STUDIES ON PULSED ELECTRIC FIELD ASSISTED EXTRACTION OF ANTHOCYANIN FROM JAMUN

By

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(2016-18-006)

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TAVANUR – 679 573

KERALA, INDIA

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THESI

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DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

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2019

DECLARATION

I, hereby declare that this thesis entitled "**STUDIES ON PULSED ELECTRIC FIELD ASSISTED EXTRACTION OF ANTHOCYANIN FROM JAMUN"** is a bonafide record of research work done by me during the course of research and the thesis had not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any University or Society.

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CERTIFICATE

Certified that this thesis entitled "**STUDIES ON PULSED ELECTRIC FIELD ASSISTED EXTRACTION OF ANTHOCYANIN FROM JAMUN"** is a record of research work done independently by Ms. Akhila J Chand, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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(Chairman, Advisory Committee

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SYMBOLS AND ABBREVATIONS

%	: Percentage
<	: Less than
ºC	: degree celcius
µs	: micro second
A	: Ampere
ANOVA	: Analysis of Variance
et al	: and others
g	: gram
hr	: hour
Hz	: hertz
LOT	: Line Output Transformer
m	: meter
min	: minute
mm	: millimeter
nm	: nanometer
PEF	: Pulsed Electric Field
PVC	: Poly Vinyl Chloride

rpm	: rotations per minute
RSM	: Response Surface Methodology
s	: second
TMAC	: Total Monomeric Anthocyanin Content
TNAU	: Tamilnadu Agricultural University
V	: Volt

CHAPTER 1

INTRODUCTION

Colour is considered as one of the major quality parameter of food. The main aim of adding colour to the foods is to enhance the quality, balancing the colour loss during processing and also to clout the consumer to buy the food products. While selecting the appropriate food colour, cost, stability and also the colour feasibility has to be taken care off. Food colours are of two types – natural colours and synthetic colours. Since the consumers are becoming aware of the various hazards of using synthetic colours, natural colours are gaining importance. Colour which are extracted from fruits and vegetables, seeds, roots and also from various microbes are called natural colours. Anthocyanins, betanins and various carotenoids and chlorophylls are used as natural colors for foods.

Anthocyanins (Greek-anthos kianos-blue) are glucosylated polyhydroxy and polymethoxy derivatives of flavylium which are water soluble pigments after chlorophylls and impart red to blue colours to various fruits, vegetables and storage organs. Normally at low pH (< 4), they have high intensity of colour. They absorb UV light and visible light within 250 – 650nm wave length range. Anthocyanins have growing interest because of different range of colours and its various health benefits. They are very much effective in coronary heart diseases and cancer treatment, improper brain functioning due to age (Lau *et al*., 2006). Traditionally, extraction of anthocyanin are by solvent extraction method, also known as liquid-liquid extraction. Solvent extraction technique is based on the selective dissolving of various constituents of the solution into a immiscible solvent.

Pulsed Electric Field (PEF) technology is an emerging non thermal food processing technology. It involves application of short duration electric field

pulses of high intensity to materials located between two electrodes. PEF induces extra membrane potential difference across the cell membrane. When the potential difference exceeds a critical value, called the breakdown potential, localised electrical breakdown of the membrane occurs resulting in pore formation on membrane and thus cell permeability increases (Zimmerman *et al.*, 1974). The permeabilisation of plant cell membrane improves the mass transfer in subsequent process such as extraction. One of the main factors influencing the efficiency of extraction process is the degree of cell membrane disintegration and cell wall rupture frees the intercellular compounds so that they can integrate to the external non-thermal pre-treatment stage to increase the extraction efficiency of intercellular liquids (Bazhal *et al.*, 2001)

In general PEF processing equipment consists of a high voltage pulse generator, a treatment chamber and controlling and monitoring equipments. Controlling and monitoring equipments include oscilloscope, temperature controlling devices etc. When high voltage pulses are passed through the food products, electric field cause an irreversible breakdown in microbial cells (Zimmerman and Benz, 1980). High voltage pulses from the pulse generator are passed through the product placed in between the electrodes at required intensity, shape and duration. Apart from sterilization due to electroporation, PEF can therefore be used for increasing the extraction yield of various plant compounds like anthocyanins, phenolic compounds, enzymes etc. The dosage of PEF can be changed by the parameters like electric field strength, number of pulses and the treatment time. PEF is highly economical and efficient in processing and also it retains the product quality.

Jamun, (*Syzygium cumini*) is also known as Indian blackberry belongs to the Myrtaceae family, is a potential source of anthocyanin. Jamun is one of the under processed minor fruit found commonly in different parts of Indian

continent (Maran *et al*., 2015). It grows in natural climate and clayey loamy soil in tropical and sub tropical regions. The fruit concentrate of jamun has a very long history of use for various medicinal purposes and currently it has been used for the treatment of chronic diarrhoea and other enteric disorders, including its use as an antimicrobial agent (Achrekar *et al*., 1991). Different parts of the jamun were reported on its antioxidant, anti-inflammatory, neurophsycological, antimicrobial, antibacterial, antifungal and anti-ulcerogic and radio-protative activities.

These beneficial effects are mainly due to the presence of anthocyanin pigments and its antioxidant properties. Ripened fruits can be used for various health drinks, preserves, squashes and wine. It contains 83.70-85.80g moisture, 0.70-0.13g protein, 14g of carbohydrate, 26.20g of sodium, 0.32-0.40h ash and various other dietary components. Fruit pulp of anthocyanin consists of anthocyanin, delphinidin, petunidin, malvidin-diglucosides (Ramteke *et al*.,2015). For the conventional extraction process like solvent extraction, large amount of solvents and long extraction time is required. The combination of effective extraction technologies and low-cost of raw materials represent an environmental and economical alternative to conventional extraction methods.

It could be hypothised from the above statements that application of PEF as a pretreatment before extraction could improve the mass transfer to the extractor medium increasing the efficiency and yield of extraction and therefore the influence of the process parameters of PEF treatments needs to be studied. Such studies on Jamun which is a potential source of anthocyanin were not found reported. Therefore similar studies would result in improved extraction of anthocyanin with increased quality, minimizes product losses with less energy and cost of extraction.

Taking the above facts into consideration, this research entitled **"Studies on Pulsed Electric Field assisted extraction of anthocyanin from Jamun"** was undertaken with following objectives.

1. To develop a Pulsed Electric Field pre-treatment chamber for extraction of anthocyanin from Jamun.
2. To investigate the influence of the Pulsed Electric Field treatment on extraction of anthocyanin from Jamun leading to the standardisation of the process parameters.
3. Characterisation of the Pulsed Electric Field assisted extracted anthocyanin in comparison with conventional solvent extracted anthocyanin.

CHAPTER II

REVIEW OF LITERATURE

This chapter comprises of review of various research works related to pulsed electric field assisted extraction of anthocyanin from jamun. Reviews on jamun, benefits of jamun, anthocyanin, conventional extraction and application of pulsed electric field etc has been presented.

2.1 **JAMUN**

Jamun (*Syzygium cumini*) of myrtaceae family also known as Indian blackberries are tropical fruits of Indian origin. They are found in tropical and subtropical regions of India and found in Thailand, Philippines and Madagascar. Also occurs in northern parts of Himalaya up to a height of 1300m and in Kumaon hills up to 1600m. Jamun are minor fruits with commercial values, but proper and exact information regarding the cultivation practices and high yielding varieties are not available. (Ramteke *et al*., 2015).

2.1.1 Growth conditions of jamun

Jamun tree is tall, evergreen and generally occur in shady regions, wind breaks and in avenues. It is also grown on wide variety of soils. In India, for high yield and growth, it is found on loamy soil which has high moisture content. The soil moisture content is beneficial for good fruiting and also it can grow under saline conditions. It can also protect soil from water logging. The yield of jamun are greater in the rainy areas, with sweet and dark colored fruits (Kubola *et al*., 2011).

2.1.2 Varieties of jamun

Syzyfium friniticosum, Syzygium zeylanica, Syzygium malaccensis, Syzygium densiflora, Syzygium cumini are some the varieties of jamun. *S.friniticosum* and *S.zeylanica* are small trees which give edible fruits. *S. malaccensis* are mostly found in south India.

S.densiflora and *S.cumini* can be used root stock in jamun and they are highly resistant to insect attack especially termites. Ram jamun is one of the common variety of jamun found in India, which give high yield in July-August months. It produces purple coloured, oblong, juicy and sweet jamun with high yield (TNAU agritech portal).

2.1.3 Benefits of jamun

Jamun is highly nutritious, as it contain variety of micro and macro nutrients which are useful for human body. Tasty and pleasantly flavored jamun can be used for desert purposes. The vinegar extracted from jamun juice can be prepared from slightly ripe fruits. Jamun juice can be used for high cooling and digestive process, used for stomachic, carminative and diuretic ailments (Srivastava *et al*., 1983).

Parts of jamun such as seeds, pulp, flower etc have high medicinal value, mainly berries are chemoprotective as it contains large amount of antioxidants, anti proliferative and anti inflammatory compounds. The effect of antioxidants resulting in the suppression of oxygen scavenging species formation by reducing the hydroperoxides and free radicals in the human body (Aqhil *et al*.,2012).

The seeds of jamun are used for different types of ailments, mainly for diabetes mellitus and can be used as feed concentrate for animals as it is rich in carbohydrates, calcium and proteins. Different parts of the jamun were also reported for its antioxidant, antiinflammatory, neuropsycho-pharmacological,

anti-microbial, anti-bacterial, anti-HIV, an-tileishmanial and antifugal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and radio-protective activities (Sagravat *et al*., 2006).

Apart from medicinal values, jamun berries can be used to produce large varieties of food products like squash, wine, vinegar and jellies. Jamun is rich in chemical compounds such as anthocyanin, glucoside, ellagic acid and other phytochemicals.

Colour is one of the most important quality of food. In the past, consumers did not care about the kind of pigments used in food colouring (natural or synthetic). But with reference to food colorants recently there is an aversion towards synthetic pigments owing to the belief such as "synthetic pigments are associated with several illnesses" and "natural pigments have pharmacological benefits" (Mustafa *et al*., 2004). The objective of adding colour is to make them appealing, augment the loss of colour during processing, to improve the quality and also to influence the consumer to buy a product (Martin *et al*., 2017).

Table 2.1.Nutritive value of Jamun (TNAU, Agritech portal., 2015)

Sl No	Nutrient	Percentage
1	Moisture	28.2
2	Protein	0.7
3	Mineral	0.4
4	Fibre	0.9
5	Carbohydrate	19.7
6	Calcium	0.02
7	Phosphorus	0.01
8	Iron	1.0
9	Calorific value	83/100g

Table.2.2.Phytochemicals present in Jamun plant (Ramteke *et al*., 2015)

Sl. No	Plant part	Phytochemicals present
1	Seeds	Jambosine, gallic acid, ellagic acid, corilagin, quercetin, β-sitoterol, 4, 6 hexahydroxydiphenoylglucose.
2	Flowers	Oleanolic acid, ellagic acids, isoquercetin, quercetin, kampferol and myricetin.
3	Fruit pulp	Anthocyanins, cyanidin, delphinidin, petunidin, malvidin-diglucosides.
4	Leaves	β-sitosterol, betulinic acid, mycaminose, crategolic (maslinic) acid
5	Essential oils	α-terpeneol, myrtenol, eucarvone, muurolol, α-myrtenal, 1, 8-cineole, geranyl acetone, α-cadinol and pinocarvone.

2.2 ANTHOCYANIN

Anthocyanins are natural pigments which provide red to blue pigmentation especially to flowers, fruits and tubers. The name anthocyanin originated from two greek words- antho means flower and kuanos means blue. Anthocyanins are highly water soluble vascular pigments belonging to the group of flavanoids, which is the subclass of largest polyphenols family. Anthocyanin appears as red pigment in acidic and blue colour in alkaline conditions (Laleh *et al*., 2006). They are found in many fruits and vegetables, hence constitute as major component in human diet. Anthocyanin pigments are nearly flavourless and taste as moderately

astringent sensation. Mostly anthocyanin found in skin of fruits and vegetables except some berries (Ktsumoto *et al*., 2007).

2.2.1 Chemical structure of anthocyanin

Anthocyanins are medium size biomolecules with molecular weight ranging from 400 to 1200. Anthocyanin's colour intensity is strictly based on the number and structure of hydroxyl grous and methoxyl groups present in it. Blueness increases with increase in hydroxyl group and redness increase with increase in methoxyl group (Ovando *et al*., 2009).

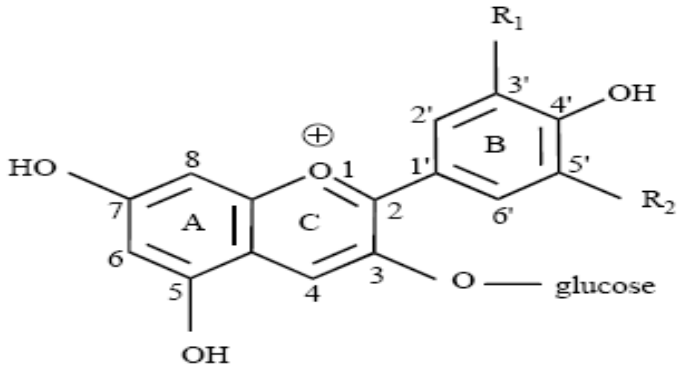


Plate 2.1 Structure of six different anthocynidins with glucose (Khoo *et al*., 2017)

2.2.2 Antioxidant property of anthocyanin

The health and therapeutic effects of anthocyanin are mainly contributed by its antioxidative activities. As reported in the literature (Bors *et al*., 1990), anthocyanin chalcones and quinoidal bases with a double bond conjugated to the keto group are efficient antioxidants in scavenging free radicals. Also, the glycosylated B-ring structure of anthocyanin contributes to the high antioxidant activity, where orthohydroxylation and methoxylation substantially increase the antioxidant activity (Wang *et al*., 1997).The antioxidant activity of malvidin-3-glucoside that was determined by metalcatalyzed lipid peroxidation models in

comparison with other antioxidants (Lapidot *et al*., 1999).The result shows that the quinoidal-base and pseudo-base of malvidin-3-glucoside significantly inhibited peroxidation of linoleate by myoglobin compared with catechin.

2.2.3 Anthocyanin as food colour

Anthocyanins are capable to impart vibrant colours to the food products. So they are of particular interest to the commercial food industry. Anthocyanins extracted from blue and purple corns are used for the production blue tortillas industrially (Shungiyama *et al*., 1977). Incorporating extracted natural anthocyanin as food colourant reduces the ill effects of artificial colours and also is very useful for human health too (Shipp *et al*., 2010).

Table 2.3.Colours produced by various anthocyanidins (Martin *et al*., 2017)

Sl.no	Anthocynidin	Produced colours
1	Delphinidin	Purple and blue
2	Petunidin	Purple
3	Malvidin	Purple
4	Cyanidin	Blue, magenta and crimson
5	Peonidin	Magenta
6	Pelargonidin	Orange salmon

2.3 CONVENTIONAL METHODS OF EXTRACTION

Chandreshekhar *et al* (2012) studied on the extraction of anthocyanin from red cabbage. The extraction was carried out with filteration method leading to the centrifugation of filterate to remove the fine particles present. The filteration was done using muslin cloth. Finally for the complete extraction, adsorption and desorption methods were tried with six different adsorbants. In that Amberlite XAD – 7HP adsorbant showed high adsorption capacity and less desorption ratio.

Lopez *et al* (2007) investigated the effect of pulsed electric field on the extraction of phenolic compounds from Tempranillo grapes. Grapes were fermented and extracted wine by the application of light pressure of 1kg/cm^2. Because of the long maceration process, the colour and the yield of phenolic compounds increased.

Martinez *et al* (2016) studied on the extraction of phenolic compounds from *Artrosphira platensis.* 1millilitre of untreated and treated samples were taken, then 19ml of distilled water was added. The samples were placed in a rotary shaker at 20ºC at 6000 rpm for 10 minutes. Complete extraction of anthocyanin was not carried out and the quality of the extracted anthocyanin was also poor.

Puertolas *et al* (2013) investigated the extraction of anthocyanin from purple fleshed potato, by incubating the samples in a water bath at different temperatures and time. Afterwards, the incubated samples were centrifuged at 6000 rpm for 10-15 minutes with water as solvent. After 480 minute and at low temperature the anthocyanin yield was estimated and it was found that the yield was similar that with ethanol as solvent.

Rajbhar *et al* (2014) made a study on different methods of extraction of polyphenols such as ultrasonic extraction, high pressure extraction, soxhlet extraction and heat reflux extraction methods and the solvents of extraction. As the polyphenols are hydrophilic in nature including aglycones, glycosides and oligomers, they are mostly extracted with solvents other than water. Polar organic solvents such as ethanol, methanol, acetonitrile or their mixture with water are the major solvents as polyphenols are stable at acidic pH.

2.4. PULSED ELECTRIC FIELD TECHNOLOGY

Thermal processing of food can be carried out only by the application of heat in the range of 60ºC to 100ºC (Jay *et al*., 1992). This may leads to the loss of

nutritive and organoleptic value of food. Also the energy utilization of the process is also high (Alwaseer *et al.*, 2003).

Non thermal processing of food is an alternative methods to reduce the nutrient losses, thermal degradation and improving safety while maintaining the food product quality. In this method, ambient or less ambient temperature only is required for the processing. Non thermal processing technologies were proposed under several principles and it requires less energy consumption.

Oscillating magnetic field technology, pulsed light technology, pulsed electric field technology, high hydrostatic pressure technology are promising non thermal technologies which retain the product quality as such (Canovas *et al*., 1999).

Pulsed electric field technology (PEF) is one of the novel non thermal technology which is found to have wide applications in the field of food industry. It can be used not only for the inactivation of microorganisms, but also for the extraction of compounds also. The basic definition of PEF technology relies on the application of high electric field in the range of 10-80kV/cm and results in the cell disintegration (electroporation). For the microbial disintegration, the applied voltage should be greater than threshold voltage (Zimmerman *et al*., 1986). The main aim of PEF technology in biological materials is disintegration of cells, plasmolysis and extraction of intercellular materials.

Chaitanya (2014) reported that to meet the demand of growing population, emerging technologies like high hydrostatic pressure, pulse electric field, sonication and membrane technologies could be potential methods for the enhanced extraction of pigments and bioactive compounds from plant or animal materials in future.

2.4.1 Principle of pulse electric field technology

Pulsed electric field technology is based on the pulsing current applied to the food product, which is placed in between the two electrodes. The electrodes are connected to non conducting materials to restrict the pulse loss. Pulsed electric field system consists of a high voltage pulse generator, treatment chamber, monitoring and controlling devices. The electric pulses generated from the pulse generator applied to the electrodes and then to the product, which is placed in between the electrode. The applied voltage which is greater than threshold voltage of 1V leads to electroporation (Zimmerman and Benz., 1980). The pulse dosage required for the food product can be adjusted with number of pulses, treatment time, electric field strength and shape of pulse. Mainly the PEF technology requires high voltage within a short span of time.

In a PEF system, the shape of pulse is determined by the components in the system. The voltage generated by the PEF system is strictly based on the treatment time and effective pulse width.

$$V(t) = V_0 e^{-t/r}$$

Where V_0 is the voltage charged in the circuit, t is the pulse duration and r is the effective pulse width from generator. V(t) can calculate from time required to decay input voltage (Zhang *et al*, 1995).

Field strength along with treatment time contributes to the effective processing of food product. Critical field strength should reach at an effective threshold value of 1V, required for the cell disintegration and death of microbes (Hulshegar *et al*, 1981). Blatt et al (1989) noted Faraday's electric field concept, it encompasses the field between two charges. When a unit positive charge is placed in a point with in an electric field produced by the electrodes experiences a force at a distance r. The potential difference (V) between two points separated by a

distance r separated by a non conducting material produces electric field strength of (E) and is directly proportional to the applied potential difference and is inversely proportional to the distance between them (d).

$$E=V/d$$

The generated pulse can be square wave, oscillatory wave or exponential decay. The pulses can be unipolar (only have positive) or bipolar (have both positive and negative). Exponential and square waves are commonly used in PEF system as they are very effective. In square and exponential waves, exponential waves are 60% more efficient than square waves at 20 pulses applied on 12kV/cm field strength. It required only 15% less energy than square wave pulses (Bushnell *et al*, 2000).

In the microbial destruction, biological factors have much effect on the treatment rate. The size and shape of the organism, growth phase and type of bacteria also considered. In gram positive and gram negative bacteria, gram positive species are more resistant to electric field, hence the applied electric field strength should be of higher value (Lubicki *et al*, 1997).

Sale *et al*, 1967 stated that the effectiveness of PEF can be increased by the application of model systems such as distilled water, phosphate buffer and Simultaneous Milk Ultra Filtrate (SMUF). The chemical factors also influence the effectiveness of PEF system such as amount of water, state of the product and constituents of the product.

The major concern of commercialization of PEF technology is its high expense and production of high voltage. But PEF technology is highly energy efficient and proved to incur less operational cost (Barbosa *et al*, 1999).

2.4.2 Methods of pulsed electric field assisted extraction

Chalermchat *et al* (2004) studied the effect of pulsed electric field assisted extraction of pigments from red beetroot slices and it was described through Fickian method of diffusion model. The release of ions after the PEF treatment indicated the permeabilisation of beetroot cells. The thin slices shows high extraction yield even with less pulsed electric field treatment which had low permeabilisation rate and the yield also increased with high electric field strength.

Fincan *et al* (2004) treated slices of beetroot in pulsed electric field system and extracted the red pigment with normal solid liquid extraction method. The degree of extraction was compared with conventional extraction method. He found that, the extraction rate was high at 270 rectangular pulses of 10µs at 1kV/cm. The energy consumption during the treatment was 7kJ/kg and 90% of pigment was extracted by PEF assisted treatment. The concentration of isotonic solution and the field strength was directly proportional to each other within 1hr of extraction. The PEF assisted extraction resulted in the higher permeability of the beet cells.

White protein was extracted from 40g of mashed alpha-alpha by pulsed electric field assisted pressure extraction. It was carried out at two different pressures – 2MPa and 4MPa. It was found that the pulse electric field assisted extraction of white protein from alpha-alpha resulted in increased yield. The increased yield was obtained at 200 pulses at 1 Hz pulse frequency. Dry matter from mashed treated sample showed 73% increased white protein content (Gashovska *et al*. 2006)

Praporscic *et al*., (2006) investigated the pulsed electric field assisted extraction of juices from two different sized apple and carrot tissues. It was extracted by filter press cell. The result showed high amount of juice at 250-400 kV/cm electric field strength. The quality parameters such as ^{o}Brix, mass and

absorbance of the extracted juice were checked. The â% for apple was 20%-30% and for carrot 20%. The yield of extracted juice depend on the size of the sample and time of treatment.

Lopez *et al.,* (2007) extracted anthocyanin and other phenolic compounds during fermentation of Tempranillo grapes after pulsed electric field treatment. Pulsed electric field treatment at two electric field strength, 5 and 10kV/cm were carried out. The anthocyanin yield increased between 5 to 10kV/cm field strength range. But the total phenolic content was high at 5kV/cm, the other paramaters such as pH, total acidity, reducing sugar had no change after the PEF treatment.

Gachovska *et al* (2010) studied on the pulsed electric field assisted extraction of anthocyanin from mashed red cabbage. The yield and other parameters of treated sample and untreated samples were compared. Experiment were carried out as batch treatment with various solvent concentrations (16 to 889µg/ml). The anthocyanin yield was very high at 2.5kV/cm field strength and 50 pulses of 15µs duration. The energy consumption of the treatment was 15.63J/g. The anthocyanin yield was 2.5 times higher than that fro conventional extraction method.

Pulsed electric field pretreatment enhanced the yield of phenolic compounds from cocoa bean shell and coffee silverskin. The yield of polyphenols and methyl xanthins were 20% higher than from conventional extraction methods and the yield varied with origin, industrial treatment and also variety. The operating parameters were optimized with RSM method (Pereira *et al*., 2018).

Puertolas *et al* (2013) investigated on the pulsed electric field extraction of anthocyanin from purple fleshed potato. Water and ethanol were chosen as solvent with different concentrations, 48% and 96%. Experiment was conducted with different treatment time (60-480 min), temperature (10-40ºC) and different electric field strength (3-10kV/cm). It was revealed that the temperature and

treatment time had well effect on the extraction yield. The extraction yield was maximum at 96% ethanol. After 96% ethanol, the results were same as that of water as solvent. The treatment with 3.5kV/cm field strength and 105µs gave maximum yield and properties.

Parniakov *et al* (2014) studied on the pulsed electric field assisted extraction of protein from mushroom (*Agaricus bisparus*). Efficiency and stability parameters of the extracts were checked by different methods of extraction. Pressure extraction was done at 5 bar and hot water extraction at 343K for 2h. Ethanol extraction was carried out at temperature 298K for 24h. It was found that pulsed electric field assisted pressure extraction increased the extraction yield with that of other extraction methods. High protein yield was showed in ethanol which was same as that of water as solvent.

Bushnell *et al*. (2000) extracted nutritionally valuable compounds from micro algae by two stages. In the first stage (E1), pulsed electric field assisted extraction (20kV/cm) was carried out with normal water as solvent. But in the second stage (E2), pulsed electric field assisted extraction was done with normal extraction methods using organic solvents. The solvents DMSO and ethanol gave high yield in the second stage. The final obtained data can be used for the optimization of normal and pulsed electric field assisted extraction for industrial purposes.

Lam *et al*. (2017) carried out the investigation on the pulsed electric field assisted extraction of protein from micro algae *Chlorella vulgaris* and *Neochloris oleoabundans*. The entire pulsed electric field extraction process were carried out as batch and continuous treatments. The application of PEF, results the disintegration of algal cells and cause high yield of protein extraction. The high yield was observed at 1-40 pulses, 0.05-5 m pulses and at 7.5-30kV/cm field

strength. After the PEF treatment, the cells release ions and resulted the permeabilisation of algal cell.

Carotenoid is a pigment which is found in the carrot and has immense biological use and it was extracted by pulsed electric assisted green extraction method. Virgin olive oil was employed as solvent and it was compared with 96% ethanol. Results showed higher yield with virgin olive oil as solvent. Anthocyanin yield of 34.16µg/g was obtained at 2.5kV/cm field strength, 1:10 solid- solvent ratio and at 10s treatment time (Putranto *et al*., 2014).

2.4.3 Applications of pulsed electric field technology

Giner *et al* (2000) studied the effect of PEF treatment to pectin methylesterase in tomato. It was found out that, the inactivation of enzyme can be performed at 24kV/cm field strength using NaCl solution as solvent media. Inactivation rate was maximum at 6% residual activity, 400 pulses and at 20µs pulse width. The pulses used were exponential decay with batch system of treatment.

Barsotti *et al* (2002) investigated on the inactivation of lactose dehydrogenase enzyme from milk. The results showed that the complete inactivation of the enzyme was done at 100% residual activity with which solvent medium was a buffer of pH 7.2. The electric field strength applied was 31.6kV/cm with band width of 0.96µs and 200 pulses. The inactivation temperature was 30ºC with batch treatment using exponential decay pulses.

Vanhoey *et al* (2002) inactivated pectin methylesterase enzyme from apple juice with batch system of treatment. The electric field strength was 7-31kV/cm with 5-40µs pulse width and 1-1000 pulses. The complete inactivation was done at 90-100% residual activity.

Peroxidase with residual activity of 65.3% was inactivated found in tomato. The PEF parameters were electric field strength of 5-25kV/cm, 1.5µs pulse width

with 201-1242 number of pulses. The shape of the pulses was exponential decay with batch treatment and continous treatment. But batch treatment showed more inactivation rate than continuous treatment (Zhang *et al*, 2005).

Polyphenol oxidase enzyme from mushroom at 60 % residual activity was inactivated using buffer solution of pH 6.5 as solvent medium employing pulsed electric field. Pulsed electric field strength was 50kV/cm. Pulse width was 2µs at 30 pulses. The pulses were exponential decay type with batch treatment (Hoe *et al*, 2007).

Castro *et al* (2009) noted that the enzyme alkaline phosphatase from raw milk can be inactivated by PEF treatment. Batch treatment of raw milk, complete inactivation of the enzyme was possible with residual activity of 40%. The pulse width of the treatment was 400µs with 70 pulses. The quality of the raw milk was found to be same after the treatment also.

Milk can be preserved by PEF treatment at 40kV/cm electric field strength with a pulse width of 80µs. The maximum temperature of treatment was 50ºC, hence increased the shelf life 2 weeks in refrigeration conditions. No physical, chemical changes were found.

Yeom *et al* (2002) performed a series of `study regarding the shelf life of orange juice. The effect of PEF with treated and untreated orange juice samples with different temperature conditions was studied. Untreated samples were undergone heat treatment. The quality of the orange juices with various parameters such as pectinmethylesterase (PME), ºbrix, vitamin c content, acidity, flavor compound index and colour were noted. For PEF treated juice, the sample had undergone 35kV/cm field strength for 59µs pulse width under 60ºC temperature. Heat treated samples was at 90ºC for 30s. He noted that the microbial growth reduced from10^3 cells/ml to 10 cell/ml. PME activity reduced by 88% and the shelf life of the product was 110 days at 4ºC or at 22ºC.

Liquid whole eggs were processed and treated in PEF at 35kV/cm field strength. 0.15% nitric acid was used as solvent medium for 20µs pulse width and

at a temperature of 45ºC. Batch type treatment was carried out and checked the quality aspects of egg were found. It was preferred over a commercial brand in an acceptance test PEF treated sample was found to superior when compared to commercial brands through acceptance test (Qin *et al*, 2000).

Hermawan *et al* (2004) could not found any changes in PEF treated scrambled eggs with untreated eggs. The eggs were treated with 25kV/cm field strength, at 55ºC for 35 min. From these investigations, we can concludIt was concluded that PEF treatment could not impart any significant changes on whole and scrambled eggs. There was no change in properties like viscosity, colour, flavanoids. The changes occurred only at low temperatures than normal temperatures.

PEF assisted extraction of apple juice was carried out with pressure extraction. Apple was grated by 6mm grater. Only19% extraction could be carried out. But after a pressure of 5 bar, the extraction rate increased with 500 pulses, 10µs pulse width PEF treatment. The yield increased at the rate of 43%, 68% and finally 79% with increase in electric field strength of 215, 300, and 427kV/cm respectively. The application of moderate pressure along with PEF increased amount of juice extraction (Bouzana *et al*, 2001).

Toepfl et al (2005) investigated on the effect of extraction of PEF treated apple juice by pressing the apple slices. Royal gala variety of apple was choosen for the study. Prior to pressing, the apple was treated with 0.8-5kV/cm field strength, 10-40 pulses. After 3kV/cm field strength and 20 pulses, eighty three percentage of the juice was obtained. For the untreated sample, macerated with enzymes the yield was only 73.5% as compared to the treated one.

Kohler *et al*., (2005) extracted protein chlorophyll and carotenoids from Spirulina and Chlorella using PEF treatment. The protein activity was calculated from PEF treated Spirulina and Chlorella. It was found that an extraction yield of 27%, 80% and 52.5% had been found as protein, chlorophyll and carotenoids respectively in Chlorella. The electric field strength was 15kV/cm with specific

energy of 100kJ/kg and the antioxidative activity was found to be increased to 100% with sample treated with PEF.

Removal of water from food products to minimise microbial attack, increased cost of operation to a great extent and significant amount of energy also wasted in food industry. Electropermeabilisation of food products by the application of PEF with modular field strength enhance the drying rate of cellular tissues.

Rastogi *et al* (2000) invstigated on the effect of PEF on the drying of food products. By the application PEF of 1.5kV/cm field strength, the fluidized bed drying time of sliced potato reduced to 75%.

Omowaye *et al* (2001) studied on the relationship between PEF and drying rate of plant based food products by the application of osmotic drying and air drying. PEF application enhanced mass transport rate by permeabilisation across cell membrane. Hence it can utilized to increase the mass transfer rate, thereby the drying rate by 10-30%. The drying rate increased with 1.0kV/cm and 5-20 pulses. While making the drying parameters constant, convective drying rate at 60ºC was also enhanced with decrease in drying time of 20-30%. The energy consumption for the removal of water from food products is mainly depend on the temperature and pressure and the overall water removal was based on the efficiency of drying system.

In urban areas, removal of waste materials and its processing are the major problems in day to day life and minimization of excessive sludge have greater importance because of legislative and ecological issues. Kopplov *et al*, (2004) studied on the various aspects of disintegration of sludge, subsequent release of intracellular material, autolysis and disintegration of cells. The application of PEF with 15kV/cm electric field strength, the autolysis sludge can be effected with input energy of 360kJ/kg. Gas production and volatile suspended solids production found to be enhanced by 8 to19% during anaerobic degradation

process after the PEF energy input of 150kJ/L applied. The heat treated sludge required an energy input of 445kJ/L.

The PEF treated sludge showed very less biological activity than heat treated samples and high organic content in water fractions. It was revealed that samples with 50% PEF treated samples showed maximum heat recovery. The major benefits of PEF treated samples are mechanical rupture.

CHAPTER III

MATERIALS AND METHODS

This chapter outlines the design and development of a PEF system and methodologies adopted for studying the effect of PEF treatment for extraction of anthocyanin from jamun. 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 system developed and optimisation of process parameters for PEF assisted extraction of anthocyanin from Jamun, the methods for determining various parameters are also explained.

3.1 DEVELOPMENT OF A PRETREATMENT PEF SYSTEM FOR EXTRACTION OF ANTHOCYANIN

In this study a PEF system would be used for pretreatment of Jamun towards the extraction of anthocyanin. The PEF induces an extra membrane potential difference across the cells and when this exceeds a critical value, localized electrical breakdown of membrane occurs resulting in pore formation and increase in cell permeability. This would increase mass transfer in the extraction process increasing the extraction efficiency. The design of a lab scale PEF unit was conceptualized and fabricated based on a thorough review of research carried out on PEF system. The developed PEF system is shown in figure 3.1 and plate 3.1 consists of

1) Outer protective chamber
2) Inlet unit
3) Pulse Generating System
4) Treatment chamber
5) Display Unit
6) Cooling System

Figure 3.1.Schematic of PEF assisted extraction unit

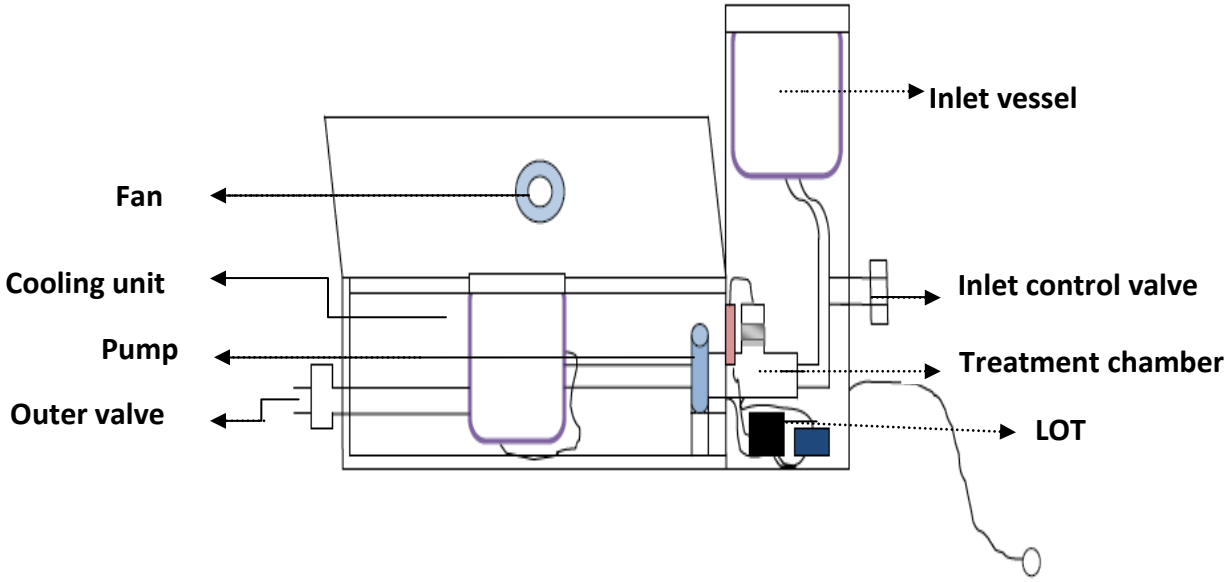


Plate 3.1 Pulsed electric field system

3.1.1 **Outer protective chamber**

The main frame and supporting structures of the PEF equipment are made of 1 inch mild steel angles. The frame is covered with 10 gauge aluminium sheets. All display and controls for monitoring and measuring parameters such as electric field strength, pulse frequency and ON/OFF switch are provided on the outer covering. Special insulation is provided so that the outer framework is isolated from the internal circuits for safety of the operator.

3.1.2 **Inlet system**

Inlet system consists of a stainless steel feed vessel of 5litre capacity, a PVC flow control valve and 2cm diameter stainless steel pipes (plate 3.2) The inlet vessel is connected to the treatment chamber through the stainless steel pipes and flow control valve. The pipe is installed in such a way that smooth flow of pulp occurs without causing blockage due to the high viscosity Jamun pulp flow. The flow control valve regulates the feed rate of the pulp to the treatment chamber.

Plate.3.2. Inlet system

3.1.3 **Pulse generating system**

The pulse generating system forms the main component of PEF system in which a high boost circuit was made to generate the pulses of voltage varying from 5 to 20kV.The input supply requirements are 230V, 12W power and a current of 5A. Pulse generation in the PEF system is achieved by a LOT (Line Output Transformer) and a controlling unit with micro controller to adjust the amplitude and frequency of pulses. LOT is used to step up the input voltage (230V) to required voltage (up to 20kV) with an input current of 5A. Embedded P was the language used to set up the micro controller.

Initially, LOT is used extensively in switched mode power supply for high voltage supplies. Using LOT, wide range of high and low voltages can be generated with a transformer with less number of windings by rectification. The primary winding of the transformer in LOT is driven by a switch from power supply. When the power supply is ON, the primary inductance causes the current to build up. The voltage in the output winding rises quickly (usually less than a second) until it is limited by load conditions. The produced current in the output is in pulsed waveform that repeats at horizontal frequency of the display.

Along with the LOT a filtering unit is also attached to check the properties of the pulses generated. Filter unit is used to filter the input current and to prevent the equipment from high voltage fluctuations. Filter unit helps for the action of DC component of the input and denies the AC input to the unit. LOT and filter unit are fabricated with circuits made of different capacitors, resistors, diodes and transformers. The circuit diagram of the LOT and filter unit are given in Fig 3.2 and 3.4. The LOT circuit produces pulses with different voltages but only required voltage pulses should be allowed to transmit to the electrodes in the treatment chamber. To ensure transmission of heavy and unwanted pulses from LOT to the electrode, an isolated feedback circuit is installed between LOT

circuit and electrode. The circuit diagram of the isolated feedback circuit is shown in Fig. 3.3.

Figure 3.2 Circuit diagram of LOT

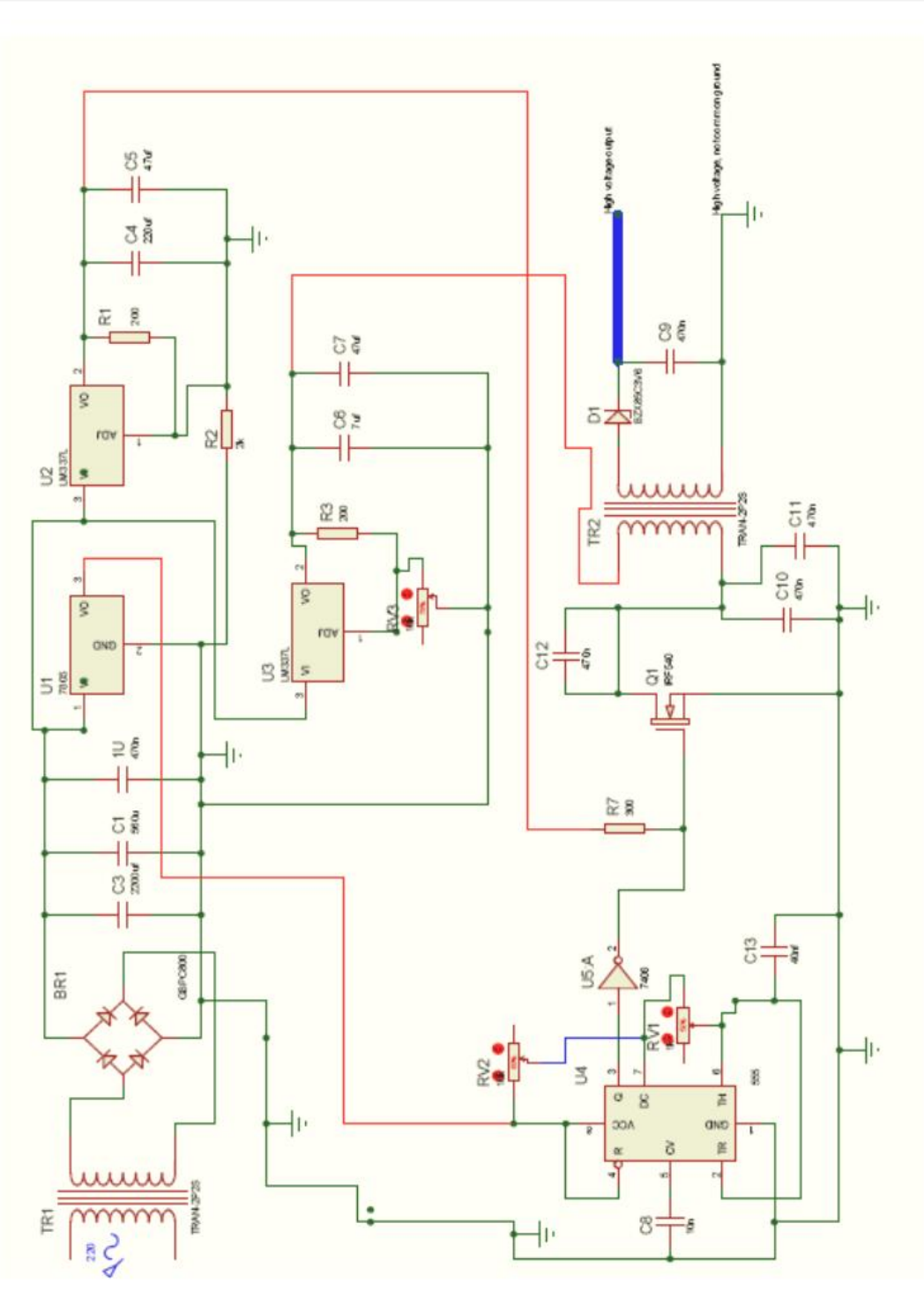


Figure 3.3 Circuit diagram of isolated feedback circuit

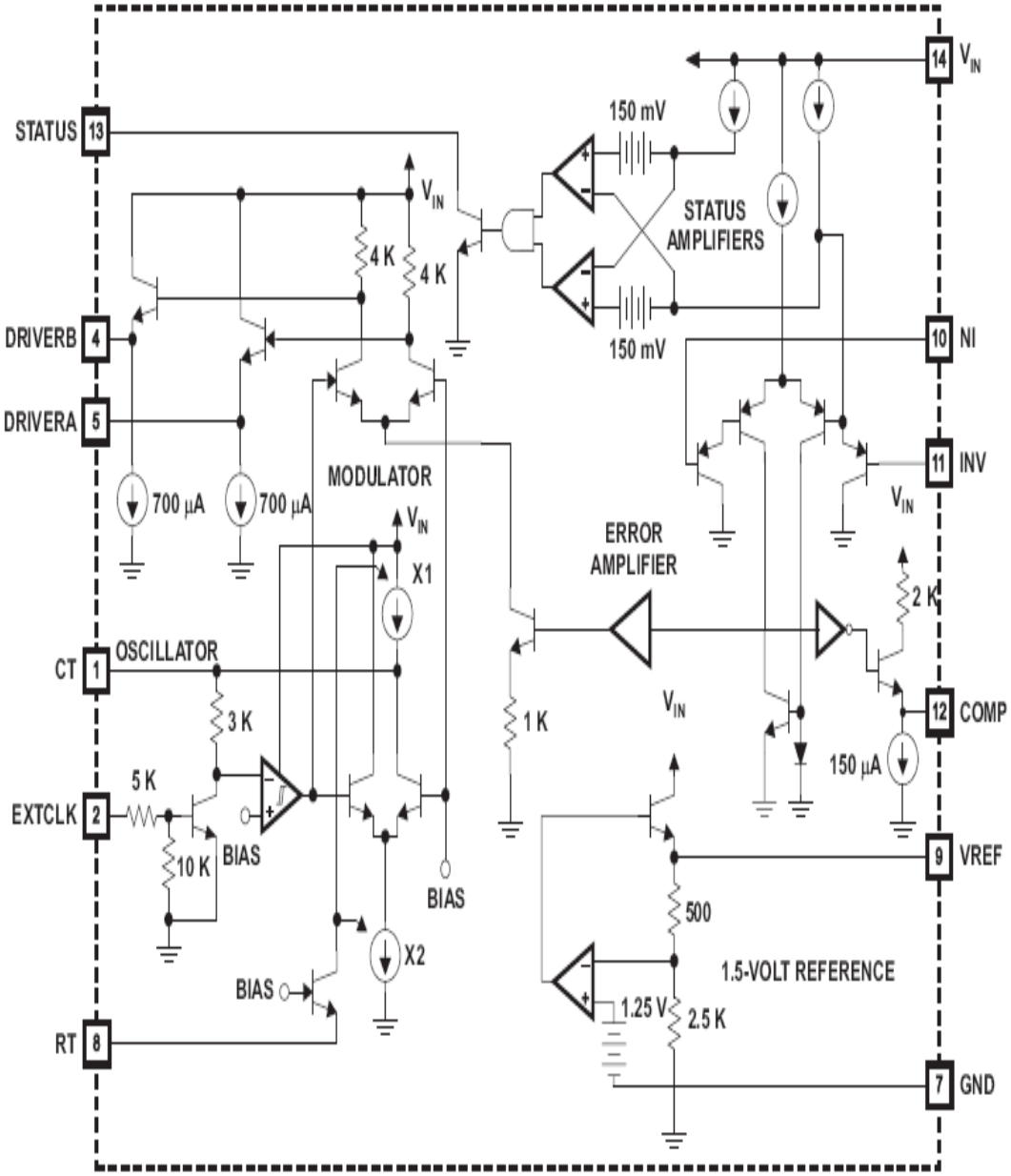
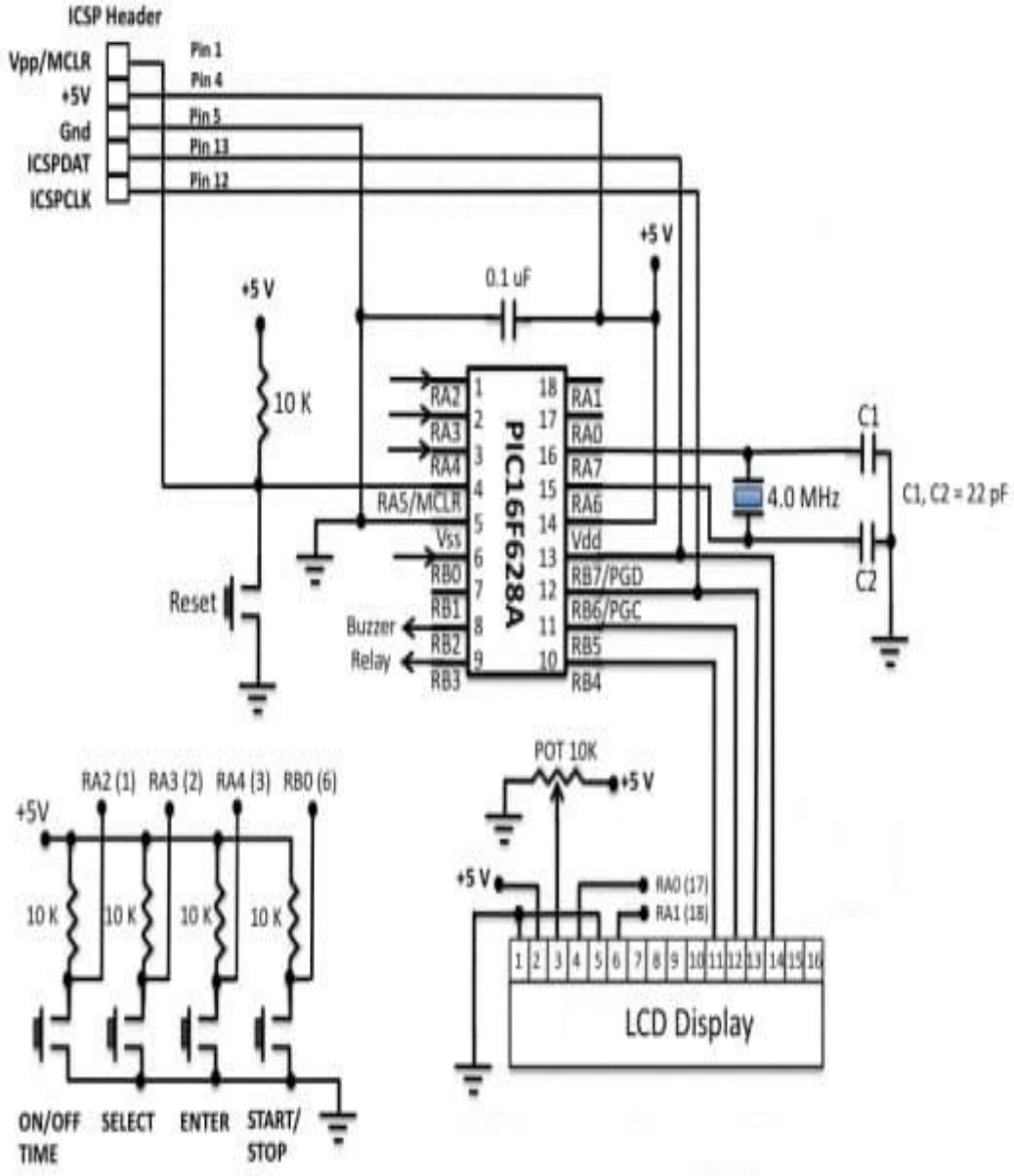


Figure 3.4: Circuit diagram of filter unit



3.1.4 **Treatment chamber**

The treatment chamber (Plate 3.3) consists of two stainless steel electrodes installed with a gap of 5cm inside a PVC chamber of inverted inverted T-shape with an inside coating of Teflon to prevent transmission of pulses to outside and to prevent electric shocks. The chamber encompasses a sensor connected to the electrodes to measure the field strength and number of pulses which is connected to the display unit. The high voltage pulses generated from the pulse generator is transmitted to the electrodes in the treatment chamber in the required modes. The Jamun pulp in the treatment chamber is pulsated with the electric field for the required time as per the experimental design by maintaining a flow rate employing flow control valve. The pulses are transmitted through the pulp via electrodes resulting in electroporation of the cells.

Table 3.1.Specifications of the treatment chamber is as follows:

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

High resistance wires with knobs were used for the connection between the treatment chamber and pulse generator. A submersible miniature pump of 2hp is used to pump out pulp from treatment chamber to the cooling unit and thus maintaining flow without blocks. All the pipes in the system were made of food grade stainless steel.

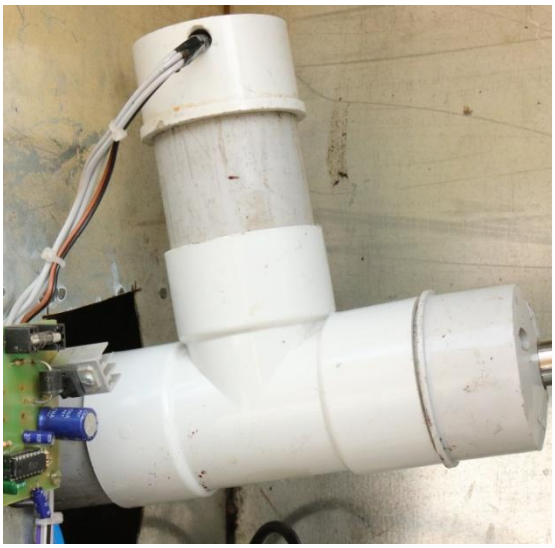


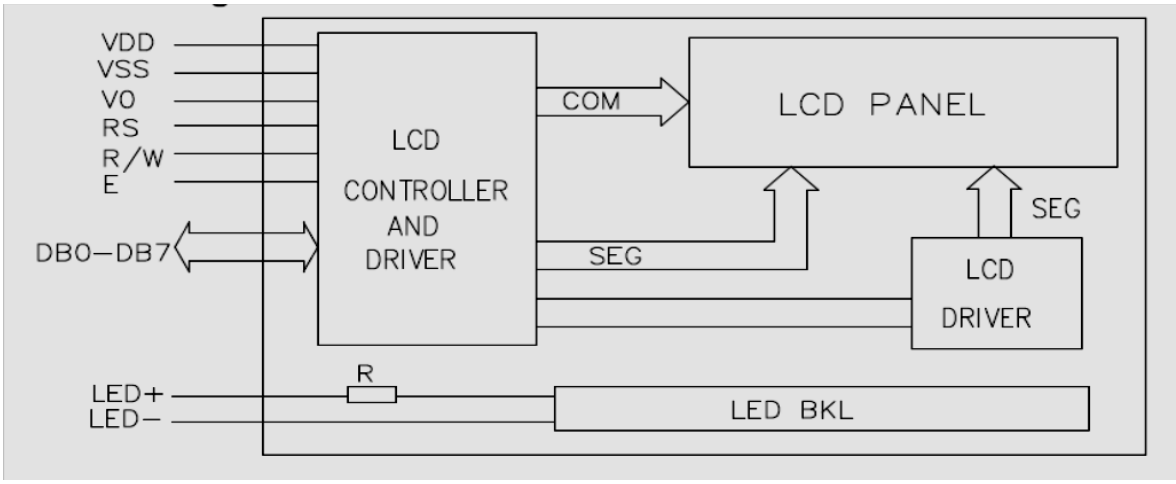
Plate 3.3. Treatment chamber

3.1.5 **Display unit**

The display unit was programmed using Embedded P language. This unit is a 5x8 dots display with cursor. The display unit shows the frequency of the generated pulses, electric field strength. The time of treatment should be calculated using a stop watch. The frequency and field strength displayed on the display unit can be adjusted with ‘up’ switch and ‘down’ switches respectively. The block diagram of the unit is shown in Figure 3.5 and display unit is shown in plate 3.4.

Plate 3.4 Display unit

Figure 3.5 Block diagram of display unit



3.1.6 Cooling system

The cooling of the treated pulp is essential to prevent degradation of the product due to sudden temperature increase because of exposure to the pulsed field. The system consists of a cooling bath made of stainless steel vessel of 5L capacity and is filled with water (Plate 3.5). The treated product is passed through a stainless pipe of 2cm diameter through the cooling bath to effect the drop in temperature. The cooling water is circulated by means of a pump for efficient cooling. Additionally, a fan is also provided on the outer frame over the top of the cooling bath which is activated by a microcontroller. The fan will be ON once the high voltage firing is initiated receiving signal from the microcontroller and OFF once the pulsating field is put off.

Plate 3.5. Cooling unit

3.1.7 Outlet system

Outlet system (Plate 3.6) consists of a PVC control valve connected to the stainless steel outlet pipe which will regulate the outflow of treated pulp.

Plate 3.6. Outlet unit

3.2 QUALITY CHARACTERISTICS OF ANTHOCYANIN

3.2.1 Anthocyanin yield

Anthocyanin yield can be determined by pH differential method and was used to find out the total monomeric anthocyanin content (TMAC) of the samples. Structural transformation in the anthocyanin content during the changes in pH can be used to measure the TMAC. pH 1.0 and 4.5 buffers were prepared by using 0.025M potassium chloride and 0.4M sodium acetate. Aliquots of the samples were mixed with equal amount of pH buffers and placed in dark for 1h. The absorbance of each samples were noted using spectrophotometer and the equipment was calibrated using distilled water. The absorbance values at 530nm and 700 nm were noted using distilled water as blank. Difference in wavelength with pH values was calculated using the following equation (Maran *et al*., 2015).

$$\mathbf{A = (A_{530nm} - A_{700nm})_{pH\ 1.0} - (A_{530nm} - A_{700nm})_{pH\ 4.5}} \quad \textbf{.....3.1}$$

TMAC was determined by

$$\mathbf{TMAC = \frac{A \times MW \times DF \times 1000}{E \times 1}} \quad \textbf{……3.2}$$

A is the absorbance of sample, MW is the molecular weight of cyanidine – 3-glucoside, DF is the dilution factor, E is the molar absorptivity of cyaniding-3-glucoside.

3.2.2 Antioxidant activity

Antioxidant activity of Jamun was determined by DPPH assay method. Accordingly 0.394 mg of DPPH was taken and mixed with 100 ml of ethanol solvent. Different dilutions of the sample were prepared (0.5 ml, 1 ml, 1.5 ml) and

add equal amount of DPPH-ethanol solution to the samples. Gallic acid was taken as control. Fifty milligrams of GA was made to 50ml with distilled water and the samples were kept in dark at 40ºC for 30 minutes. The OD values of the samples were taken at 517 nm in terms of GA antioxidant activity and the inhibition rate is calculated using the following formula

$$I\% = \frac{A_C - (A_i - A_j)}{A_C} \qquad \text{.....3.3}$$

Where I denotes the DPPH scavenging rate, A_C indicates the absorbance of blank, A_i denotes absorbance in presence of sample, A_j denotes the absorbance of sample without DPPH (Ge and Ma., 2013).

3.2.3 Colour

The colour of the Jamun pulp was found using a Hunter lab colour flex meter (Hunter Association Lab, Inc., Reston, Virginia, USA; model: Hunter lab's colour flex Ez). It consists of a sample port, opaque cover and a display unit. This colour flex meter works with the principle of focusing the light on the sample and measuring the amount of energy reflected from the sample across the visible spectrum.

Hunter lab model describes the colour of the sample by matching a sequence of colour across the visible spectrum. Then the sample was placed on the sample port and it was covered with an opaque cover to prevent interference. It reads the colour of the samples as L, a*, b* values. L indicates luminance forms the vertical axis that indicates whiteness to darkness. Chromatic portions of the solids is described by a (+) as redness, a (-) as greenness, b (+) as yellowness and b (-) as blueness.

3.2.4 Refractive index

The refractive indeces of the samples were determined using Newton's ring apparatus by reflected system of Newton's ring method. Newton's ring apparatus composed of an optically plane glass plate denoted as P and a convex lens of larger focal length is placed on the top of the glass plate. Another glass plate denoted as G is placed 45º on the top of the lens. When the lens is place on the glass plate, air is filled in between the space.

If we consider m^{th} and $(m+k)^{th}$ ring which have diameter D_m and D_{m+k} respectively.

Then,

$$D_m^2 = 4mR\lambda$$

$$D_{m=k}^2 = 4(m+k)R\lambda$$

$$D_{m+k}^2 - D_m^2 = 4kR\lambda$$

Where λ is the wavelength of the light and R is the radius of curvature of the lens used. With a thin film of the transparent liquid in between the glass plate and lens, if D_m and D_{m+k} are the diameters of the m^{th} and $m+k^{th}$ rings

$D_{m+k}^2 - D_m^2 = 4KR\lambda/n$, where n = refractive index of the liquid.

From these two equations,

$$n = D_{m+k}^2 - D_m^2 / D_{m+k}^2 - D_m^2 \qquad \text{…….. (3.4)}$$

Light from a sodium vapour lamp S is rendered parallel by a short focus convex lens. The parallel rays fall on the glass plate G, inclined at 45º to the horizontal, gets reflected and then fall normally on the convex lens L placed over on the glass

plate P. A system of bright and dark concentric circular rings are observed through a microscope M, arranged vertically above the glass plate G. The microscope is properly focused so that alternate bright and dark circular rings can be visible clearly.

By working the fine adjustment screw of the microscope makes sure that there are about 25 clear rings on either side of the centre. Starting from the centre of the fringe system, the microscope is moved towards the left so that the cross wire is tangential to the m^{th} (20^{th}) dark ring. The microscope reading on the horizontal scale was taken. By working the fine screw adjustment, the microscope was moved towards the right. The cross wire was adjusted to the tangential to the 18^{th}, 16^{th} etc. dark ring is succession up to the second dark ring on the left and the corresponding readings were taken. Then the cross wire was made tangential to the second dark ring on the right side. Readings were taken corresponding to the 2^{nd}, 4^{th} …. 20^{th} dark ring, as before. The difference between the readings on the left and right of each ring gives the diameter D of the respective ring. Hence $(D_{m+k}^2 - D_m^2)$ was calculated.

A drop of sample is placed on the plane glass plate and the lens was placed over it. The lens and the glass plate were pressed together so that a thin film of liquid without any air bubble is formed between them. The experiment was repeated as before and D_m and D_{m+k} were measured. The refractive index of the liquid was then calculated.

Refractive index (n) = Mean of $(D_{m+k}^2 - D_m^2)$ / Mean of $(D_{m+k}^2 - D_m^2)$ … (3.5)

3.3 EXPERIMENTAL DESIGN

In this study, PEF treatment is applied as a pretreatment prior to the extraction process to verify the influence of the treatment over the anthocyanin extraction yield for Jamun.

The process parameters which would influence the anthocyanin yield such as field strength, treatment time and pulse frequency were taken as independent variables and their ranges for the study were fixed based on thorough review of literature and the preliminary studies conducted. Physical quality characteristics of extracted anthocyanin were taken as dependent variables.

3.3.1 Independent variables

a) Field Strength (V):

1) V_1 : 5kV/cm

2) V_2 : 10kV/cm

3) V_3 : 15kV/cm

b) Treatment time (t):

1) T_1: 2min

2) T_2 :3 min

3) T_3 :4 min

c) Pulse frequency (F):

1) F_1: 60 pulse/s

2) F_2: 80 pulse/s

3) F_3: 100 pulse/s

3.3.2 Dependent variables

a) Anthocyanin yield

b) Colour

c) Antioxidant activity

d) Refractive index

3.4 STATISTICAL ANALYSIS

Statistical optimization was carried out for effective interpretation of the interaction of process variables and generating an authentic process response compared to that of conventional empirical optimization. Response surface method (RSM) experimental design was taken to optimize the process variables as it emphasizes the modeling and analysis of the problem in which responses is highly influenced (Montgomery., 2001). The main advantage RSM is to reduce the number of experimental treatments needed to give sufficient information for the statistically acceptable results. The three process variables such as electric field strength, pulse frequency and treatment time at three different levels were chosen based on the preliminary trials conducted.

Process variables were determined and after the selection of dependent, independent variables and their ranges, experiments were carried out based on the Box-Behnken design. Box-Behnken designs for three variables of three different levels at three centre point combinations for each were determined. This design was chosen as it fulfilled the requirements needed for optimization of pulsed electric field assisted extraction process. For statistical process calculations, variables were coded as -1, 0, +1 level and designed in X form coding as shown in table 3.2 (Gopika and Ghuman., 2014).

Table 3.2.Values of independent variables at three levels of Box-Behnken design

Independent variable	Symbol		Level	
	Coded	Uncoded	Coded	Uncoded
Electric field strength (V/cm)	X_1	E	-1 0 1	5 10 15
Pulse frequency (Pulses/s)	X_2	F	-1 0 1	60 80 100
Treatment time (min)	X_3	T	-1 0 1	2 3 4

According to BBD, total experiments to be conducted were seventeen for three independent variables. Seventeen experiments with three variables at three levels were conducted as shown in Table 3.3.

Table 3.3.Experimental design used for the PEF assisted extraction of anthocyanin from Jamun

Standard order	Run	Coded variables			Un-coded variables		
		Electric field strength (V/cm)	Pulse frequency (Pulses/s)	Treat-ment time (min)	Electric field strength (V/cm)	Pulse frequency (Pulses/s)	Treatment time (min)
1	6	-1	-1	0	5	60	3
2	13	1	-1	0	15	60	3
3	15	-1	1	0	5	100	3
4	17	1	1	0	15	100	3
5	2	-1	0	-1	5	80	2
6	5	1	0	-1	15	80	2
7	1	-1	0	1	5	80	4
8	4	1	0	1	15	80	4
9	12	0	-1	-1	5	60	2
10	8	0	1	-1	5	100	2
11	11	0	-1	1	5	60	4
12	9	0	1	1	5	100	4
13	3	0	01	0	5	80	3
14	16	0	0	0	5	80	3
15	7	0	0	0	5	80	3
16	14	0	0	0	5	80	3
17	10	0	0	0	5	80	3

After performing the experiments, in order to predict the optimal point, a second order regression equation was fitted to co-relate the relationship between independent variables and their responses. It accounts for the variations caused by linear, quadratic and interactive effect of the process variables (Lee *et al.,* 2006).

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3$$

Where,

Y is the response variable

b_0, b_1, b_2 and b_3 are regression coefficients of linear terms

b_{11}, b_{22}, b_{33} are regression coefficients of quadratic terms

b_{12}, b_{13} and b_{23} are regression coefficients of cross-products terms

X_1, X_2 and X_3 are coded values of the independent variables X i.e. electric field strength (X_1), pulse frequency (X_2) and treatment time (X_3) respectively. R^2, coefficients of determination was used for the quality of fit of the second order equations and F-test was used for its statistical significance. P value was used to determine the significance of regression coefficient. The coefficients of the equation were determined by employing Design Expert Version 9.0 a (State – Ease, Inc. USA). Analysis of variance (ANOVA) was carried out for the final predictive equation using Design Expert Software. Using this software, the response surface equation was optimized for the response variables. The response

surface and contour plot analysis were performed by fixing one independent variable at the middle level while changing the other two variables.

3.5 EXPERIMENTAL PROCEDURE

To evaluate the effect of PEF treatment on the yield of anthocyanin from Jamun and to optimize the process parameters, experiments were conducted in K. C. A. E. T Food Engineering lab as per the design of experiments as stated in section in 3.3. Freshly harvested ripe Jamun of variety Ram Jamun were purchased from local market at Thrissur, washed and stored at 4-8ºC in domestic refrigerator were used for the study. The required quantity was taken for treatment. The seeds were removed manually and pulped using a 750 W domestic blender.

3.5.1 Preliminary studies on extraction of anthocyanin from Jamun

As very few literature is available on the extraction of anthocyanin from Jamun studies were carried out to find out the suitable solid –solvent ratio, temperature and treatment time of the hot water bath extraction. The hot water extraction of anthocyanin from Jamun was carried out at three different temperature (40, 60, 80ºC), treatment time (1, 1.5, 2hr) and solid-solvent ratio (1:5, 1:10, 1:15). The results were then analysed and the treatment combinations which resulted in maximum yield of anthocyanin was taken for PEF treatment analysis. In this study a solid-solvent ratio of 1:15, treatment temperature of 40ºC and treatment time of 90 minutes resulted in highest anthocyanin yield. Similar results were also reported by Maran *et al*., (2015).

3.5.2 PEF assisted extraction of anthocyanin

Before starting the experiment, the machine supply is put on and the parameters such as electric field strength and pulse frequency were set through the configuring unit on the display panel. The Jamun pulp is supplied to the PEF treatment chamber via inlet flow control valve. The outlet valve is closed. Once the pulp has occupied the treatment chamber. The inlet flow control valve is closed. Now the pulse generator is switched on and the pulp is allowed to receive the electric pulses of set electric field strength and pulse frequency for the treatment time as required. After that the outlet valve is opened and the treated Jamun pulp is cooled through the cooling system and collected in glass jars for anthocyanin extraction and quality analysis of the extracted anthocyanin.

3.5.3 Anthocyanin extraction from PEF treated Jamun

The PEF treated anthocyanin undergone same procedure of preliminary studies to extract anthocyanin from Jamun as explained in the section 3.5.1.

3.6 DETERMINATION OF QUALITY CHARACTERISTICS OF PEF ASSISTED EXTRACTED ANTHOCYANIN

3.6.1 Colour

The colour of the PEF assisted extracted anthocyanin was measured using Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virginia, USA). Colour of the extracted anthocyanin was measured by L, a*, b* colour values. Extracted anthocyanin was filled in the measuring port of the colourimeter up to the mark provided on the colour port and L, a*, b* colour values were noted as in section 3.2.3

3.6.2 Antioxidant activity

Antioxidant activity of the PEF assisted extracted samples were determined using the procedure in section 3.2.2

3.6.3 Anthocyanin yield

Anthocyanin yield of the extracted anthocyanin was measured using the procedure in section 3.2.1

3.6.4 Refractive index

Refractive index of the anthocyanin extracted from jamun was determined by using the procedure in section 3.2.4

CHAPTER IV

RESULTS AND DISCUSSION

This chapter outlines the results on the development of pulsed electric field assisted extraction system and evaluation of developed system towards the extraction of anthocyanin from Jamun. The outcome of the experiments laid out for the evaluation of the process parameters leading to their standardization are discussed in detail. Also the effect of various process variables on the physical quality characteristics of the PEF assisted extracted anthocyanin are analysed, discussed and compared with conventional extraction process.

4.1 DEVELOPMENT OF PULSED ELECTRIC FIELD ASSISTED EXTRACTION SYSTEM

A pulsed electric field assisted extraction system for extracting anthocyanin was fabricated as shown in plate 3.1. It consists of an outer protective chamber, inlet unit, pulse generating system, treatment chamber, display unit and a cooling system (Figure 3.1). The main frame which is made of mild steel angles and special insulation provided to isolate the outer casing for the safety of operators from electric shocks. All display and controls for monitoring and measuring parameters are provided on outer covering.

The inlet system consists of a stainless steel feed vessel of 5 litre capacity, a control valve of 2cm diameter and connected to stainless steel pipes. Pulse generating system forms the main component of PEF system in which a high boost circuit was made to generate the pulses of voltage varying from 5-20kV with an input supply of 230V, 12W and 5A.

A LOT for the pulse generation and a filtering unit to check the properties of formed pulses is provided inside the system which is connected to the treatment chamber. LOT circuit produces pulses with different voltages but only the

required voltage pulses should be allowed to transmit to the electrode. An isolated feedback circuit is installed between LOT unit and electrode for controlling the voltage of pulses proceeding to treatment chamber.

The treatment chamber consists of two stainless steel electrodes installed inside an inverted T shaped PVC chamber to which high voltage pulses from pulse generator is transmitted in the required modes. The pulses are transmitted to the pulp via electrodes. A submersible miniature pump is used to pump out the pulp to cooling unit.

The display unit as shown in Plate 3.4 was programmed using Embedded P language. The unit displays the electric field strength, pulse frequency and the treatment time can be measured using a stopwatch.

The cooling system (Plate 3.5) consists of a cooling bath made of a stainless steel vessel of 5 litre capacity and is filled with water. Additionally a fan is also provided on the outer frame on the top of the cooling bath which is activated by a microcontroller. The fan will be on over the high voltage firing is initiated, receiving signal from the microcontroller and off over the pulsating field is put off.

Outlet system consists of a control valve which will regulate the outflow of treated pulp.

4.2 STANDARDISATION OF THE PROCESS PARAMETERS OF THE PEF ASSISTED EXTRACTION

In order to evaluate the fabricated PEF system towards the extraction of anthocyanin from Jamun and for optimization of the process parameters, a series of experiments were conducted. As very few literatures is available on the extraction of anthocyanin from Jamun, preliminary studies were carried out to

find out the suitable solid-solvent ratio, temperature and treatment time of conventional extraction method using a water bath. Based on the studies, temperatures of 40, 60 and 80ºC, solid-solvent ratios of 1:5, 1:10, 1:15 and treatment time of 60, 90 and 120 minutes were selected for further analysis. The results which yielded maximum anthocyanin were taken for PEF assisted extraction studies. Accordingly, a solid –solvent ratio of 1:15, temperature of 40ºC and treatment time of 90 minutes were taken as optimum and were used for further studies. PEF assisted extraction studies were conducted at an electric field strength of 5, 10, 15kV/cm, pulse frequency of 60, 80 and 100 pulses/s and treatment time of 2, 3 and 4 minutes as explained in section 3.5.

For optimizing the parameters, anthocyanin yield, antioxidant activity and colour were taken as responses and listed as per the experimental design. Seventeen experimental data were used in the design to optimize the parameters as per response surface methodology. In order to relate the dependent and independent process variables, a second order quadratic model was used. In the second order polynomial equation, the coefficient of each term was determined using multiple regression analysis through Design Expert software.

The experimental data was fitted to selected models to obtain the regression co efficient. Analysis of variance (ANOVA) was carried out to examine each response for statistical significance of the terms in the regression equation. The adequacy and significance of the quadratic model was ascertained. To check the significance of each of the coefficients ‘p’ values were used as a tool. The adequacy of regression model was checked by R^2, Adjusted R^2, Adequate Precision and Fisher’s F-test (Montgomery, 2001).

Adjusted R^2 is a measure of amount of variation around the mean, adjusted for the number of terms in the model. As the number of terms in the model increases, the adjusted R^2 decreases if those additional terms do not add

value to the model. Adequate precision compares the range of predicted values at design points to the average prediction error.

The significance of all terms in the polynomial was judged statistically by computing the F-value at probability (p) of 0.1 to 0.01. A complete second order quadratic model was employed to fit the data and adequacy of the model was tested by considering R^2, Adjusted R^2, predicted R^2 (a measure of how good the model predicts a response value) and Fischer F-test (Haber and Runyon., 1977). During the explanation of variation in behavior, the smaller the value of R^2, the less importance the dependent variables in the model have. Partial differentiation of the process parameters was done for optimization of the model with respect to each parameter. The resulting function is solved by equating the equation to zero.

4.3 OUTPUT CHARACTERISTICS OF PEF SYSTEM

4.3.1 Anthocyanin yield

The anthocyanin yield extracted from Jamun during various combinations of process parameters are presented in Table 4.1.

Table 4.1 Effect of anthocyanin yield on extraction of anthocyanin from jamun

Sl. No	Sample	Anthocyanin Yield (mg/100g)
1	$E_1 P_2 T_3$	8
2	$E_1 P_1 T_2$	8.5
3	$E_1 P_3 T_2$	5.8
4	$E_1 P_2 T_1$	8.3
5	$E_2 P_1 T_3$	10.9

6	E_2 P_1 T_1	11.9
7	E_2 P_2 T_2	9.8
8	E_2 P_2 T_2	10.92
9	E_2 P_3 T_3	9.05
10	E_2 P_2 T_2	10.68
11	E_2 P_2 T_2	10.23
12	E_2 P_2 T_2	10.54
13	E_2 P_3 T_1	8.5
14	E_3 P_2 T_3	6.53
15	E_3 P_3 T_2	7.29
16	E_3 P_2 T_1	6.78
17	E_3 P_1 T_2	7.32

The anthocyanin yield varied between 5.8 to 11.9 mg/100g of the sample. The maximum anthocyanin yield was obtained at an electric field strength of 10kV/cm, pulse frequency of 60 pulses/s and extraction time of 2 minutes.

Response surface methodology was used to enquire the relationship between the independent and dependent variables. The ANOVA table for the response "anthocyanin yield" is given in Appendix (Table 1). The second order non-linear regression equation was fitted between dependent and independent variables using the experimental values. Following regression model was obtained to predict the anthocyanin yield of PEF assisted extraction of Jamun.

$$\text{Anthocyanin yield} = 10.43 - 0.34\,A - 1.00\,B - 0.12\,C + 0.67\,AB + 0.013\,AC + 0.39\,B - 2.95A^2 - 0.26\,B^2 - 0.086\,C^2 \quad \ldots\ldots\ldots\ldots 4.1$$

Where A = Electric field strength

B = Pulse frequency

C = Treatment time

From Table 1, it can be concluded that the values of R^2, R^2-adj and R^2-pred for the anthocyanin yield were 94.39, 87.18, and 31.18 percent respectively. The coefficient of determination (R^2) of the regression model for anthocyanin yield was 94.37 percent which implies that the model could account 94.39 percent variability in data. Lack of fit was insignificant and F-value suggested that model was significant at 1 percent and 5 percent level of significance. The adequate precision (11.53) value for anthocyanin yield indicates that the model can be used to predict the response within the design space. An adequate response of 4 is a prerequisite for reliable prediction using mathematical models (Montgomery, 2001). Therefore, second order model was adequate in describing the extracted anthocyanin yield from Jamun.

It is evident from the Equation 4.1 that the anthocyanin yield was in positive relation with electric field strength, treatment time and a negative relation with pulse frequency. The anthocyanin yield increases with increase in electric field strength up to 10kV/cm and above 10kV/cm, the anthocyanin yield was found to decrease. With increase in pulse frequency, decrease in anthocyanin yield was noted. Table 1 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on anthocyanin yield at 1 percent ($p<0.001$) level of significance.

4.4.2 Antioxidant activity

The antioxidant activity of anthocyanin extracted from Jamun during various combinations of process parameters are presented in Table 4.2. The antioxidant activity varied between 58.4 to 97.2%. The maximum antioxidant activity was

obtained at an electric field strength of 10kV/cm and a pulse frequency of 80pulses/s. Though the antioxidant activity varied slightly with treatment time, the variation is statistically insignificant.

Table 4.2 shows the antioxidant activity of extracted anthocyanin

Sl. No	Sample	Antioxidant activity (%)
1	$E_1 P_2 T_3$	62.1
2	$E_1 P_1 T_2$	70.3
3	$E_1 P_3 T_2$	68
4	$E_1 P_2 T_1$	65
5	$E_2 P_1 T_3$	96.5
6	$E_2 P_1 T_1$	97.2
7	$E_2 P_2 T_2$	81.3
8	$E_2 P_2 T_2$	90.3
9	$E_2 P_3 T_3$	72.1
10	$E_2 P_2 T_2$	75.4
11	$E_2 P_2 T_2$	85.2
12	$E_2 P_2 T_2$	89.2
13	$E_2 P_3 T_1$	65.4
14	$E_3 P_2 T_3$	58.4
15	$E_3 P_3 T_2$	64.3
16	$E_3 P_2 T_1$	62.5
17	$E_3 P_1 T_2$	66

The ANOVA table for the response "antioxidant activity" is given in Appendix (Table 2). The second order non-linear regression equation was fitted between dependent and independent variables employing the experimental values. Following regression model was obtained to predict the antioxidant activity of PEF assisted extraction of Jamun.

$$\text{Antioxidant activity} = 84.28 - 1.77\,A - 7.52\,B - 0.13\,C + 0.15\,AB - 0.3AC + 1.85\,BC - 18.97A^2 + 1.83B^2 - 3.31C^2 \quad \ldots\ldots\ldots\ldots 4.2$$

From Table 2, it may be inferred that the values of R^2, R^2-adj are 80.33, 55.03 percent respectively and adequate precision for the antioxidant activity was 5.233. The coefficient of determination (R^2) of the regression model for antioxidant activity was 80.33 percent which implies that the model could account 80.33 percent variability in data. Lack of fit was insignificant and F-value suggested that model was significant at 1 percent and 5 percent level of significance. The adequate precision (5.233) value for antioxidant activity indicates the model can be used to predict the response within the design space. Therefore, second order model was adequate in describing the extracted antioxidant activity from Jamun.

It is evident from the Equation 4.2 that the antioxidant activity was in positive relation with electric field strength, treatment time and a negative relation with pulse frequency. The antioxidant activity increases with increase in electric field strength up to 10kV/cm and above 10kV/cm, the antioxidant activity was found to decrease. With increase in pulse frequency, decrease in anthocyanin yield was recorded. Increase in treatment time showed increase in antioxidant activity till 3 minutes and thereafter antioxidant activity found to decrease. Table 2 shows that, the linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on antioxidant activity at 1 percent ($p<0.001$) level of significance.

4.4.3 Colour

The colour of the PEF assisted extracted anthocyanin from Jamun is determined by using Hunter lab colour flex meter as explained in 3.6.4. The values of L, a and b obtained for various samples are given in Tables 4.3.

Table 4.3 shows the effect of colour in extraction of anthocyanin from Jamun

Sl. No	Sample	Color		
		L	a*	b*
1	$E_1 P_2 T_3$	4.36	0.98	-0.89
2	$E_1 P_1 T_2$	2.9	1.48	-1.42
3	$E_1 P_3 T_2$	3.56	1.29	-1.41
4	$E_1 P_2 T_1$	4.2	1.48	-1.54
5	$E_2 P_1 T_3$	2.1	2.1	-1.96
6	$E_2 P_1 T_1$	2.2	2.36	-2.23
7	$E_2 P_2 T_2$	2.44	1.95	-1.84
8	$E_2 P_2 T_2$	2.56	2.01	-2.05
9	$E_2 P_3 T_3$	2.78	1.76	-1.61
10	$E_2 P_2 T_2$	2.5	1.96	-1.9
11	$E_2 P_2 T_2$	2.45	1.91	-1.89
12	$E_2 P_2 T_2$	2.62	1.98	-1.94
13	$E_2 P_3 T_1$	2.69	1.84	-1.7
14	$E_3 P_2 T_3$	4.61	0.86	-0.93
15	$E_3 P_3 T_2$	3.15	0.86	-0.98

16	E_3 P_2 T_1	4.35	1	-1.05
17	E_3 P_1 T_2	3.45	1.36	-1.3

The ANOVA table for the responses "L, a*, b*" are shown in Appendix (Table 3, 4, 5). The second order non-linear regression equation was fitted between dependent and independent variables employing the experimental values. Following regression model was obtained to predict the L, a*, b* values of colour of PEF assisted extraction of Jamun.

$$L = 2.51 + 0.68\,A + 0.19\,B + 0.051C - 0.24\,AB + 0.025AC + 0.047\,BC + 1.34A^2 + 0.59B^2 + 0.52C^2 \quad \ldots\ldots\ldots\ldots 4.3$$

$$a^* = 1.96 - 0.14\,A - 0.19\,B - 0.12\,C - 0.077AB + 0.090\,AC + 0.045BC + -0.82\,A^2 + 0.11B^2 - 0.057\,C^2 \quad \ldots\ldots\ldots\ldots 4.4$$

$$b^* = -1.92 + 0.12\,A + 0.15\,B + 0.14C + 0.078AB - 0.13AC - 0.045BC + 0.71A^2 - 0.063\,B^2 + 0.11\,C^2 \quad \ldots\ldots\ldots\ldots 4.5$$

From Appendix Table 3, it can be inferred that the values of R^2, R^2-adj and R^2 pred for L value are 98.7, 97.12, 82.89 percent respectively. Adequate precision for the L (whiteness/darkness) value was 21.38. The coefficient of determination (R^2) of the regression model for L value was 98.7 percent which implies that the model could account 98.7 percent variability in data. Predicted R^2 (82.89) is in reasonable agreement with coefficient of determination (R^2) 98.7 percent. Lack of fit was insignificant and F-value suggested that model was significant at 1 percent and 5 percent level of significance. The adequate precision (21.38) value for L value indicates the model can be used to predict the response within the design space. For a*(redness/blueness) value (Appendix table 4), R^2, predicted R^2 and R^2-adj were 99.43, 93.01 and 98.69 and adequate precision 37.054.

The coefficient of determination ($R^{2)}$ value was 99.43 implies that the model could account 99.43 percent variability in data. Predicted R^2 (93.01) is in reasonable agreement with R^2 (99.43). From Table 5, it can be inferred that for b*(yellowness/blueness) value, R^2, predicted R^2, R^2-adj and were 97.04, 65.15 and 93.23 respectively. Adequate precision was 15.249 For all the colour values, lack of fit was found to be insignificant and F-value suggested that the models were significant at 1 percent and 5 per cent level of significance. Therefore, all the second order models were adequate in describing the extracted anthocyanin yield from Jamun.

It is evident from the Table 4.3 that the value of L decreases with increase in electric field strength till 10kV/cm and increases with further increase in electric field strength. Decrease in L value indicates the increase in darkness of the sample. Increase value of a* indicates the increase in red colour in the sample and increase in table 3 and 4, the colour values have much effect on the input parameters and linear (A, B, C), interactive (AB, BC, AC) and quadratic (A^2, B^2, C^2) terms had a significance effect on the L, a* and b* values at 1 per cent ($p<0.001$) level of significance.

4.4.4 Refractive index

The values of refractive index of PEF assisted extracted anthocyanin from Jamun obtained in various experiments are shown in Table 4.4.

Table 4.4.Effect of process variables towards refractive index of anthocyanin

Sl.No	Sample	Refractive index
1	$E_1 P_2 T_3$	1.34
2	$E_1 P_1 T_2$	1.37
3	$E_1 P_3 T_2$	1.34

4	$E_1 P_2 T_1$	1.35
5	$E_2 P_1 T_3$	1.38
6	$E_2 P_1 T_1$	1.36
7	$E_2 P_2 T_2$	1.35
8	$E_2 P_2 T_2$	1.34
9	$E_2 P_3 T_3$	1.33
10	$E_2 P_2 T_2$	1.35
11	$E_2 P_2 T_2$	1.34
12	$E_2 P_2 T_2$	1.35
13	$E_2 P_3 T_1$	1.34
14	$E_3 P_2 T_3$	1.32
15	$E_3 P_3 T_2$	1.33
16	$E_3 P_2 T_1$	1.33
17	$E_3 P_1 T_2$	1.34
18	CE	1.37

The values of refractive index varied between 1.33 and 1.38. The values are close to the conventionally extracted anthocyanin i.e. 1.33 to 1.37 as reported by Maran *et al*., (2015).

The ANOVA table for the response “refractive index” is given in Appendix table 6. The second order non-linear regression equation was used to relate between

dependent and independent variables. Following model was fitted to predict the refractive index of anthocyanin.

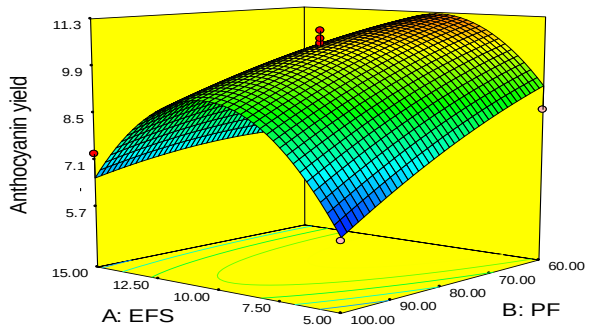
$$\text{Refractive index} = 1.35 - 002A - 0.011B - 003C - 003AB + 0.000\,AC - 003BC - 6.750E - 003A^2 - 003B^2 + 003\,C^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (4.6)$$

From Appendix Table 6, it could be inferred that the values of R^2, R^2–adj and R^2-pred for the refractive index were 94.4, 87.19, 77.3 respectievely. Adequate precision (14.32) value for refracive index indicates that the model can be used to predict the response within the design space. The coefficient of determination, R^2 of the regression model for refractive index was 94.4 per cent which implies that the model could account for 94.4 per cent variability in data. Lack of fit was insignificant and F- value suggested that the model was significant at 1 per cant and 5 per cent.

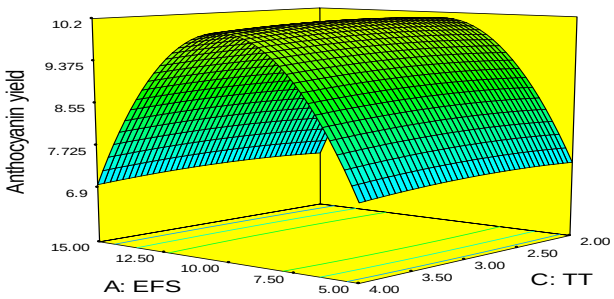
It is evident from Equation 4.6 that the variation of refractive index of anthocyanin by PEF assisted extraction with variations in input variables are insignificant. Appendix Table 6 shows that, the linear (A, B, C), interactive (AB, BC and AC) and quadratic (A^2, B^2, C^2) terms had a significant effect on refractive index at 1 per cent ($p<0.001$) level of significance.

4.5.1 Effect of process parameters on the anthocyanin yield

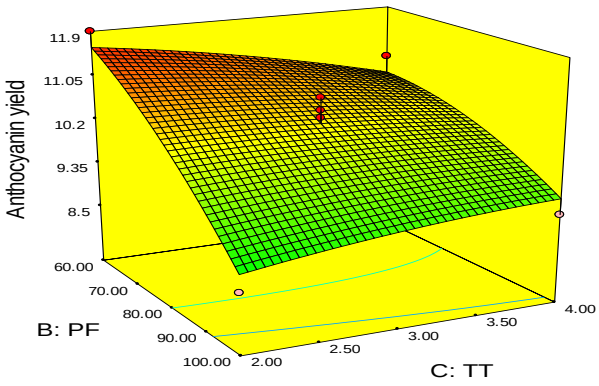
The relationship between independent (Electric field strength, pulse frequency and treatment time) and the dependent variables are illustrated by plotting 3D graphs representing the response surface (anthocyanin yield) generated by the model (Equation 4.1) comprised of 3 graphs a, b, c as given in Figure 4.1.



a



b



c

Figure 4.1 Effect of process parameters on anthocyanin yield

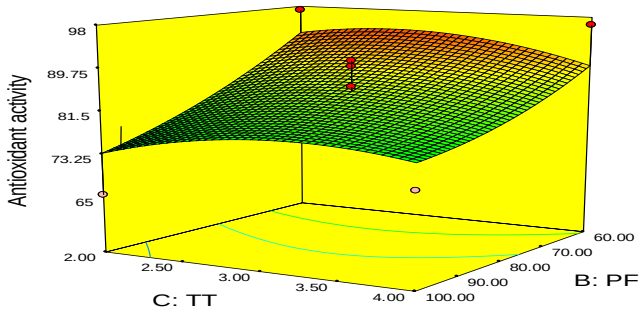
It could be perceived from the Fig. 4.1 (a) and (b) that anthocyanin yield increased when electric field strength was increased from 5kV/cm to 10kV/cm. Further increase in electric field strength resulted in the decrease of anthocyanin yield. This might be due to the fact that primary increase in the electric field strength helps to attain the cell threshold potential that causes cell electroporation process but beyond certain electric field strength, the anthocyanin present in sample gets disintegrated showing reduction in anthocyanin yield (Lopez *et al*., 2007).

The total anthocyanin yield varied from 5.8 to 10.92 mg/100mg of the sample. The least anthocyanin yield amount was obtained when the experiments performed with electric field strength of 5kV/cm. It may be observed from the Fig. 4.1 (b) and (c) that as treatment time increased from 2 min to 4 min, there is only a slight increment in the total anthocyanin yield was observed. This indicates that treatment time has an insignificant effect on the anthocyanin yield. This was also evident from the surface plot (Fig. 4.1 (b) and (c)). Similar findings were also reported by Alessandro and Ramiro (2017) on phenolic compounds.

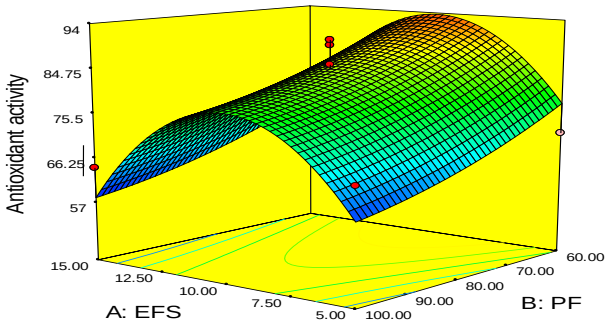
Also, from Fig. 4.1 (a) and (c) it may be concluded that at low pulse frequencies the anthocyanin yield was found to be high. With increase in pulse frequency, the total anthocyanin yield decreases. The decrement in the anthocyanin yield might be due to the fact that, increase in pulse resulted in heat generation within the raw material which disintegrates the anthocyanin molecules, as the anthocyanin molecules are heat sensitive (Zhou *et al*., 2015).

4.5.2 Effect of process parameters on antioxidant activity

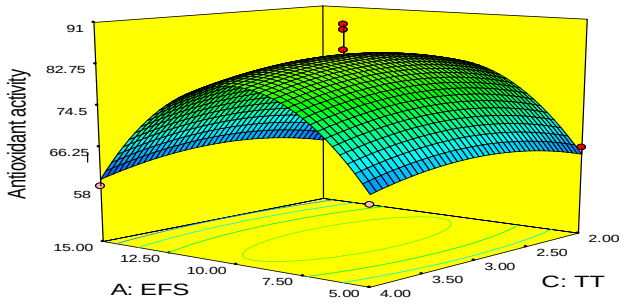
The relationship between electric field strength, pulse frequency and treatment time on the antioxidant activity of the extracted anthocyanin is illustrated by plotting 3D graphs representing the response surface generated by the model (Equation 4.2). The 3D responses were shown in Fig. 4.2, (a), (b) and (c).



a



b



c

Figure 4.2.Effect of process parameters on antioxidant activity

From Fig 4.2 (b) and (c) it may be derived that electric field strength has a significant effect on antioxidant activity. At a low electric field strength 5kV/cm, the antioxidant activity of anthocyanin was found to be less and at 10kV/cm in antioxidant activity was found to be 96.5%. Also, further increase of electric field strength resulted in decrease of antioxidant activity as evident from the fact that antioxidant activity at 15kV/cm was found to be 66%. This trend might be due to the instability of anthocyanin with increase in electric field strength and also increase in electric field strength causes disintegration of anthocyanin molecule due to excess heat generation (Jaya *et al*., 2018).

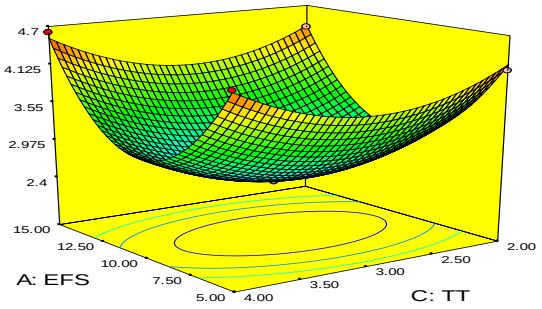
From equation 4.2 shows that pulse frequency had an insignificant effect on antioxidant activity of anthocyanin. This was also supported by surface plot in Fig 4.2 (a) and (b) similar results were also recorded by Asavasanti *et al*., (2010).

From Fig 4.2 (a) and (c) it may be inferred that treatment time has a significant effect on antioxidant activity. As the treatment time increased, antioxidant activity was also found to increase. After 3 minutes of treatment time, antioxidant activity showed no significant difference. This might be due to the fact that after 3 minutes of treatment time, there is no mass transfer of anthocyanin to the outside media. For a single membrane, if the time of treatment exceeds the characteristic time, the rupture process is complete and the permiability of the anthocyanin to the solvent is bare minimum (Lebovka *et al*., 2001).

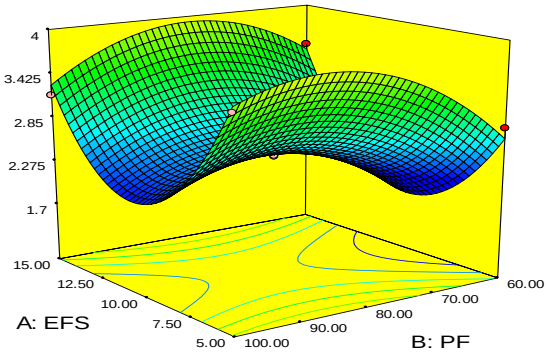
4.6.4 Effect of Process Parameters on Colour of anthocyanin

The effect of electric field strength, pulse frequency an treatment time on the colour of extracted anthocyanin is illustrated by plotting 3D graphs representing the response surface generated by the model Equations 4.3, 4.4, 4.5. The 3D responses for L, a* and b* were shown in Fig. 4.3, 4.4 and 4.5 comprising of three graphs a, b and c each.

4.6.3 Effect of Process Parameters on Colour of Anthocyanin



a



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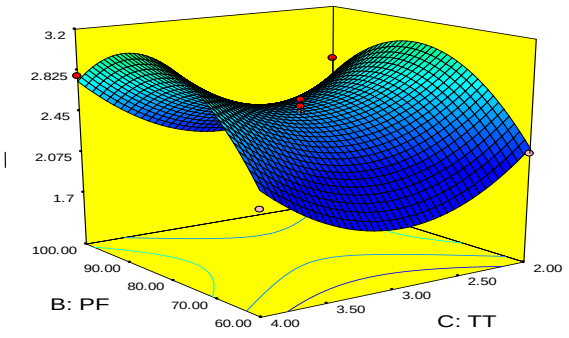
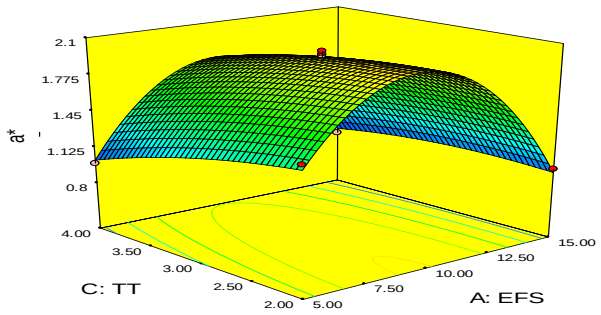
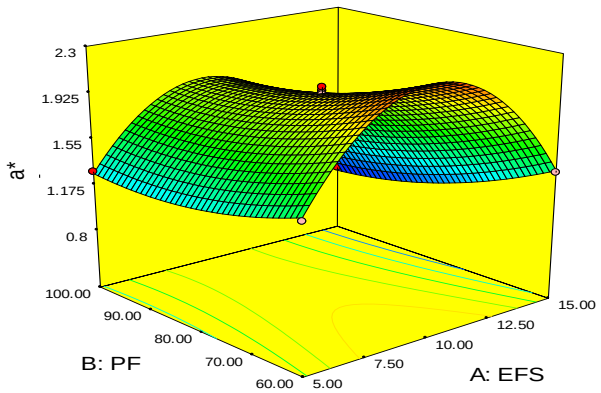
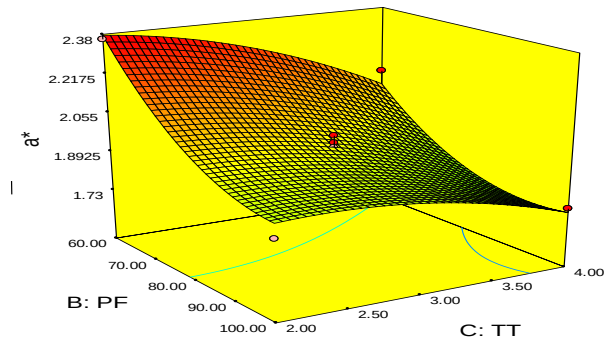


c

Figure 4.3.Effect of process parameters on lightness of anthocyanin

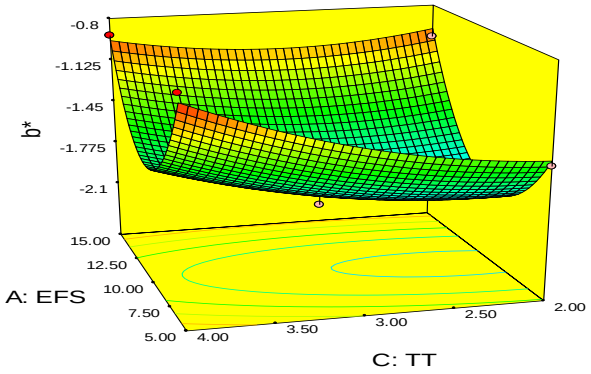






C

Figure 4.5.Effect of process parameters on a*



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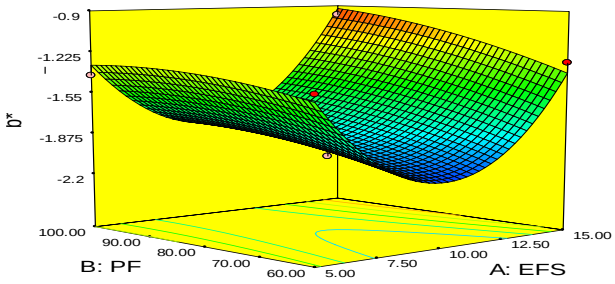

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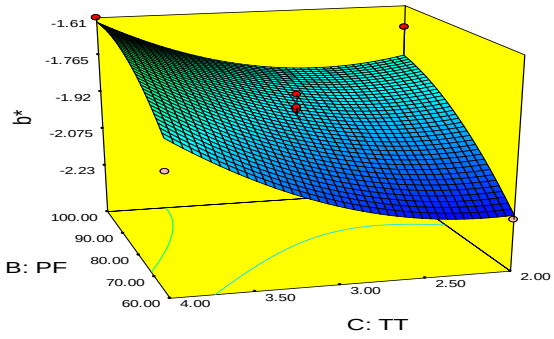


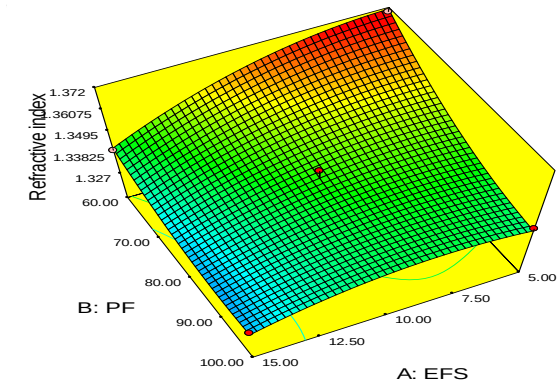
C

Figure 4.5 Effect of process parameters on b*

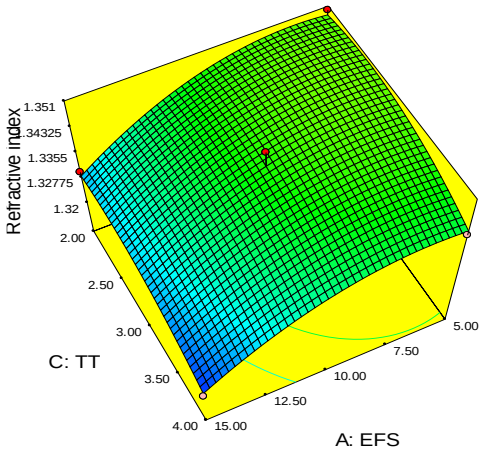
From Fig. 4.3, 4.4, 4.5 (a), (b) and (c), it may be desired that the process parameters had a significant effect on L, a and b values of PEF assisted extracted anthocyanin from Jamun. Increase in electric field strength and pulse frequency resulted in increase in electroporation process. This results in the maximum extraction of colour and associated suspended particles from the pulp. But an increase in electric field strength and pulse frequency beyond 10kV/cm and 80 pulses/s resulted in biochemical instability which causes increase of L values, decrease of a* values and increase of negative value of b* which are not characteristic of the anthocyanin colour. Similar results were also reported by Teusdea *et al*., (2017). Decrease in Lightness (L) value indicates increase in darkness indicating the maximum extraction of colour. L value ranges between 2.1 and 4.61. Increase in "a" values indicates increase in redness of the sample. In this study, "a" values ranges from 0.86 to 2.36 showing increase in redness. From Fig 4.5 (a), (b) and (c), it may be concluded that treatment time and pulse frequency had insignificant effect on "b*" values than electric field strength. The results indicated that the extracted anthocyanin are more dark coloured than conventionally extracted athnocyanin. Plate 4.1 shows the difference between the anthocyanins extracted by conventional and PEF assisted extraction process.

4.6.5 Effect of process parameters on Refractive index of anthocyanin

The effect of electric field strength, pulse frequency and treatment time on the refractive index is illustrated by plotting 3D graphs representing the response surface generated by the model Equations 4.6. The 3D responses for refractive index was shown in 4.6 comprising of graphs a, b and c.



a



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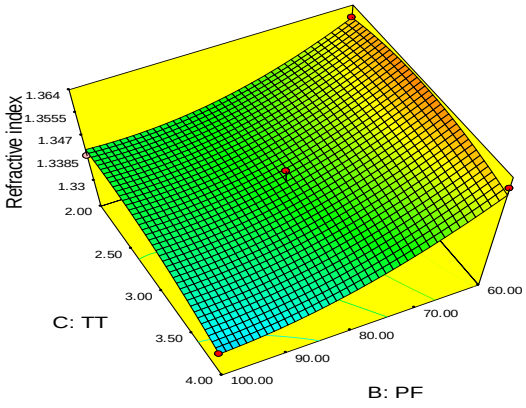


c

Figure 4.6 Effect of process parameters on refractive index

The treatment time had no effect on the refractive index of the essential oil. The electric field strength and pulse frequency have insignificant effect on the refractive index of the anthocyanin. As the electric field strength increases, refractive index was also found to increase. This might be due to the rise in temperature in the system decreases the red colour of anthocyanin and increases of blue colour (darkness) as refractive index is the ratio of speed of light in vacuum to the speed of light in medium (Anon, 2014). But when compared with the refractive index of conventional extraction of anthocyanin, the refractive index of anthocyanin also falls within the range of 1.330 to 1.371. Therefore, the refractive index was found to be similar for anthocyanin obtained in both the processes (Maran et al., 2015).

4.5 Desirability

Desirability analysis was performed by employing the design expert software version 7.0.0. Desirability changes from zero to one for any given response. The program combines individual responsibilities in to a single number and then searches for the greatest overall desirability. A value of one represents the ideal case. Zero indicates that one or more responses fall outside desirable limits (Myers *et al*., 2009).

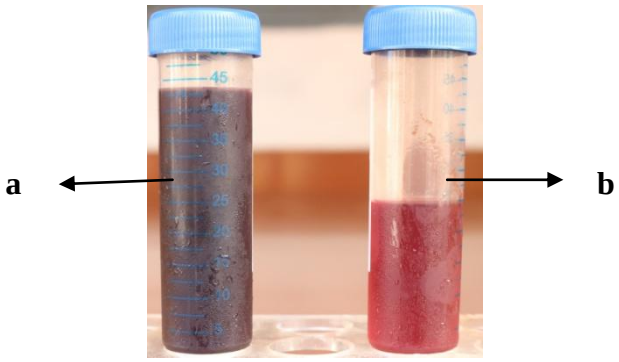
From the desirability analysis, the optimal level of various parameters were found and listed in Table 4.5. From the analysis, electric field strength of 9.48kV/cm ; pulse frequency of 60 pulses/s; and treatment time of 2.24 minutes were found to be optimum values. The anthocyanin yield, antioxidant and colour values at this optimum process parameter levels for PEF assisted extraction process of Jamun were found to be 11.5836mg/100g, 93.024%, 2.010(L), 2.365(a*), -2.22(b*) respectively and the same were found to be 8.91mg/100g, 77.23%, 2.91(L), 1.65(a*) and -1.84(b*) respectively for conventional extraction

process. The desirability of the optimization was found to be 0.967. As the desirability value is close to 1.0, the optimized values could be considered ideal.

Table 4.5.Optimal level obtained from the desirability analysis

Sl.No	Response	Desirabiity	Optimal level	Low level	High level
1	Anthocyanin yield	Maximise	11.583	11.421	11.644
2	Antioxidant activity	Maximise	93.024	92.441	93.896
3	L	Minimise	2.010	1.781	2.099
4	a*	Maximise	2.365	2.320	2.369
5	b*	Minimise	-2.222	-2.193	-2.224
6	Refractive index	Is in range	1.352	1.330	1.371

Plate 4.1 Anthocyanin extracted from Jamun through PEF assisted & conventional extraction process



a - PEF assisted extracted anthocyanin & b- conventionally extracted anthocyanin

CHAPTER V

SUMMARY AND CONCLUSION

Colours are used in food to enhance the quality to clout the consumer and also for balancing the colour loss during processing. Colours are classified in to natural or artificial colours. Natural colours are obtained from plants or plant products. Anthocyanins are water soluble pigments which can impart red to blue colours to fruits and vegetables. Anthocyanins have gained their importance not only because of its wide range of colour spectrum, but also because of its health benefits. They are generally extracted by conventional methods.

The commonly used conventional extraction methods are solid-solvent extraction, adsorption extraction etc. But these methods possess disadvantages such as low extraction efficiency and high extraction time.

Pulsed Electric Field (PEF) technology is an emerging non thermal food processing technology. It involves application of short duration electric field pulses of high intensity to materials located between two electrodes. PEF induces extra membrane potential difference across the cell membrane. When the potential difference exceeds a critical value of 1V, called the breakdown potential, localised electrical breakdown of the membrane occurs resulting in pore formation on membrane and thus cell permeability increases. The permeabilisation of plant cell membrane improves the mass transfer in subsequent process such as extraction. One of the main factors influencing the efficiency of extraction process is the degree of cell membrane disintegration and cell wall rupture that frees the intercellular compounds resulting in increased extraction efficiency of intercellular liquids.

This study envisages development of pulsed electric field assisted extraction system for extracting anthocyanin from Jamun. The developed extraction system

consists of an outer protective chamber, inlet unit, pulse generating system, treatment chamber, display unit and cooling system. The main frame was made of mild steel angles as two supporting structures. All display and controls for monitoring and measuring parameters are provided on the outer covering which is fabricated out of aluminium sheets. The inlet system consists of a stainless steel feed vessel of 5 litre capacity, flow control valve and stainless steel pipes. The inlet vessel is connected to treatment chamber via steel pipes of 2cm diameter. Pulse generating system forms the main component of PEF system in which a high boost circuit was made to generate pulses of voltage 5-20kV with an input supply of 230V, 12W power and a current of 5A. It consists of LOT, controlling unit and a microcontroller. LOT is used to produce pulses with different voltages. The controlling unit and micro controller consists of a filter unit that adjust the amplitude and frequency of the pulses. Treatment chamber consists of two stainless electrodes to which high voltage pulses from pulse generator is transmitted. An isolated feedback circuit is attached to electrodes to ensure only required pulses to the system. The pulses are transmitted through the pulp via electrodes resulting electroporation of the cells. Display unit was programmed using Embedded P language. This unit displays the frequency and electric field strength of generating pulses. Cooling of the treated pulp was carried out in the cooling unit to prevent the degradation of the product due to sudden temperature increase due to the pulsed field. Cooling unit consists of water cooling system and a cooling fan. Outlet system consists of a PVC control valve which will regulate the outflow of treated pulp.

In order to evaluate the developed system towards extraction of anthocyanin from Jamun, the process parameters like electric field strength, pulse frequency and treatment time were chosen as independent variables. Anthocyanin yield, antioxidant activity and colour were chosen as dependent variables. Based on the preliminary studies the three levels of process parameters were selected such as

electric field strength of 5, 10 and 15kV/cm, pulse frequency of 60, 80 and 100 pulses/s and treatment time of 2, 3 and 4 minutes.

The experiments were performed by supplying Jamun pulp to the PEF equipment and the process parameters such as electric field strength and pulse frequency were set through the configuring unit. The outlet valve is closed, once the pulp has occupied the treatment chamber, the inlet valve is also closed. Now the pulse generator is switched on and the pulp is allowed to receive the electric pulses of set field strength and pulse frequency for the treatment time as required. After that the outlet valve is opened and the treated pulp is cooled and collected in glass jars for conducting yield and quality evaluations.

For optimization of the process parameters and to check the efficiency of the experimental design, the second order non-linear regression equation was fitted between dependent and independent variables. Analysis of variance (ANOVA) for the final predictive equation was carried out using Design Expert Software. RSM was adopted and Box-Behnken design of three variables and three levels, each with three centre point combinations was used. The response surface equation was optimized for the response variables using the above software.

The results showed that with increase in electric field strength from 5kV/cm to 10kV/cm, the anthocyanin yield was found to increase. Also, with increase in pulse frequency anthocyanin yield was found to decrease. Increase in treatment time up to 3 minutes, the yield increased. Further increase in treatment time the anthocyanin yield and other quality parameters were found to be reduced. Refractive index has insignificant effect on the quality parameters of anthocyanin. Pulsed electric field assisted extraction of anthocyanin resulted in an anthocyanin yield of 11.584 mg/100g of sample, with an antioxidant activity of 93.188% and colour values of 2.013 (L), 2.365 (a*) and -2.22(b*) where as the conventional extraction yielded an anthocyanin yield of 10mg/100g of sample, antioxidant

activity of 82.3%, colour values are L of 3.1, a* of 1.23 and b* of -1.35 and refractive index of 1.37. Thus it could be concluded that PEF assisted extraction of anthocyanin resulted in increase in anthocyanin yield and quality parameters.

The optimized conditions of electric field strength, pulse frequency and treatment time for PEF assisted extraction of anthocyanin from Jamun were found to be 9.47 kV/cm, 60 pulses/s and 2.24 minutes. It is evident from the study that PEF assisted extraction of anthocyanin from Jamun resulted in increased extraction of high quality anthocyanin.

The following are suggestions for future research work on the pulsed electric field assisted extraction.

1. Installation of instrumentation for sensing temperature in the treatment chamber for analyzing the heat generation may be explored.
2. The position of feed control valve may be changed for accurate control of treatment time.
3. This process is essentially a batch process. Conversion of the process to a continuous one may be explored for increased capacity requirements.

REFERENCES

Achrekar, S., Kakliji, G.S., Pote, M.S., and Kelkar, S.M.1991. Hypoglycemic activity of eugenia jambolana and ficus bengalensis: mechanism of action. *In vivo* 5(2):143–147.

Alessandro, D., and Ramiro, A.C. 2017. Fermentation assisted by PEF and ultrasound: A review. *Fermentation*. 2-12.

Alwazeer, D., Delbeau, C, Divies, D., and Cachon, R, 2003, Use of redox potential modification by gas improves microbial quality, colorretention, and ascorbic acid stability of pasteurized orangejuice, Int. 1. *Food Microbiol*. 89: 21-29.

[Anonymous]. 2014 [Online]. Available: https:// www.quora.cpm/Does the refractive index - relate- to temperature inversely – or- directly [17 May - 2014].

[Anonymous]. 2015 [Online]. Available: https:// nutritive value of Jamun –TNAU Agri. tech portal [17 June -2015].

Aqil, F., Gupta , A., Munagala, R., and Jeyabalan, J.2012. Antioxidant and antiproliferative activities of anthocyanin/ellagitannin-enriched extracts from *Syzygium cumini* . *HAAC Open Acc*.64(3): 428–438.

Asavasanti, S., Ersus, S., Stroeve, W., and Basette, D. 2010. Critical electric field strength of onion tissues treated by pulsed electric field. *J. Food Sci*.75(7): 433-443.

Barbosa, C.G.V., Gongora-Nieto, M. M., Pothakamury, U. R., and Swanson, B. G.1999. Preservation of Foods with Pulsed Electric Fields, *Academic Press*. 56-61.

Barsotti, L., Merle, P.P., and Cheftel, J., 2002, Food processing by pulsed electric fields, I. Physical aspects, Food Rev. Int. 15(2): 163-180.

Bazhal, M., and Vorbier, E. 2001. Electrical treatment of apple cossettes for intensifying juice pressing. *J. Sci. Food Agri*. 80: 1668-1674.

Blatt, E.1989. Principles of Physics, 3rd edn., *Allyn and Bacon*, 355-361.

Bors, W., Heller, W., and Michel, C.1990. Flavonoids as antioxidants: determination of radical-scavenging efficiencies. *Methods Enzy.mol.* 186:343–355.

Bouzana,H., and Vorobiev, E., 2001, Beet juice extraction by pressing and pulsed electric fields,*Int. Sugar1*. 102(1216): 194-200.

Bushnell, A., Dunn, J., Clark, R., and Lloyd, S. 2000. High strength electric field pumpable food product treatment in a serial electrode treatment cell, *US Patent*. 410-423.

Canovas, B.G.V,. Nieto, G. Pothakamury, U.R., and Swanson, B.G. 1999. Preservation of Foods with Pulsed Electric Fields. *Academic Press: Lon*. 0-12.

Castro, A.1., Swanson, B.G., Barbosa-Canovas, G. V., and Meyer, R. 2009. Pulsed electric field modification of milk alkaline phosphatase activity. *J. Bio. Sci*. Washington State University.

Chaitanya, G. 2014. Food Colouring: The natural way. *Res. J. Chem. Sci*. 4(2): 87-96.

Chandrasekhar, J., Madhusudhan, M.C., and Raghavarao, K.S.M.S. 2012. Extraction of anthocyanins from red cabbage and purification using adsorption. *Food Bioprod Proc*. 90(4): 615–623.

Chalermchat, Y., Fincan, M., and Djmek, P. 2004. Pulsed electric field treatment for solid–liquid extraction of red beetroot pigment: Mathematical modelling of mass transfer. *J. Food Eng*. 64. 229–236.

Fincan, M., De-Vito, F., and Dejmek, P. 2004. Pulsed electric field treatment for solid–liquid extraction of red beetroot pigment. *J. Food Eng.* 64: 381–388.

Gachovska, T.K., Ngadi, M.O., and Raghavan. G.S.V. 2006. Pulsed electric field assisted juice extraction from alfalfa. *Canadian Biosystems Eng*. 48.

Gachovska,T., Cassada, D., Subbiah, J., Hanna, M., Thippareddi, H., and Snow, D. 2010. Enhanced Anthocyanin Extraction from red cabbage using pulsed electric field processing. *J. Food Sci*. 75(6): 323–329.

Ge, D., and Ma, B. 2013. Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. *Inn. Food Sci. Emerging Technol*. *9*(1): 85–91.

Giner, J., Gimeno, v., Espachs, A., Elez, P., Barbosa-Canovas, G. v., and Martin Belloso, 0. 2000. Inhibition of peach polyphenol oxidase by pulsed electric fields. *European Conference on Emerging Food Science and Nutrition*. 109.

Gopika, T. and Ghuman, B.S. 2014.Optimisation of microwave assisted process for extraction of celery seed phenolic compounds. *J. Agric. Eng*. 51(2): 9-18.

Haber, A. and Runyon, R. 1977. *General Statistics* (3rd Ed.). Addison-Wesley, reading, MA.

Hoe, D.F., Perira, B.N and Serita, V. 2007. Extraction of polyphenol oxidase from mushroom. *J. Food Eng*. 65: 23-27.

Hermawan, N., Evrendilek, G. A., Dantzer, W R., Zhang, Q. H., and Richter, E. R., 2004. Pulsed electric field treatment of liquid whole egg inoculated with *Salmonella Enteritidis*. *J. Food Sal.* 24: 71-85.

Hulsegher, H., Pottel, J., and Niemann, E. G. 1981. Killing of bacteria with electric pulses of high field strength, *Radiat. Environ. Biophys*. 20: 53-65.

Jay, J.G., Kutsyr, P.Y., and Fukui, U.I. 1992. Applications of pulsed electric field technology. *J. Food proc*. 57: 45-62.

Jaya, P.L., Katser, H.J., and Uherty, P. 2018. Recent Advances and Applications of Pulsed Electric Fields (PEF) to Improve Polyphenol Extraction and Color Release during Red Wine making. *Beverages*.124: 35-38.

Katsumoto, Y., Fukuchi-Mizutani, M., and Fukui, Y. 2007. Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiol*. 48(11):1589–1600.

Khoo, H. E., Azlan, A., Tang, S.T., and Lim, S.M. 2017. Anthocyanidins and anthocyanins : colored pigments as food, pharmaceutical ingredients and the potential health benefits. *Food Nutrition Res*. 61(1).

Koehler, E., Toepfl, S., Knorr, D., and Pulz, 0., 2005, Unconventional procedures for the production and stabilization of extracts with active agents, *In: 6th EuropeanWorkshop Micro algal Biotechnol*. Potsdam, Germany.

Kopplov, 0., Barjenbruch, M., and Heinz, v., 2004, Sludge pre-treatment with pulsed electric fields, WaterSci. Techno!' 49(10): 123-129.

Kubola, J., Siriamornpun, S., and Meeso, N. 2011. Phytochemicals, vitamin c and sugar content of that wild fruits. *Food Chem*. 126(3): 972-981.

Laleh, G.H., Frydoonfar, H and Heldary, R. 2006. The effect of temperature, pH and species on stability of anthocyanin pigments in four *Berberis* species. *Pak. J . nutri.* 90-92.

Lam, G. P., Postma, P. R., Fernandes, D. A., Timmermans, R. A. H., Vermuë, M. H., Barbosa, M. J., Olivieri, G. (2017). Pulsed Electric Field for protein release of the microalgae Chlorella vulgaris and Neochloris oleoabundans. *Algal Res. 24*:181–187.

Lapidot, T., Harel, S., and Akiri, B. 1999. PH-dependent forms of red wine anthocyanins as antioxidants. *J. Agric. Food Chem.* 47(1):67–70.

Lau, A.K 2006. Evaporators. *Encyclopedia Food Sci. Technol.*, 2: 751–765.

Lebovka, N.I., Bazhal, M.I., and Vorobiev, E. 2001. Pulsed electric field breakage of cellular tissues: Visualization of percolative properties. *Innov. Food Sci. Emerg. Technol*. 2: 113-125.

Lee, P.Y., Tangh, N.J., and Leong, S.Y. 2006. Pulsed electric field processing and its consumer opportunities. *Technol. sci.* 65-72.

Lopez, N., Puertolas, E., Condon, S., Alvarez, I., and Raso, J. 2007. Effects of pulsed electric fields on the extraction of phenolic compounds during the fermentation of must of Tempranillo grapes. *Innovative Food Sci and Emer Technol. 9*(4): 477–482.

Lubicki, P.,andJayaram, S., 1997,High voltage pulse application for the destruction of the Gram-negative bacterium Yersinia enterocolitica, Bioelectrochem. Bioenerg. 43: 135-141.

Maran, J.P., Sivakumar, V., Thirugnanasambandham, K., and Sridhar, R. 2015. Extraction of natural anthocyanin and colors from pulp of jamun fruit. *J. Food Sci. Technol*. 52(6): 3617–3626.

Martín, J., Navas, M.J., Moreno, A.M.J and Asuero, A.G. 2017. Phenolic Compounds - Importance and Applications. *The Open Food Sci. J.* 7-22.

Martinez, M., and Sowbhagya, H. B. 2016. Technological advances in food colours. *Chem. Industry Digest*. 79-88.

Montgomery, D.C. 2001. Design and Analysis of Experiments. *New York Wiley*. 416-419.

Mustafa, L.F., Girty, M.J., and Haberlson ,J.F. 2004.Extraction, evolution and sensory impact of phenolic compounds during red wine maceration. *Ann. Rev. Food Sci. Technol*. 5: 83–109

Myers, R.H. 2009. Response surface methodology. *Ann Arbor, Edwards Brothers Inc.*

Omowaye, B. I. 0., Eshtiaghi, N., and Knorr, D. 2001. Impact of high intensity electric field pulses on cell permeabilisation and as pre-processing step in coconut processing, *lnnov. Food Sci. Emerg. Technol.* 1(3): 203-209.

Ovando, A.C., Nyati, M., and Hernandez, G.H. 2009. Chemical studies of anthocyanins: A review. *Food chem.*859-871.

Parniakov, O., Libovka, N and Hecke, E.V. 2014.Pulsed electric field assisted pressure extraction ad solvent extraction from mushroom (*Agaricus biosporus*). *Microbio. J.*72: 54-59.

Pereira, B.L., Guglielmetti, A., and Zeppa, G. 2018. Pulsed electric field assisted extraction of bioactive compounds from cocoa bean shell and coffee silverskin. *Food Bioprocess Technol*. 11(4): 818-835.

Praporscic, I., Shynkaryk, M., Lebovka, N.I., and Vorobiev, E. 2006. Analysis of juice colour and dry matter content during pulsed electric field enhanced expression of soft plant tissues. *J. Food Eng.* 79(2): 662-670.

Puertolas, E., Saldana, G., Alvarez, I., & Raso, J. 2013. Experimental design approach for the evaluation of rose wines obtained by pulsed electric fields. Influence of temperature and time of maceration. *Food Chem. 126*. 1482–1487.

Putranto, A.W., Argo, B.D., and Wijana, S. 2014. Green pulsed electric field-assisted extraction method of total carotenoid carrot pulp using olive oil as solvent. *Indonesian Green Technol. J.* 3(1): 233-248.

Qin,B.L., Barbosa-Canovas,G.v., Swanson,B., Pedrow,P.D., and Olsen,R.G., 2000.Inactivating microorganisms using a pulsed electric field continuous treatments system. *IEEE Trans. Ind. Appl.* 34: 43-50.

Rajbhar, H., Cruz, C.D., Porantes, R.M. 2014. Antioxidant and antiproliferative activity of blue corn and tortilla from native maize. *Chemi. Central J*. 110: 11-14.

Raman, B., Petroyi, J.K., and Kumaer, U. 1994. Exponential and square wave pulses. *J. Electrical Engg*. 4: 56-72.

Ramteke, V., Kurrey, V., and Kar, S. 2015. Jamun: A traditional fruit and medicine. *Pop. Kheti* 3(3): 4–9.

Rastogi, N., Eshtiaghi, M., and Knorr, D., 2000,Accelerated mass transfer during osmotic dehydration of high intensity electricfieldpulse pre-treatedcarrots,1. Food Sci. 64: 1020-1023.

Sagravat, H., Mann, A., and Kharya, M. 2006. Pharmacological potential of eugenia jamuna: A Review. *Pharm J*. 96-104.

Sale, W., and Hamilton, J., 1967, Effects of high electric fields on microorganisms, Biochem. Biophys. Acta 148:789-800.

Shipp, J and Abdel, E.M. 2010. Bentham Food Applications and Physiological Effects of Anthocyanins as Functional Food Ingredients. *J.Food Sci*. 53-59.

Shungiyama, P.O ., Gutysert, K.M and Fridenst, K. 1977. Effect of Pulsed electric field treatment on blue tortillas and corns. *J. Biosci*. 16: 34-39.

Srivastava, Y., Venkatakrishna-Bhatt, H., and Gupta, O.P. 1983. Hypoglycemia induced by *Syzygium cumini* linn seeds in diabetes mellitus. *Asian Medical J*. 26(7): 489-493.

Teusdea, C., Bandici, L., Kordiaka, R and Vicas , S. 2017. The effect of different pulsed electric field treatments on producing high quality wines. *Notulae Bota.Horti*.540-547.

Toepfl,S.,Heinz,V.,andKnorr,D.2005.Overviewofpulsedelectricfieldprocessingforfood,In:Emerging Technologies for Food Processing (D.-W.Sun,ed.), Elsevier,Oxford,pp.67-97.

Vanhoey, A. B. v., and Hendrickx, M., 2002, Effects of high electric pulses on enzymes, Trends FoodSci. Technol. 12: 94-102.

Wang, H., Cao, G., and Prior, R.L. 1997. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* 45(2): 304-309.

Yeom, H. w., Zhang, Q. H., and Chism, G. W., 2002, Inactivation of pectin methyl esterase in orangejuicebypulsed electric fields, 1. FoodSci. 67: 2154-2159.

Zhang, Q., Canovas, B., and Swanson, B.G. 1995, Engineering aspects of pulsed electricfield pasteurization. *J.Food Eng*. 25(2): 261-281.

Zhang H, Tsao R. 2005. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr. Opin. Food Sci.; 8:33–42.

Zhou, W., Katiyo, W and Yang, R. 2015. Optimisation of combined pulsed electric field and mild temperature processing conditions for mushroom polyphenol oxidase inactivation. *Adv. J. Food sci. technol*. 638-646.

Zimmermann, U., and Benz, R. 1980. Dependence of the electrical breakdown voltage on the charging time in *Valonia utricularis*. *L Membr.Bio*. 53: 33-43

Zimmerman, U., Pilwart, G., and Riemann, F. 1974. Dielectric breakdown in cell membrane. *Biophys J.* 14: 881-889.

Zimmermann,U.,1986,Electricalbreakdown,electropermeabilization and electro fusion. *Rev. Physiol. Biochem.Pharmacal*. 105: 176-257.

APPENDIX 1

Table 1: ANOVA for anthocyanin yield

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	48.93	9	5.44	13.09	0.0013	Significant
A: Electric field strength	0.90	1	0.90	2.16	0.1850	
B: Pulse frequecy	7.96	1	7.96	19.16	0.0032	
C: Treatment time	0.12	1	0.12	0.30	0.6004	
AB	1.78	1	1.78	4.29	0.771	
AC	6.250E-004	1	6.250E-004	1.505E-003	0.9701	
BC	0.60	1	0.60	1.45	0.2683	
A^2	36.54	1	36.54	87.95	<0.001	
B^2	0.29	1	0.29	0.69	0.4338	
C^2	0.031	1	0.031	0.075	0.7927	
Residual	2.91	7	0.42	-	-	
Lack of fit	2.16	3	0.72	3.83	0.1139	**Not significant**
Pure error	0.75	4	0.19	-	-	
Cor total	51.84	16				

R^2 - 0.9439 Adj R^2 - 0.871

Adeq Precision-11.531

APPENDIX 2

Table 2: ANOVA for antioxidant activity

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	2088.94	9	231.88	3.18	0.0709	Significant
A: Electric field strength	25.20	1	25.5	0.35	0.5753	
B: Pulse frequecy	453	1	453	6.20	0.0416	
C: Treatment time	0.13	1	0.13	1.712E -003	0.9682	
AB	0.09	1	0.09	1.232E -003	0.9730	
AC	0.36	1	0.36	4.930E -003	0.9460	
BC	13.69	1	13.69	0.19	0.6781	
A^2	1514.41	1	1514.41	20.74	0.0026	
B^2	14.18	1	14.18	0.19	0.6728	
C^2	46.27	1	46.27	0.63	0.4522	
Residual	511.16	7	73.02	-	-	
Lack of fit	362.14	3	120.71	3.24	0.1429	**Not significant**
Pure error	149.03	4	37.26			
Cor total	2598.1	16				

R-Squared - 0.8033 Adj R-Squared-0.5503

Adeq Precision-5.233

APPENDIX 3

Table 3: ANOVA for L value of colour

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	10.70	9	1.19	60.87	<0.001	Significant
A: Electric field strength	0.036	1	0.036	1.87	0.2141	
B: Pulse frequecy	0.29	1	0.29	14.98	0.0061	
C: Treatment time	0.21	1	0.21	1.08	0.3341	
AB	0.23	1	0.23	11.80	0.010	
AC	2.50E-003	1	2.50E-003	0.13	0.731	
BC	9.025E-003	1	9.025E-003	0.46	0.5185	
A^2	7.61	1	7.61	389.62	<0.001	
B^2	1.48	1	1.48	75.89	0.001	
C^2	0.031	1	0.031	58.75	0.001	
Residual	*0.*14	7	0.02			
Lack of fit	0.11	3	0.038	6.55	0.0505	**Not significant**
Pure error	0.023	4	0.19			
Cor total	10.83	16				

R-Squared – 0.987 Adj R-Squared-0.9712

Adeq Precision-21.38

APPENDIX 4

Table 4: ANOVA for a*value

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	3.57	9	0.40	13.09	0.0013	Significant
A: Electric field strength	0.17	1	0.17	2.16	0.1850	
B: Pulse frequecy	0.30	1	0.30	19.16	0.0032	
C: Treatment time	0.12	1	0.12	0.30	0.6004	
AB	0.024	1	0.024	4.29	0.771	
AC	0.032	1	0.032	1.505E -0.03	0.9701	
BC	8.10E-0.03	1	8.10E-0.03	1.45	0.2683	
A^2	2.86	1	2.86	87.95	0.001	
B^2	0.051	1	0.051	0.69	0.4338	
C^2	0.014	1	0.014	0.075	0.7927	
Residual	0.021	7	2.95E-0.003	-	-	
Lack of fit	0.015	3	0.015	3.83	0.1139	**Not significant**
Pure error	5.4E-0.003	4	5.4E-0.003	3.69	0.1197	
Cor total	51.84	16				

R^2 - 0.9943 Adj R^2 - 0.9301

Adeq Precision-37.054

APPENDIX 5

Table 5: ANOVA for b* value

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	32.45	9	32.45	13.09	0.0012	Significant
A: Electric field strength	0.19	1	0.19	2.16	0.150	
B: Pulse frequecy	0.32	1	0.32	19.16	0.0032	
C: Treatment time	0.22	1	0.22	0.30	0.005	
AB	0.029	1	0.029	4.29	0.771	
AC	0.029	1	0.029	1.505E -0.03	0.9701	
BC	6.10E-0.03	1	6.10E-0.03	1.45	0.2683	
A^2	3.86	1	3.86	87.95	0.001	
B^2	0.051	1	0.051	0.69	0.4288	
C^2	0.015	1	0.015	0.075	0.7927	
Residual	0.021	7	3.55E-0.003	-	-	
Lack of fit	0.015	3	0.015	3.83	0.1139	**Not significant**
Pure error	6.5E-0.003	4	6.5E-0.003	3.69	0.1148	
Cor total	41.84	16				

R^2 - 0.9704 Adj R^2 - 0.9323

Adeq Precision-11.531

APPENDIX 6

Table 6: ANOVA for refractive index

Source	Sum of squares	df	Mean square	F value	p-value Prob>F	
Model	12.35	9	12.35	15.21	0.0012	Significant
A: Electric field strength	0.21	1	0.21	1.16	0.150	
B: Pulse frequecy	0.24	1	0.24	15.16	0.0032	
C: Treatment time	0.22	1	0.22	0.25	0.005	
AB	1.505E -0.03	1	1.505E -0.03	3.25	0.771	
AC	0.031	1	0.029	0.031	0.9701	
BC	0.013	1	0.013	1.45	0.223	
A^2	3.86	1	3.86	87.95	0.001	
B^2	0.045	1	0.045	0.69	0.4288	
C^2	6.10E-0.03	1	6.10E-0.03	0.075	0.7927	
Residual	0.021	7	3.55E-0.003	-	-	
Lack of fit	0.015	3	0.015	3.83	0.1231	**Not significant**
Pure error	6.5E-0.002	4	6.5E-0.002	3.9	0.1214	
Cor total	26.54	16				

R^2 - 0.944 Adj R^2 - 0.8719

Adeq Precision-14.32