1	2.13E-07	0.958904	0.3441	
B-PEF time	1.73E-05	1	1.73E-05	77.67123	< 0.0001	
C-MW power density	2.13E-07	1	2.13E-07	0.958904	0.3441	
D- Time of exposure	0.108871	1	0.108871	489359.1	< 0.0001	
AB	0	1	0	0	1.0000	
AC	6.4E-07	1	6.4E-07	2.876712	0.1120	
AD	0	1	0	0	1.0000	
BC	0	1	0	0	1.0000	
BD	0	1	0	0	1.0000	
CD	0	1	0	0	1.0000	
A²	4.61E-09	1	4.61E-09	0.020733	0.8876	
B²	1.95E-07	1	1.95E-07	0.875972	0.3652	
C²	4.61E-09	1	4.61E-09	0.020733	0.8876	
D²	4.97E-07	1	4.97E-07	2.23172	0.1574	
Residual	3.11E-06	14	2.22E-07			
Lack of Fit	1.07E-06	10	1.07E-07	0.208333	0.9798	not significant
Pure Error	2.05E-06	4	5.12E-07			
Cor Total	0.108893	28				

Table B.2. Values obtained from ANOVA table for energy consumption

Std. Dev.	4.717E-004
Mean	0.19
C.V. %	0.24
PRESS	9.344E-006
R-Squared	1.0000
Adj R-Squared	0.9999
Pred R-Squared	0.9999
Adeq Precision	568.648

**STUDIES ON COMBINED TECHNOLOGIES OF PULSED
ELECTRIC FIELD AND MICROWAVE ASSISTED PROCESS
FOR EXTRACTION OF PECTIN FROM JACKFRUIT RIND
AND CORE**

By

NANDHU LAL A. M.

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ABSTRACT OF THESIS

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KELAPPAJI COLLEGE OF AGRICULTURAL

ENGINEERING AND TECHNOLOGY

TAVANUR - 679 573

KERALA, INDIA

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ABSTRACT

Value addition of inedible parts of Jackfruit such as rind and core using efficient and environment friendly methods would reduce wastage, and its disposal problem and also fetch additional profit to farmers. Pectin, a secondary food ingredient used as gelling, stabilizing and emulsifying agent in food products is such a valuable by-product having nutritional as well as health benefits. Conventional extraction method includes direct boiling using acidified water, which is time consuming and degrades quality pectin. Application of combined novel technologies might help in conquering the inadequacies of conventional methods.

In this study, a pulsed electric field and microwave assisted extraction system for extracting pectin from Jackfruit rind and core was developed. To evaluate the developed system towards pectin extraction, the effect of process parameters influencing pectin yield and energy consumption such as PEF strength (5, 10 and 15 kV/cm); PEF treatment time (2, 4 and 6 min); microwave power density (450, 550 and 650 W/g) and time of exposure (5, 10 and 15 min) were studied. The physicochemical and quality parameters of extracted pectin such as moisture content, ash content, protein content, viscosity, solubility, colour, equivalent weight, methoxyl percentage, galacturonic acid and degree of esterification of the pectin were analyzed and compared with that obtained through conventional extraction.

A PEF strength of 11.98 kV/cm, PEF treatment time of 5.46 min, microwave power density of 647.55 W/g and time of exposure of 5 min were found to be the optimized process variables of the combined treatment. High methoxyl pectin of good quality was obtained through the combined process. The moisture content, viscosity, ash content, protein content, equivalent weight, methoxyl percentage, galacturonic acid and degree of esterification of the combined PEF and microwave treated samples were 8.95 %, 39.78 cP, 6.78 %, 3.283 %, 557.473 g/mol, 8.37 %, 69.44 % and 68.43 % respectively with light brown colour whereas that of conventional extracted pectin were respectively 10.04 %, 38.14 cP, 7.27 %, 9.98 %

466.905 g/mol, 9.376 %, 67.85 % and 78.45 % with dark brown colour pectin. Scanning Electron Micrographs of jackfruit rind and core powder samples before and after combined treatment and conventional extraction revealed an increase in rupture and severing of parenchymal cells of the combined treated samples indicating better extraction efficiency. It was concluded that combined pulsed electric field and microwave treatment resulted in increased extraction of high quality pectin from Jackfruit rind and core.