Ultrasound-Assisted Extraction of Bioactives from Horticultural Produces and their Encapsulation with Ion Gelation Method

Thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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December, 2022

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Dedicated

to

my parents and my family members

Department of Chemical Engineering
Indian Institute of Technology Guwahati
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CERTIFICATE

It is certified that the work contained in this thesis entitled **"Ultrasound-Assisted Extraction of Bioactives from Horticultural Produces and their Encapsulation with Ion Gelation Method"** submitted by **Mr. Khalid Mehmood Wani** for the award of the degree of Doctor of Philosophy has been carried out in the Department of Chemical Engineering, Indian Institute of Technology Guwahati under my supervision. To the best of my knowledge, the Ph.D. thesis work has not been submitted elsewhere for the award of a Degree or Diploma.

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DISCLAIMER

The experimental, characterization and optimal data presented in the Ph.D. thesis was performed by me and were reported after due verification. To the best of my knowledge, the work summarized in this Ph.D. thesis has not been submitted elsewhere for the award of a Degree or Diploma.

(Mr. Khalid Mehmood Wani)

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Abstract

The globalization of the food sector has resulted in a significant increase in demand for functional foods, value addition, and agricultural waste reutilization. Bioactive substances from fruits and vegetables (leafy and non-leafy sources) that act as free radical scavengers have recently piqued researcher's curiosity. Bioactives are ubiquitous substances that are found in all plants and belong to a varied group of secondary metabolites. These phytochemicals have been demonstrated to have strong antioxidant capabilities, allowing them to protect the human body from various health issues such as cancer, inflammation, critical bacterial, hypoglycaemic effects etc.,

Till date, various extraction and purification processes have been deployed to obtain liquid extraction of bioactive constituents from plants and their by-products. Traditional techniques such as maceration, hydro-distillation, soxhlet extraction, and hot water extraction have been deployed to extract valuable bioactive compounds from plant sources. These techniques suffer with the basic limitations of long extraction time, use of expensive and toxic solvents and degradation of heat sensitive compounds. On the other hand, the application of ultrasound assisted extraction (UAE) has been mostly explored in food and pharmaceutical processing schemes for the needful extraction of bioactive components. This is due to its simplicity, lower cost, lower processing time, lower solvent requirements, effective extraction efficiency, lower energy, higher reproducibility, scalability, and environmentally friendly nature.

Despite being a host of all above mentioned nutritional benefits, the bioactive compounds suffer with few limitations. These include sensitivity towards heat and light, quicker degradation due to processing, gastrointestinal and other environmental conditions, low water solubility, and a high metabolic rate in free form that fosters their easy excretion from the human body. Therefore, it is necessary to stabilize these compounds in order to facilitate their storage, processing, and applications. The incorporation of bioactive extracts into polymer matrices is often targeted for their stability and bioavailability. Among various available methods, encapsulation is an outstanding technique for improved bioactive constituent delivery

systems through a prolonged or controlled release of the bioactives. Henceforth, sustainable bioactivity can be achieved along with their stability.

The objective of this study was twofold. Firstly, it involved the identification of most effective of leafy and non-leafy sources that ensure abundance of bioactives such as TPC for their aqueous extract. Such an extraction does not on its own facilitate its customization into food products prepared with processes such baking and air frying. Hence, encapsulation of bioactives with an inexpensive method shall be targeted so as to realize possible scope for practical applications. Thus, the secondary directive was to encapsulate the TPC containing extract using ion gelation method. As highlighted in the thesis, alginate has few drawbacks as a capsule wall material. Therefore, in this study, pectin extracted from pomelo peel was used as a supportive wall material to encapsulate the TPC extracted from papaya leaves. The conducted study with papaya leaf extract and sodium alginate-pectin base wall material and ion gelation based encapsulation can serve as a model study for the needful exploration of other relevant research frontiers.

Considering these issues, the Ph.D. thesis devotes towards the fulfilment of five major objectives. Among these, the first three objectives refer to aqueous ultrasound-assisted extraction (normal UAE (NUAE) and pulsed UAE (PUAE)) and hot water extraction (HWE) of bioactives from various leafy and non-leafy sources of North-East India (guava leaves, papaya leaves, raw papaya peel (PPE) and pulp(PPU)). The fourth objective involved citric acid based (CA) aqueous NUAE, PUAE and acidic hot water extraction (AHWE) for the pectin extraction from pomelo peel. Finally, the fifth objective targeted ion gelation based encapsulation of bioactives extracted into the aqueous phase from the papaya leaf system and with calcium alginate (Ca-Al) pectin beads. For the first three objectives, the design of experiments approach has been followed to address the optimality of extraction temperature (ET), sonication time (ST) and solid to liquid ratio (Loading ratio or LR). Thereby, the maximization of total polyphenolic content (TPC), total flavonoid content (TFC), Vitamin C (VITC) and antioxidant activity (AA) were targeted. Similarly, in the fourth objective, the solution pH and ST were varied for fixed choice of LR and ET, and the maximization of pectin yield (PY) and degree of esterification (DE) were targeted. Consequently, the pectin product obtained with best process condition was subjected to various characterization such as

proximate analysis (moisture, fat and ash), nutritional (TPC, TFC and AA), functional (pectin solubility, water holding capacity (WHC), oil holding capacity (OHC) and emulsification properties), functional group (FTIR), morphological (FESEM), thermal (DSC and TGA) and structural characterization (XRD). The last objective of the thesis targeted the optimality of sodium alginate concentration, calcium chloride concentration and flow rate of syringe pump for the maximization of encapsulation efficiency of aqueous papaya leaf extract for a fixed choice of pectin concentration and needle size. Consequently, the optimally encapsulated system was subjected to characterization studies such as nutritional, (antioxidant activity and moisture), functional group (FTIR), morphological (FESEM), and thermal characterization (DSC) and bead size and storage stability analysis. Finally, the last objective also addressed the release kinetics of total phenols extracted with water from papaya leaf extract encapsulated Ca-Al pectin beads.

With the adopted research methodology, the PhD thesis involved studies with wider sets of independent degrees of freedom for the realization of useful insights into the response variables. In the first objective, the ST, ET and LR have been varied from 5 – 20 min, 40 – 70 °C and 0.02 – 0.2 g/mL, respectively and with the face centered design (FCD) based response surface methodology (RSM) design model. Thereby, the responses have been evaluated to alter as 15.5 – 68.8 mg GAE/g TPC, 50.1 – 80.3 % AA, 5.7 – 25.7 mg/100g VITC and 44.7 – 289.77 mg QE/g TFC for NUAE and 19.49 – 72.62 mg GAE/g TPC, 30.08 – 86.07 % AA, 6.15 – 18.83 mg/100g VITC and 28.08 – 288.13 mg QE/g TFC for PUAE.

In the second objective, the ET, ST and LR have been altered from 5 – 20 min, 40 – 70 °C and 0.02 – 0.2 g/mL respectively and with the FCD-RSM design model. Thereby, the response for the processes have been evaluated. For the NUAE, the TPC, TFC and % AA varied from 32.83 – 148.15 mg GAE/g , 47.9 – 531.5 mg QE/g and 18.65 – 86.3% respectively. For the PUAE, these varied as 52.92 – 158.88 mg GAE/g, 56.18 – 547.41 mg QE/g and 26.82 – 89.24 % respectively.

In the third objective, the ET, ST and LR have been altered from 5 – 20 min, 40 – 70 °C and 0.2 – 0.5 g/mL respectively and with the FCD-RSM design model. Thereby, the response for the processes have been evaluated as 53.35 - 145.21 mg GAE/g TPC, 16.44 - 139.5 mg/100g VITC, and 35.25 - 85.83 AA %

for NUAE of PPU, For the NUAE of PPE and with similar variation of process parameters, the responses altered as 53.68 - 126.6 mg GAE/g sample, 70.67 - 302 mg QE/g, and 47.67 - 80.36 % AA. Likewise, the responses for the PUAE of PPU varied as 80.87–169.16 mg GAE/g TPC, 31.29 – 92.9 % AA, and 28.52–164.94 mg/100 VC and as 97.46 – 145.44 GAE/g TPC, 97.36–292.2 mg QE/g TFC, and 46.51–89.17% AA for the PUAE of PPE.

In the fourth objective, the ST and pH have been varied from 1.5 – 3.5 and 5-30 min respectively and with the FCD – RSM design model. Thereby, the responses have been evaluated as 3.0 – 35.3 % PY and 40.73 – 84.9 % DE for NUAE. For PUAE, the corresponding responses varied as 5.5 – 40.41 % and 47.6 – 88.6 % respectively. In the fifth objective, sodium alginate concentration, $CaCl_2$ concentration and flow rate have been varied from 1 – 3 % w/v, 3 – 9 % w/v and 0.5 – 3.5 mL/min, respectively. Thereby, the best trend of encapsulation efficiency altered as 32.52 – 85.6 % for the Ca-Al pectin beads.

From the thesis findings, the following have been achieved. Firstly, the comparative and best efficiency of NUAE, PUAE and HWE has been established for the aqueous extraction of guava leaves, papaya leaves, raw papaya pulp and peel. Thereby, the optimal combinations of process conditions and response variables have been identified. Secondly, the morphological, thermal, nutritional and functional characteristics of PUAE based pectin product have been determined to quantify upon the confidence levels of the produced pectin product. Thirdly, the efficacy of the ion gelation method and its process parameters has been assessed. Fourthly, the possible scope for bioactives encapsulated Ca-Al pectin bead system towards functional food application has been delineated from the release kinetics data.

In summary, the PhD thesis provided useful insights towards the promising performance of NUAE and PUAE processes for the production of aqueous bioactives extract from leafy and non-leafy sources of North-East India, affordable pectin product generation from pomelo peel and successful encapsulation of aqueous bio-extracts extract in the ion gelation based Ca-Al and pectin beads system. All investigated processes namely PUAE, NUAE, HWE, AHWE and ion gelation are simple to envisage upon and are susceptible for cost effective scale up. The reported findings in the thesis are expected to

further enhance the application of mentioned technologies for bioactives extract production and their encapsulation for various food and medicinal applications. Thereby, the thesis can provide useful guidelines for the cost effective processing and development of bioactives and pectin based functional foods.

Novelty Statement

The Ph.D. thesis involved the realization of five major objectives. Among these, the first three objectives refer to aqueous ultrasound-assisted extraction (normal UAE (NUAE) and pulsed UAE (PUAE)) and hot water extraction (HWE) of bioactives from various leafy and non-leafy sources of North-East India (guava leaves, papaya leaves, raw papaya peel (PPE) and pulp(PPU)). The fourth objective involved citric acid based (CA) aqueous NUAE, PUAE and acidic hot water extraction (AHWE) for the pectin extraction from pomelo peel. Finally, the fifth objective targeted ion gelation based encapsulation of bioactives extracted into the aqueous phase from the papaya leaf system and with calcium alginate (Ca-Al) pectin beads. Specific novelties of the thesis will be briefly presented in the following paragraphs.

The first three objectives involved following novelties: (a) criticality of LR which was not addressed in the prior art (b) degree of non-linear influence of any two process parameters on all measured responses and their rank and (c) sensitivity of the PUAE in conjunction with the NUAE and (d) comparative performance of HWE for aqueous bioactives extraction. Among these the data from HWE performance studies can provide useful guidelines for agro-rural product development.

The fourth objective involved the following novelties: (a) criticality of CA based extraction and the role of pH and ST (b) RSM based optimization of NUAE and PUAE processes (c) comparative pectin extraction efficiency for alternate acids and (d) pectin product characteristics with PUAE and AHWE processes.

The final objective involved the following novelties: (a) RSM customized optimization of ion gelation based aqueous papaya extract encapsulation in Ca-Al pectin beads (b) comparative assessment of various properties of bioactives encapsulated Ca-Al and Ca-Al pectin beads.

However, the critical novelty of the conducted research was not only based on the RSM but upon the effectiveness of bioactives aqueous extraction from mentioned plant sources with the UAE. Such quantification requires the sensitive identification of best process parametric and response characteristics. The RSM may not be reliable for cases that are two complex to represent mathematically and for systems

without statistical correlation. Hence, the conducted research cannot be considered as an absolute framework to infer upon the indicated inferences and conclusions.

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Nomenclature

Abbreviations

MT	Million tonnes
UAE	Ultrasound-assisted Extraction
MPa	Mega Pascal
FW	Fresh Weight
NE	North-East
OHC	Oil holding capacity
WHC	Water holding capacity
NUAE	Normal UAE
PUAE	Pulsed UAE
CA	Citric acid
SWE	Sub-critical water extraction
DES	Deep eutectic solvent
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid
G	Gauge
MPE	Mulberry pomace extract
Ca-Al	Calcium alginate
TE	Trolox equivalent
TPC	Total Polyphenolic content
TFC	Total Flavonoids content
R^2	Coefficient of determination
GAE	Gallic acid equivalents
QE	Quercetin equivalents

CE	Catechin equivalents
PPU	Papaya pulp
PPE	Papaya peel
FCD	Face Centered Design
PLE	Papaya leaf extract
PL	Papaya leaf
ASE	Aescin equivalents
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DCPIP	2, 6-dichlorophenol indophenol sodium salt
DW	Dry weight
AOAC	Association of Official Analytical Chemists
MR	Moisture ratio
HHPAE	High hydrostatic pressure assisted extraction
HWE	Hot water extraction
AHWE	Acidic HWE
HSME	Hot solvent microwave extraction
HPLC	High-performance liquid chromatography
MS	Mass spectrometry
MW	Molecular weight
AE	Agitation extraction
UMAE	Ultrasound-microwave assisted extraction
CCD	Central Composite Design
CME	Conventional maceration extraction
MAE	Microwave assisted extraction
RSM	Response Surface Methodology

ANOVA	Analysis of Variance
C.V.	Coefficient of Variation
ET	Extraction temperature
LR	Loading ratio
DI	Distilled water
ST	Sonication time
FCR	Folin ciocalteu reagent
BSA	Bovine Serum Albumin
kDa	Kilo-dalton
DOE	Design of Experiments
DE	Degree of Esterification
NHB	National Horticulture Board
PS	Pectin Solubility
ES	Emulsion stability
EC	Emulsion capacity
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared spectroscopy
FDA	Food and drug administration
HCl	Hydrochloric acid
GE	General Electric
XRD	X-ray diffraction
HNO_3	Nitric acid
$CaCl_2$	Calcium chloride
FESEM	Field Emission Scanning Electron Microscopy
TGA	Thermogravimetric Analyzer

PY	Pectin Yield
AA	Antioxidant Activity
EE	Encapsulation efficiency
VITC	Vitamin C
BBD	Box-Behnken design
EALB	Non-encapsulated Ca-Al beads
ALPOW	Alginate powder
EALPB	Non-encapsulated Calcium alginate-pectin beads
EP	Extracted pectin
ALEXPB	Calcium alginate pectin beads with PL extract
ALEXB	Calcium alginate beads with PL extract

Nomenclature

Y	Desired response variable
X_i and X_j	Independent variables ($i \neq j$).
β_0, β_i, β_{ii}, and β_{ij}	Coefficients of intercept and terms of linear, squared, and their interaction in the regression,
B_1 beads	Beads stored with PL extract
B_0 beads	Beads stored without PL extract
B_{1REF} and B_{0REF}	Beads samples stored at refrigerated conditions with and without PL extract, respectively
B_{1RT} and B_{0RT}	Beads samples stored at room temperature conditions with and without PL extract, respectively
A_c and A_s	Absorbance of control and sample respectively

Notations

W_d	Weight of the sample obtained
W_s	Weight of dried sample
W_a	Weight of ash (g)
W_f	Weight of fat (g)
A_s, A_{std}	Absorbance of the diluted and blank samples respectively
V_1, V_2	Volume of ascorbic acid and sample extract consumed (mL)
A_c	Absorbance of control sample
k_0, k_H, k_P, k_d, k_r, n, m	Release kinetics model parameters
E_C	Electric consumption (kW h)
P	Power consumption (W)
E_{CO2}	CO_2 emission (kg)
M_0 *and* M	Weights of extracted dried pectin and dried pomelo peel powder respectively.
V_1 *and* V_2	Volumes of NaOH consumed after first and second titration step
V_R *and* V_I	Remained emulsion layer and initial volume of the system respectively
V_F *and* V_I	Emulsion layer and total volume of the system respectively
T_e *and* T_i	Encapsulated TPC in beads and PLE respectively
M_t *and* M_∞	Mass fraction of polyphenols released at time t, and infinite time ($t = \infty$) respectively.
k_0, k_H *and* k_p	Zero-order release, Higuchi rate and Korsmeyer kinetic constants
k_d *and* k_r	Fickian and relaxation kinetic constants respectively
n *and* m	Release exponent and Fickian diffusion exponent respectively

Chapter 1:

Introduction and Literature Review

Chapter 1

Introduction and Literature Review

Background and Orientation

In this chapter, section 1.1 presents a brief overview of the bio-resources of North East (NE) India, followed with the functional utility of certain portions of few underutilized leafy and non-leafy vegetable sources of NE India. Thereafter, the section focuses upon alternate extraction techniques being adopted for bioactives extraction from underutilized leafy and non-leafy vegetable sources. Finally, the section addresses the response surface methodology (RSM) based ultrasound-assisted extraction (UAE) of bioactives from leafy and non-leafy sources and encapsulation of bioactives.

Section 1.2 elaborates upon the relevant prior-art. This section focusses upon two perspectives. Among these, the first refers to the literature findings associated to UAE of the bioactives from leafy and non-leafy sources and optimization of process parameters using RSM. The second perspective in the prior-art has been devoted towards the encapsulation of the extracted bioactives using ion gelation method and optimization of the process parameters for maximum encapsulation efficiency. Subsequently, section 1.3 of the thesis outlines the relevant gaps in the chosen domains of study and elaborates on the potential scope for future research. Thereby, the thesis objectives being listed in section 1.4 corroborate with the broader subjective gaps based on the relevant prior art. Finally, the thesis overview and organization have been addressed in section 1.5 of the chapter.

1.1 Preamble

1.1.1 Bio-resources of NE India

With its rich and variegated soil, climate and topography, the North-East (NE) India is well known as a bio-diversity hot spot of the country and for this reason, the region hosts to many leafy and non-leafy horticultural bio-resources that contribute profusely to the human nutrition. In India, the NE region

produces 6.79% of total horticultural produces of which vegetables alone contribute about 51.83% ((Data source: NHB State Department report of 2010-11). Due to lack of appropriate know how, of preservation, processing and value addition, a large proportion of such resources remain underutilized. During 2014 – 15, the horticulture sector growth rate rose from 6.6% (2013-2014) to 7.6% in Assam State (Data source: Ministry of Agriculture and Farmers Welfare).

1.1.2 Overview of Some Underutilized Leafy and Non-Leafy Sources of NE India

Among numerous underutilized leafy and non-leafy vegetable sources, the primary attention of the thesis has been upon few resources being extensively cultivated in the region. These are the notable horticultural produces such as guava, papaya and pomelo fruit. Horticultural produces such as guava leaves, papaya leaves, papaya and pomelo fruit are rich in the constitution of bioactive components such as polyphenols, flavonoids and antioxidants. These have been well documented for their diverse applications in medicinal (herbal supplements) and additive system for the treatment of dysentery, vertigo, skin disorders, jaundice, brain diseases, diabetes, antispasmodic, mellitus and sedative disorders due to their anti-inflammatory, anti-diarrheic, antihypertension, anti-obesity and other abilities) and further, they also serve as nutritional supplements.

1.1.3 Extraction Techniques for Plant-Derived Bioactive Compounds Extracted from Underutilized Sources of Leafy and Non-Leafy Sources

Till date many conventional techniques (soxhlet extraction, solid-liquid extraction and maceration) have been studied for the extraction of bioactives compounds from various fruits, vegetables and other plant portions (Yin et al., 2018). However, these methods suffer with the basic limitations of reduced yield, higher cost, prolonged sonication time (ST), higher organic solvent consumption, and most critically the associated degradation of heat sensitive bioactive constituents due to prolonged heating periods (Zakaria et al., 2021). On the other hand, non-conventional techniques such as microwave assisted extraction [MAE], pulsed electric field extraction [PEFE], supercritical fluid extraction [SFE], ultrasound assisted extraction [UAE] etc., have been widely employed in food and pharmaceutical industries for the recovery of bioactives (Kumar et al., 2020). Among these, UAE is gaining substantial

research interest in bioactives extraction due to its simplicity, lower cost, lower processing time, lower solvent requirements, effective extraction efficiency, lower energy, higher reproducibility, scalability, and environmentally friendly nature (Wen et al., 2018).

1.1.4 RSM based UAE of Bioactives from Leafy and Non-Leafy Sources

While conventional trial and error based experimental design approach has been simple, it is well known for its significant inventories of resources and time and does not customize towards a greater understanding into associated interaction between key and non- key process parameters (Li et al., 2019). Response surface methodology (RSM), on the other hand, is a more reliable method due to its consideration of the interactive influence of the diverse process parameters (linear, quadratic, and interaction impacts on response variables) and its promising ability to foster a set of optimum conditions through a reduced set of the experimental trials (Mohamed Ahmed et al., 2020). Till date, RSM based UAE of bioactives from plant sources has been addressed only in fewer investigations (Lara-Abia et al., 2022; Zeng et al., 2020; Li et al., 2019; Kong et al., 2015; Liu et al., 2014). Needless to mention, the prior art mostly relies on trial and error but not on RSM design-based experimental studies to infer upon the optimal process parametric and response data of the UAE of bio-resources for bioactives extraction. Hence, the deduced inferences at times do lack confidence and requires appropriate research methodology variations for the optimization of bioactive constituent properties associated to varied range of experimental data.

1.1.4.1 Principle of UAE

Ultra-sonication process propagates mechanical waves being transmitted through an elastic medium and through a set of cycles of compression (higher pressure) and rarefactions (lower pressures) (Mohammadpour et al., 2019). Primarily, UAE involves two phenomena namely cell wall diffusion and cell content rinsing after breaking the cell wall (Fig. 1.1). Involving sounds waves at a frequency above 20kHz, the UAE can foster the extraction and subsequent preservation and value addition of bioactives from plant bio-resources. Due to the reduction in extraction time, the UAE can reduce the exposure of the bioactive compounds to mentioned degradative factors. This results in the improved preservation of

the bioactive compounds due to their greater bioactivity. The temperature, power, pressure, frequency, and time are critical factors that alter UAE system performance (Chemat et al., 2017).

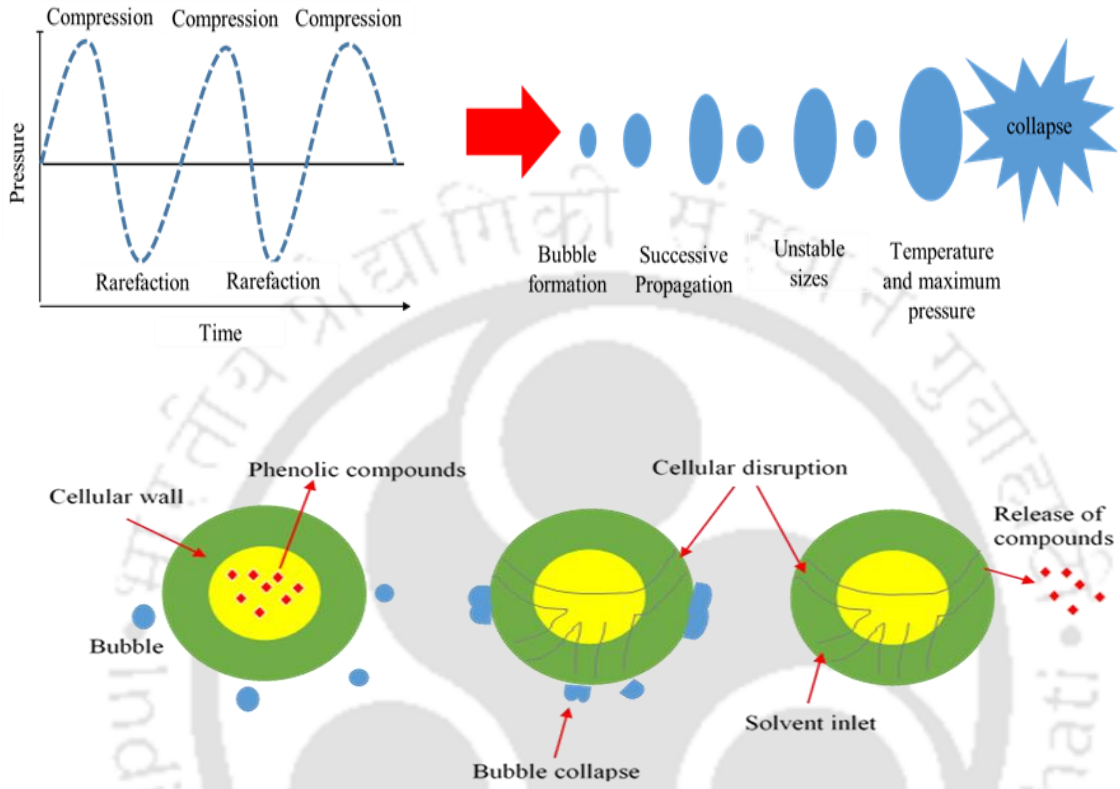

Figure 1.1: Principle of ultrasound assisted extraction.

1.1.4.2 Mechanism of UAE

The primary process mechanism of UAE (Fig. 1.2) is based on cavitation phenomena, which involves the formation, growth, and implosive collapse of microbubbles inside the liquid phase. The implosion of these microbubbles in ambient fluid system results in cell wall disruption and surface cleaning to subsequently foster a greater penetration of solvent into the sample matrix. Consequently, enhanced extraction rate of the targeted bioactive compounds can be achieved (Zeng et al., 2020). The propagation of ultrasonic waves customizes bubble oscillation and collapse and subsequent thermal, mechanical, and chemical effects (Kumar et al., 2021).

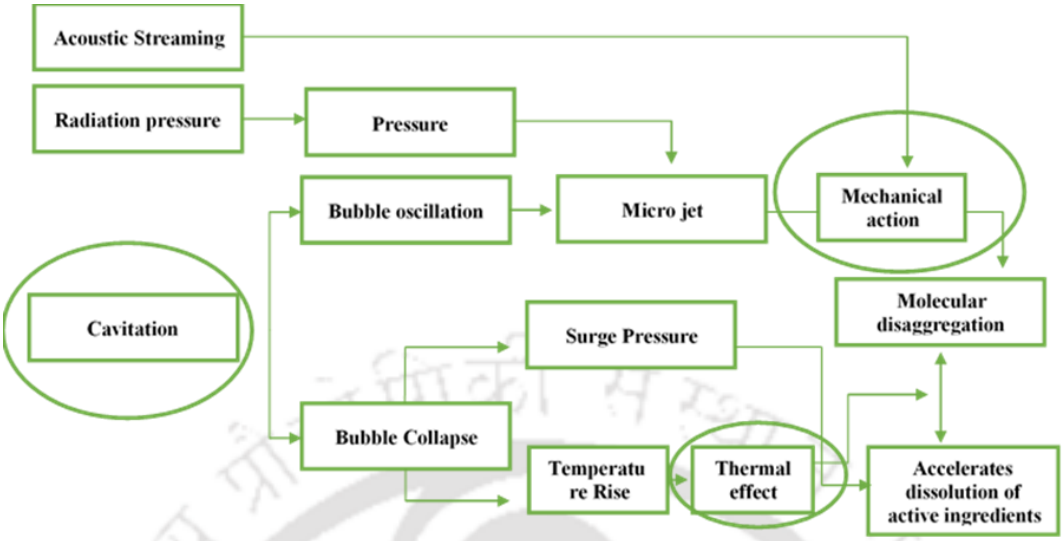

Figure 1.2: Mechanism of ultrasound-assisted extraction.

1.1.5 Need for Encapsulation of Bioactives Extracted using UAE from Leafy and Non-Leafy Sources

Extracted bioactives from plant resources are prone to undergo degradation due to various conditions such as influence of oxidative enzymes, higher temperature, pH, and presence of light and oxygen. Recently, encapsulation through extrusion technology has been successfully performed in order to increase the stability of polyphenols and enhance bioactive compounds preservation. The encapsulation technique can be applied to a wide variety of materials, including plant extracts as well as other materials such as drugs, enzymes, and nutrients. Commonly deployed techniques for encapsulation of bioactives include spray drying (Akbarbaglu et al., 2021), freeze drying and electrospinning (Machado et al., 2018), coacervation (de Souza et al., 2020), liposome entrapment (Mozafari et al., 2008), inclusion complexation (Shi et al., 2021), co-crystallization (Chezanoglou & Goula, 2021), nano-encapsulation (Rehman et al., 2019), yeast encapsulation (Young & Nitin, 2019), and emulsions (Lohith Kumar & Sarkar, 2018). These techniques require specialized equipment, higher processing temperature and as well as demand higher consumption of organic solvent. On the other hand, ionic gelation method has been increasingly used to encapsulate bioactive compounds. This is due to its

promising features such as lower cost of operation, operation at mild conditions, i.e., at room temperature, and elimination of organic solvents. The principle of ionic gelation refers to the formation of insoluble gels using anionic polymers such as alginate and pectin in a divalent ionic system (such as calcium) (Najafi-Soulari et al., 2016).

1.1.5.1 Alginate based Encapsulation of Bioactives

Alginate, is a naturally occurring anionic polymer and comprises (1,4)-linked -D-mannuronate (M) and L-guluronate (G) residues. The alginate obtained from brown algae has been extensively used encapsulant due to its biocompatibility, low cost, simplicity to use, chemical stability, lower toxicity, lower immunogenicity, availability, and ease of gelation in the presence of Ca^{2+} (Ćorković et al., 2021). Several limitations exist for the usage of alginate for the encapsulation of bioactive materials. Firstly, alginate hydrogels have poor mechanical strength. This leads to hydrogel rupture and subsequent release of the encapsulated bioactive material. This can be particularly challenging in applications that demand greater ability of the hydrogel to withstand mechanical stresses. These applications refer to tissue engineering or drug delivery. Secondly, alginate hydrogels exhibit poor stability and swelling behavior in physiological conditions. These in turn affect the long-term release and efficacy of the encapsulated bioactive material and thereby limits their utility in drug delivery applications. Thirdly, alginate hydrogels are susceptible for rapid degradation through enzymatic processes. Such degradation is prominent in the presence of calcium ions. This restricts their applications that demand their long term stability. This can be effectively mitigated through the appropriate blending of alginate with another polymer or with pectin. In this regard, several studies such as encapsulation of lycopene (Sampaio et al., 2019), mulberry pomace phenolics (Zhang et al., 2021), grape pomace phenolics (Romanini et al., 2021), lemon balm antioxidants (Najafi-Soulari et al., 2016), cocoa extract (Lupo et al., 2015), β-carotene (Rutz et al., 2016) etc., have been addressed to infer that the encapsulation of bioactives through ionic gelation method can be effectively targeted through a combination of alginate and pectin as a base wall material. Such a system ensured higher high encapsulation efficiencies and thereby offered good protection to bioactive constituents in adverse circumstances (Arriola et al., 2016).

1.1.6 Targeted Leafy and Non-Leafy Sources

Among various available leafy and non-leafy bioactive sources with pharmacological and nutritional properties, the thesis devotes towards few leafy and non-leafy sources. The basis of their selection has been upon their extensive cultivation, rich bioactive constitution and under-exploration. The sources targeted in this work are guava leaves, papaya leaves, papaya pulp and peel and pomelo fruit peel.

Guava leaves *(Psidii guajava folium*) are the dark green dry leaves and verdant shoots of the guava tree (Jiang et al., 2020). The leaves have been used in folk medication and have been documented to possess many bioactive compounds such as catechin, quercetin, gallic acid, vescalagin, hyperoside, peltatoside, guaijaverin and iso-quercetin (Liu et al., 2014). Guava leaf extract have been traditionally used for curing many diseases such as antispasmodic, mellitus, sedative, anti-inflammatory, anti-diarrheic, antihypertension, antiobesity, and antidiabetic properties (Jiang et al., 2020).

Papaya fruit (*Carica papaya Linn*.) is a member of the *Caricaceae* family and is widely cultivated in India. All parts of papaya plant (pulp, leaves and peel) contribute to various nutritional benefits as well as various pharmacological applications (Uribe et al., 2015). Unripe papaya pulp (PPU) is consumed for its agreeable flavor and nutritional as well as pharmacological applications such as laxative, antifertility agent, and meat tenderizer (Sharma et al., 2020).

Phytochemical analyses confirmed that young papaya leaves possess greater quantities of alkaloids, saponins, tannins, flavonoids, polyphenols, and glycosides. For this reason, they are well-known for their therapeutic properties such antibacterial, anti-inflammatory, dengue, antiviral, hypoglycaemic, hepatoprotective, anti-plasmodial, and antitumor characteristics (Heung et al., 2021).

Papaya peel (PPE) is one of the most common by-products of papaya processing, and accounts for about 12% of the total weight of the fruit (Jiang et al., 2022). Polyphenols, flavonoids, fibers, carbohydrates, proteins, fatty acids, tannins, and minerals are few value-enhanced bioactive substances that exist in the PPE (Dotto & Abihudi, 2021).

Pomelo fruit (*Citrus grandis (L.) Osbeck*) is the largest fruit among the citrus family. Its peel makes upto approximately 40% of its fruit weight (Methacanon et al., 2014). The peel of the fruits has been

analyzed to possess many useful bioactives such as cellulose, flavonoids, essential oil, pectin etc. Thereby, the peel has been considered as a useful resource for the production of extracts having extensive application in pharmaceutical, health, cosmetic, food, and feed industries (Liew et al., 2016).

1.2 Prior Art

The primary goal of the thesis is to target and address a mature research methodology and framework for the UAE of bioactive compounds from mentioned leafy and non-leafy vegetable resources of the North-east India. Thereby, five broader inter-connected areas of research have been targeted. These are:

a) Ultrasound assisted extraction of bioactives from guava leaves.

b) Ultrasound assisted extraction of bioactives from papaya leaves.

c) Ultrasound assisted extraction of bioactives from papaya pulp and papaya peel.

d) Ultrasound assisted extraction of pectin from pomelo fruit peel.

e) Encapsulation of papaya leaf extract using ion gelation method.

1.2.1 Ultrasound Assisted Extraction of Bioactives from Guava Leaves

Extraction is a vital process that ensures upon the needful release of vital components from the plant matrix into the extract media (Ciric et al., 2020). While hot water and soxhlet extraction are low cost technologies to customize leafy source extracts, they suffer with the basic limitation of reduced yield and degradation of heat sensitive bioactive constituents due to prolonged heating periods (Mohammadpour et al., 2019). On the other hand, the application UAE has been mostly explored in food and pharmaceutical processing schemes for the needful extraction of bioactive components. Till date, several studies have been conducted to provide useful insights in this area of research. A brief account of the available information in the literature have been presented in the following paragraphs.

The influence of different solvents (water, ethanol, methanol, and different concentrations of hydro-ethanolic solvents in the ratios of 70:30, 50:50, 30:70, and 10:90 (v/v)) on the extraction of total polyphenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) from guava leaves was addressed by Seo et al. (2014). Among various solvents, water indicated higher retentions

of extracted phenolic compounds (140 mg GAE/100g) from guava leaves in comparison with pure ethanol and methanol extracts. However, phenolic content being extracted using hydro-ethanolic solvent (50:50 % v/v) was higher (180mg GAE /100g) than that being extracted with water. Thereby, 50 % (v/v) hydro-ethanolic was inferred to be the most effective solvent due to its higher constitution of anti-oxidants. The authors also affirmed that the antioxidant ability of guava leaf extracts has a strong relationship with phenolic compound content but not flavonoid content (Seo et al., 2014).

Further, Liu et al. (2014) investigated guava leaf extraction using the normal UAE (NUAE) and reported that the TPC of the guava leaf extract varied from 4 – 26.1% for an appropriate variation in extraction temperature (ET) (45 – 70 °C) and sonication time (ST) (3.5 – 6 min) and solid to liquid ratio or loading ratio (LR) (9 – 15 g/mL). The author affirmed that the extracts possessing maximum TPC yield (26.12 %) and anti-hyperglycaemic activity (50.5 µg/mL and 34.6 µg/mL for α-amylase and α-glucosidase, respectively) can be obtained for the optimal parametric choice of 59.8°C ET, 5.1 min ST and LR of 12.1 g/mL.

To optimize the extraction of polysaccharide compounds from guava leaves, Kong et al. (2015) carried out UAE of guava leaves using RSM based Box behnken design (BBD) central composite design (CCD). The corresponding independent variables were ET (20 – 60 °C), ST (20 – 40 min) and power (200 – 350 W). Thereby, the authors inferred that the extracts possessing maximum polysaccharide yield (0.51 %) were achieved with optimal process conditions of 55°C ET, 30 min ST, and 240 W power.

Considering the UAE method for the aqueous extraction of flavonoids from *Psidium guajava* leaves using BBD based RSM, Li et al. (2019) inferred the optimal extraction parameters to obtain the highest total flavonoid yield were ultrasonic power of 407.41 W, ST of 35.15 min, and ET of 72.69 °C. For these conditions, the authors inferred that the average flavonoids extraction rate could reach 5.12 %.

The influence of NUAE process parameters such as ultrasonic power (250 – 450 W), ST (25 –45 min), and ET (50 –70 ºC) on the TPC yield from guava leaves was addressed by Zeng et al. (2020) using

RSM. Thereby, for a fixed choice of LR (0.025 g/mL), the authors reported that the optimal product and process characteristics as 59.82 mg GAE/g TPC, 38.38 min ST, 390.68 W power and 63.23 °C ET.

A critical summary of the prior art data related to the optimization of UAE process for bioactives extraction for the guava leaves – water system has been presented in **Table 1.2.**

Table 1.2: Literature data summary of bioactives extraction from guava leaves.

S.No	Author	Target	Parameters studied	Independent variables and their ranges	Dependent variables and their ranges	Optimal process-product characteristics
1	Li et al. (2019)	Extraction of Flavonoid	▪ ST, ET and power	▪ Power (W): 200 - 400 ▪ ST (min): 5 - 45 ▪ ET (℃): 20 - 100	▪ Extraction rate of flavonoids (%) : 4.16 -5.12	▪ Extraction power (W): 407.41 ▪ ST (min): 35.15 ▪ ET(°C): 72.69 ▪ Yield (%): 5.12
2	Zeng et al. (2020)	Polyphenol and Antioxidant activity	▪ ET, ET, Power, LR	▪ Power (W): 250 - 450 ▪ ST (min): 15 - 55 ▪ ET (°C): 20 - 100 ▪	▪ Polyphenols (mg GAE/g): 53.77-59.82 ▪ IC_{50} (µg/mL) : 4.12	▪ Extraction power (W): 390.68 ▪ ST (min): 38.38 ▪ ET(°C) :63.23
3	Liu et al. (2014)	Total phenols with anti-hyperglycemic activity	▪ ST, ET, LR	▪ ST (min): 2-10 ▪ ET(°C): 20 -100 ▪ LR (g/mL): 5-20	▪ Polyphenols (%): 18 - 26.12	▪ ET(°C): 59.8 ▪ ST (min): 5.1 ▪ LR (g/mL): 12.1 ▪ Yield (%) : 26.12

1.2.2 Ultrasound-Assisted Extraction of Bioactives from Papaya Leaves

Till date, little research has been conducted on the recovery of bioactive components from papaya leaf extracts using both conventional hot water extraction (HWE) and UAE methods in terms of process parametric and response optimization. A brief account of the available prior-art in this area of research has been outlined in the following paragraphs:

In a study, Vuong et al. (2013) considered fixed choice of higher ET (70 °C), moderate extraction time (20 min) and lower LR (7.5 %) during HWE and thereby evaluated the efficacy of alternate solvents such as acetone, ethanol, methanol and water. The authors inferred that highest levels of polyphenols (23.06 mg GAE/g) and flavonoids (17.07 mg CE /g) could be obtained with water and acetone, respectively but not the saponins, which were achieved as the best using with the ethanol solvent (82.88 mg Aes/g). Also, water as an extractant yielded maximum amount of antioxidants (166.66 µg TE/g) in comparison to other solvents.

In another study, Vuong et al. (2015) studied antioxidant and anticancer capacity of saponin-enriched papaya leaf extracts using either water or ethanol as an extracting solvent. Optimization was achieved using RSM. The authors considered combinatorial effects of temperature, time and water-to-leaf ratio on extraction efficiency. A Box–Behnken factorial design with three center points has been used for the experimental design. During their investigations, the authors varied temperature, time and water to leaf ratio from 70 – 90 °C, 5 – 25 min and 1:10 – 1:100 g/mL, respectively. Based on extensive investigation, the authors inferred that the optimal aqueous extraction conditions were 85 °C, 25 min. and a water-to- leaf ratio of 1:20 g/mL at which highest saponin content of 31.16 mg ASE/g was obtained. However, the author affirmed that 80 % (v/v) ethanol was more effective than water in extracting saponins from papaya leaf. Also, it was observed that both water and ethanol saponin – enriched extracts possessed similar antioxidant, free radical scavenging and ion-reducing capacity at concentrations ranging from 25 to 100 µg/ mL.

Addressing the extraction efficacy of UAE, room temperature reflux and agitation methods towards the preparation of methanol based papaya leaf extract, Soib et al. (2020) adopted fixed choice of process

parameters such as lower LR (7.5 %), and moderate ST (20 min). The authors investigated the extraction efficacy in terms of the phytochemical constitutions, antioxidant capacity and wound healing efficacy. Among all methods, the reflux based extraction provided highest antioxidant activity (IC_{50} of 0.236 mg/mL), and 85 % cell growth during active human skin fibroblast proliferations. Incidentally, the UAE achieved only 41% better cell growth and lower DPPH activity (IC_{50} of 0.377 mg/mL). The authors also addressed HPLC-MS based phytochemical characterization. However, HWE was not targeted and so was the optimization of the process parameters associated with either HWE or UAE or both.

Comparing the extraction efficiency of microwave assisted extraction (MAE), UAE and conventional maceration extraction (CME) using ethanol as a solvent, Abdel-Halim et al. (2021) targeted extraction of polyphenols, flavonoids and anti-hyperlipidemic activity of papaya leaves. To do so, the authors adopted fixed choice of process parameters such as higher LR (0.25 g/ml), moderate ST (20 min) and moderate ET (60 ^{o}C). The authors concluded that MAE (44.46 mg GAE /g DW, TPC and 14.40 mg QE /g DW, TFC) indicated the best results, followed by UAE (43.46 mg GAE /g DW, TPC and 13.40 mg QE/g DW, TFC and the CME (43.33 mg GAE/g DW, TPC and 13.28 mg QE/g DW, TFC). The HPLC study also affirmed highest level of polyphenols in the MAE (29.99 mg/g), followed by UAE (24.75 mg/g) and CME (22.64 mg/g).

Table 1.3 provides a brief summary of the available research pertaining to the optimization of UAE of bioactives from papaya leaf – solvent systems.

Table 1.3: Literature data summary of bioactives extraction from papaya leaves.

S.No	Author	Target	Parameters studied	Independent variables and their ranges	Dependent variables and their ranges	Optimal process-product characteristics
1.	Vuong et al. (2013)	Extraction of polyphenols, flavonoids and antioxidants	▪ Temperature, time, LR	▪ Temperature (°C): 50 -100 ▪ Time (min): 5 - 30 ▪ LR (g/mL):10:100)	▪ Polyphenols (mg GAE/g): 23.06 - 9.43 ▪ Flavonoids (mg CE/g): 6.44 - 11.96 ▪ Scavenging activity (µg TE/g):105.62-67.38 ▪ Total antioxidants (µg TE/g):166.66 -122.47	▪ Temperature (°C): 70 ▪ Time (min): 20 ▪ LR (g/mL): 7.5: 100 ▪ Polyphenols (mg GAE/g): 23.06 ▪ Flavonoids (mg CE/g): 6.44 ▪ Scavenging activity (µg TE/g):105.62 ▪ Total antioxidants (µg TE/g):166.66
2.	Vuong et al. (2015)	Antioxidant and anticancer capacity of saponin-enriched *Carica papaya* leaf extracts.	▪ Temperature, time LR	▪ Temperature(°C) :50-100 ▪ Time(min) :5-25 ▪ LR (g/mL): (1:10 -1:100)	▪ Extraction efficiency (mg ASE g-1): 25.50-31.62 ▪ Total antioxidant capacity (%) :5-15 ▪ DPPH free radical scavenging activity (%) :0-10	▪ Temperature(°C): 85 ▪ Time (min): 25 ▪ LR (g/mL): 1:20 ▪ Extraction efficiency ((mg ASE g^{-1}): 31.62 ▪ Total antioxidant capacity (%):15 ▪ DPPH free radical scavenging activity (%):10

1.2.3 Ultrasound-Assisted Extraction of Bioactives from Papaya Pulp and Papaya Peel

According to the reported literature, very few studies exist with the respect to the extraction of bioactive components from the papaya pulp (PPU) and papaya peel (PPE). For the extraction of bioactives from PPU/PPE mixture system, Uribe et al. (2015) targeted the effect of various extraction techniques such as high hydrostatic pressure extraction (HHPE), UAE, agitation extraction (AE) and their combination for the PPU/PPE mixture system. To conduct this study, the authors chose methanol (80 % v/v) as an extracting solvent for a fixed LR of 1:4 g/mL. The authors performed HHPE at 500 MPa for 10 min, UAE and AE for 30 minutes, and combined HHPE-UAE and HHPE-AE extractions for 5 min and 15 min, respectively. Thereby, the authors reported that the maximum TPC (129.1 mg GAE/100 g FW), antioxidant capacity (20.6 mM TE/100 g FW) and vitamin C (VITC) content (74 mg/100 g FW) for the HHPE-UE process. Thus, the authors did not consider the influence of ST and water as an extracting solvent in due course of the extraction yield evaluation of the bioactives.

Adopting MAE for the extraction of bioactives (polyphenols, flavonoids and antioxidants) from a mixture of PPU and PPE, Vallejo-Castillo et al. (2020) targeted variations in MAE power (130 – 390 W), LR (1:40 to 1:80 g/mL), solvent percentage (ethanol, 30–70%, v/v) on the response variables. Thereby, the authors inferred that the optimal process conditions for the maximum extraction of polyphenols (1186.39 ± 44.49 mg GAE/100g FW, flavonoids (43.88 ± 6.94 mg CE/100G FW) and antioxidants (3920.48 ± 31.97 µmol TE/100G FW) as 340W (power), 23.34% (v/v) (ethanol to water ratio) and 1:80.60 g/mL (LR).

Lara-Abia et al. (2022) considered the application of different levels of pressure, time and temperature for hydrostatic high pressure-assisted extraction (HHPAE) process in due course of extraction of carotenoids from PPU and PPE. The authors utilized soybean and sunflower oil as extracting solvents. To conduct this study, the authors considered variant range of temperature, pressure and time (20 – 40 °C, 300 – 500 MPa and 2 – 8 min) through a central composite experimental design (CCD). The reported optimal best extraction conditions for PPU using soybean oil correspond to 400 MPa, 40.5°C for 5 min with the maximum (all-E)-lycopene extraction yield of 99.1%. For PPE extracts, the authors reported that the maximum (all-E)-β-carotene (14.0 %) and (all-E)-β-cryptoxanthin (19.3%) extractant yields

correspond to 500 MPa, 35°C for 2 min with soybean oil and 400 MPa, 27.5°C for 5 min with sunflower oil. Thereby, the authors inferred that at low pressures (300 – 400 MPa), carotenoids yield from PPU and PPE enhanced significantly.

A summary of the critical findings associated to the optimization of UAE of bioactives from PPU and PPE systems has been presented in **Table 1.4.**

Table 1.4: Literature data summary of bioactives extraction from papaya pulp and papaya peel.

S.No	Author	Target	Parameters studied	Independent variables and their ranges	Dependent variables and their ranges	Optimal process-product characteristics
1.	Vallejo-Castillo et al. (2020)	MAE of Polyphenols and Flavonoids	▪ LR, microwave power, ethanol-water mixture	▪ Microwave power (W): 130 - 390 ▪ LR (g/mL): 1:40 - 1:80 ▪ Ethanol-water (%): 30 - 70	▪ Polyphenols: 1100 - 1186.39 mg GAE/100g FW ▪ Flavonoids: 31 - 43.88 mg CE/100g FW ▪ Antioxidants : 3920.48 ± 31.97 µmol TE/100g FW	▪ Extraction power (W): 340 W ▪ LR g/mL): 1:80.6 ▪ Ethanol-water (%): 23.34 ▪ Polyphenols: 1186.39 ± 44.49 mg GAE/100g FW ▪ Flavonoids: 43.88 ± 6.94 mg CE/100g FW ▪ Antioxidants : 3920.48 ± 31.97 µmol TE/100g FW

1.2.4 Ultrasound-Assisted Extraction of Pectin from Pomelo Fruit Peel

Presently, few authors targeted research upon the pectin recovery from pomelo peel using UAE based RSM approach. Considering hot water-acid extraction (AHWE) of pectin from pomelo peel, Methacanon et al. (2014) evaluated efficacy of extraction parameters towards pectin yield (PY) and its characteristics using single factor and CCD based RSM. To conduct this study, the authors varied ET, ST and concentration of calcium chloride solution from 70 – 90 °C, 60 – 200 min and 5 – 20 mM for a fixed choice of LR (1:30 (w/v)) and pH (2 using nitric acid). In this study, the authors identified the best conditions at a pH 2 (with HNO3) as 90 °C ET, 5mM calcium chloride concentration and 180 min of ST. Under these conditions, the yield of pectin was achieved about 27.63 %. Also, best values for the degree of esterification (DE) and molecular weight (MW) values of the extracted pectin were 55.8 % and 279 kDa, respectively. From this study, the authors have affirmed a strong influence of pH and ET on the PY. In contrast, the addition of calcium chloride was inferred to be insignificant. The authors also affirmed that nitric acid was better solvent than hydrochloric acid for pectin solubilization.

In another study, Liew et al. (2016) targeted the optimization of PY from pomelo peel using BBD based RSM. The authors adopted ultrasound-microwave assisted extraction (UMAE) method using citric acid (CA) at a fixed LR of 1:29 g/mL. During their investigation, the authors considered the effect of pH (1.7 – 2.3), ST (12 – 28 min), microwave power (350 – 650) and irradiation time (4 – 12 min) on the response variables. Thereby, the authors have affirmed that the highest PY (38%) and DE (56.88%) was achieved under optimized conditions of pH 1.80, 27.52 min sonication followed by 6.40 min microwave irradiation at 643.44 W power. The authors also compared various extraction methods (UMAE, MUAE, MAE, UAE) at their respective optimized conditions and inferred that among all of them, the PY through UMAE was highest. The order of the processes in terms of PY was UMAE (36.33%) > MUAE (30.50%) > MAE (27.65%) > UAE (14.25 %).

Chen et al. (2016) employed hot-solvent-microwave extraction method (HSME) for the extraction of pectin from pomelo peel and at a fixed LR (1:18 g/mL). For optimization purpose, the authors applied BBD based RSM approach. The authors targeted microwave power (390 – 650 W), ST (3 – 7 min) and pH (1 – 3) value for the optimization of the PY. Thereby, the authors identified optimal conditions to

be 520 W microwave power, 1.5 pH, and 5.6 min ST. Under these optimal conditions, the PY was obtained as 3.29 %. From the study, the authors also affirmed that the variations in pH and ST had major influence upon PY. However, microwave power exhibited a strong quadratic influence on PY. Also, HSME outperformed the conventional acidic extraction process (3.11%) in terms of pectin extraction efficiency and yield.

To optimize the yield and DE of the pectin from pomelo peel, Liew et al. (2018) adopted acid and deep eutectic solvent (DES) extraction methods using CA media. For optimization purpose, the authors targeted BBD based RSM methodology. In their investigations, the authors varied pH (1.50 – 2.50), ET (65 – 90 °C), ST (40 – 180 min) and LR (1:20 – 1:30 g/ mL). From the study, the authors indicated that the pH had a pronounced influence on the DE and PY. For the optimized condition of 1.80 pH, 141 min ST, 88°C ET and 1:29 g/mL LR, a high-methoxyl PY of 39.72% and a DE of 57.56% were obtained. The authors also investigated various acidic DES for pectin extraction and affirmed that the lactic acid–glucose–water-DES system at a ratio of 6:1:6 produced the highest yield of pectin (23.04 %). However, the authors inferred that the CA was the best to achieve optimal yield and energy cost reduction than the DES.

Comparing the extraction efficiencies of conventional heating extractive method with acid (CE), sequential ultrasound-microwave assisted technique (UMAE) and dynamic sub-critical water extractive technique (SWE) for the extraction of pectin from pomelo peels, Liew et al. (2019) inferred that the CE achieved the highest pectin extraction efficiency of 39.13% followed by UMAE (36.33%) and SWE (19.63%). The reported DE values for CE, UMAE and SWE were 59.85 %, 59.23%, and 40.1 % respectively. One of the possible reason for reduced DE in SWE is the lack of the acid in the extraction method and also due to a sustained high operating temperature used in SWE. In terms of energy usage and efficiency, while SWE consumed the highest energy at 1.05 kWh, the UMAE achieved the highest extraction efficiency of 0.49 g/h.

The influence of various acids (citric acid (CA), acetic acid and lactic acid) on the high methoxyl pectin extraction yield, chemical structure and antioxidant capacities were investigated by Van Hung et al. (2021) for three different varieties of pomelo peels. The authors conducted the extraction of pectin at 3

pH, 90°C for 90 min and for a fixed choice of LR (1:30 g/mL). Among three different deployed acids, CA yielded highest amount of pectin (6.5 – 9.0%) followed by acetic acid (6.2 – 8.2%) and lactic acid (6.1 – 8.0%). Besides, all cases referred to of high methoxyl pectin with the DE ranging from 51.1 – 62.7 %. Thereby, the authors inferred that using the CA as an extractant could yield galacturonic acid yield of ranging 76.5 –85 % from alternate varieties of pomelo peels. The antioxidant activity of pectin extracted with CA (25 – 39.8%) and lactic acid (24 – 37.2 %) was much higher than that of pectin being extracted with acetic acid (17.3 – 32.6%).

Table 1.5 outlines a brief summary of the critical research findings of the investigations that targeted UAE of pectin from pomelo peel system.

Table 1.5: Literature data summary of bioactives extraction from pomelo peel.

S.No	Author	Target	Parameters studied	Independent variables and their ranges	Dependent variables and their ranges	Optimal process-product characteristics
1.	Methacanon et al. (2014)	Hot water acidic extraction of pectin from pomelo peel	▪ Extraction time, ET and concentration of calcium chloride	▪ ET(°C): 70 – 90 ▪ Time (min): 60 – 200 ▪ Concentration of calcium chloride (mM) : 5 – 20	▪ Pectin Yield (%): 15.09 – 27.63 ▪ Degree of esterification (%): 52.54 – 59.67 ▪ Molecular weight (kDa): 218 – 918	▪ ET(°C): 90 ▪ Time (min): 180 Concentration of calcium chloride (mM): 5 ▪ Pectin Yield (%): 27.63 ▪ Degree of esterification (%): 55.8 ▪ Molecular weight (kDa): 279
2.	Liew et al. (2016)	UMAE of pectin from pomelo peel	▪ Microwave power,ST, irradiation time and pH	▪ Microwave power (W): 350-650 ▪ ST (min): 12 – 28 ▪ Irradiation time (min): 4 – 12 ▪ pH: 1.7 to 2.3	▪ Pectin Yield (%): 10.79 – 37.52 ▪ Degree of esterification (%): 53.14 – 68.78	▪ Microwave power (W): 643.44 ▪ ST (min): 27.52 ▪ Microwave irradiation time: 6.40 ▪ pH: 1.80 ▪ Pectin Yield (%): 38 ▪ Degree of esterification (%): 56.88

1.2.5 Encapsulation of Bioactives from Papaya Leaf Extract by Ion Gelation Method

The incorporation of bioactive extracts into polymer matrices is often targeted for their stability and bioavailability. Thereby, it is a well-known proven method to custom serve the health benefits associated to the bioactives. Among various available methods, microencapsulation is an outstanding technique for improved bioactive constituent delivery systems through a prolonged or controlled release of the bioactives. Henceforth, sustainable bioactivity can be achieved along with their stability.

Till date, significant investigations did not target the encapsulation of bioactives from leafy extracts. A brief account of the prior art is as follows:

Zhang et al. (2021) targeted extrusion based encapsulation of mulberry pomace extracts (MPE) in calcium alginate (Ca-Al) beads. The authors prepared two types of Ca-Al loaded MPE beads through the ionotropic gelation method. For this, 1.5% (w/v) sodium alginate and 5 % (w/v) calcium chloride ($CaCl_2$) solution were used. Thereby, 22 G stainless steel needle based beads were prepared and were classified as those with and without MPE. Subsequently, lyophilization was conducted for both types of beads. Thereby, the authors inferred that in comparison to beads without MPE, the lyophilized MPE beads possessed more TPC (81.456 ± 0.333 GAE/g dry weight of beads) and had maximum encapsulation efficiency (EE) of 43.40% ± 0.18%. Also, from the study, the authors concluded that the lyophilized beads stored in MPE exhibited favorable stability indices for the TPC during 30-day storage at 4 °C in comparison to the beads stored without MPE. Besides, it was observed that for lyophilized beads, the TPC release was fast during the initial time period (0-30 min) and was slow in the later time period (30-180 min).

Targeting the encapsulation of α-tocopherol by ion gelation method, Singh et al. (2018) reported the optimal conditions associated to the α- tocopherol beads being prepared with a combination of sodium alginate (0.5 – 2.0% w/v) as primary wall material, 5% w/w $CaCl_2$ and 2% w/v pectin filler. 25G stainless needle was used for the investigations that considered fixed choice of α-tocopherol (1% w/v). For optimization purpose, trial and error method have been followed by the authors. Thereby, the optimal conditions for the maximization of EE (59.91 %) corroborated to 1.5% w/v sodium alginate

and 2.0% w/v pectin cases. The beads were also evaluated for other characteristics such as moisture content (5.23%), swelling index (1.03%), average particle size (0.58 mm), bulk density (0.53 mg/mL), Carrs index (11.56) and free % Vitamin E in beads (0.04 %).

Sampaio et al. (2019) targeted the influence of type of polymer material (2% w/v sodium alginate and 4 % w/v pectin) on the EE of the watermelon concentrate and drying stability of lycopene-rich beads obtained through ionic gelation with 2% w/v $CaCl_2$. To do so, the authors have used a 22G stainless steel needle at a dropping distance of 20 cm with 2% w/v $CaCl_2$ solution. The authors evaluated wet and dried beads for lycopene retention, particle size distribution, morphology and thermogravimetric analysis. Based on extensive experimental investigations, the authors concluded that the dried beads were superior to retain lycopene content (higher than 80%) in comparison with the wet beads (29 and 21 % for alginate and pectin, respectively). Besides, the dried alginate beads (1.9 – 2.3 mm diameter) with better spherical shape characteristics was effective in terms of lycopene stability in comparison with the elongated pectin beads (2.6 –3.3 mm) being prepared at non-optimal conditions.

In other study, Pasukamonset et al. (2016) carried out microencapsulation of phenolic extracts of *Clitoria ternatea* (CT) petal flower through extrusion method using alginate and calcium chloride ($CaCl_2$). In due course of the investigations, the authors varied CT content (5–20% w/v), sodium alginate (1– 2% w/v), and $CaCl_2$ (1.5 – 5% w/v) solution concentrations. The authors used 21 G needle for the extrusion process and the syringe pump flow rate was maintained at 0.5 mL/min. For the optimization purpose, the authors adopted a trial and error method. Thereby, the authors inferred that the maximum combinations of antioxidant activity (11.76 ± 0.07 mg GAE/g beads) and encapsulation efficiency (84.83 ± 0.40%) was achieved for the optimized conditions of 10% w/v CT, 1.5% w/v alginate, and 3% w/v $CaCl_2$. Also, the authors affirmed that the obtained beads possessed a smoother surface shape with an average particle size distribution of 985 ± 0.53 mm and an improved thermal stability upto 188 ºC.

Vallejo-Castillo et al. (2020) targeted the preparation of alginate/pectin microcapsules for the encapsulation of papaya leaf (PL) extract. To do so, the authors mixed the sodium alginate-pectin system (30:70, 55:45 and 80:20 w/w ratios) with the gallic acid solution (fixed choice of 1:1 volume

ratio). The experiment was conducted using fixed diameter sterile syringe needle (29G), 10 cm dropping distance and 0.1 M $CaCl_2$ hardening solution (1:4 g/mL). The PL extract encapsulated beads were prepared using the optimum ratio of the alginate to pectin ratio and gallic acid constitution. Thereby, the authors affirmed that the optimized combination of alginate to pectin ratio for gallic acid encapsulation was 55:45 and 61:39 w/w for in-situ and two-step encapsulation method respectively. Corresponding gallic acid yield was 28.1 mg GAE/g for the capsules being prepared with the papaya exocarp extract.

A concise summary of the important findings of the carried out research in the field of bioactives encapsulation from leafy extracts using sodium alginate based ion gelation method has been presented in **Table 1.6.**

Table 1.6: Literature data summary of encapsulation of leafy and vegetable extract system by ion gelation method.

S.No	Author	Target	Parameters studied	Independent variables and their ranges	Dependent variables and their ranges	Optimal process-product characteristics
1.	Pasukamonset et al. (2016)	Microencapsulation of phenolic extracts of *Clitoria ternatea* (CT) petal flower	*Clitoria ternatea* (CT) concentration, sodium alginate concentration, calcium chloride concentration	▪ *Clitoria ternatea* (CT) concentration (%): 5 – 20 ▪ Sodium alginate concentration (%): 1– 2 ▪ Calcium chloride concentration (%) : 1.5–5%	▪ Encapsulation efficiency (%): 74.17 –84.83	▪ *Clitoria ternatea* (CT) concentration (%): 10% ▪ sodium alginate concentration (%): 1.5 ▪ calcium chloride concentration (%): 3 ▪ Encapsulation : 84.83 ± 0.40%
2.	Singh et al. (2018)	Encapsulation of α- tocopherol	α-tocopherol concentration, sodium alginate concentration, calcium chloride concentration and pectin concentration	▪ α-tocopherol concentration (%): 1% ▪ Sodium alginate concentration (%): 0.5–2 ▪ Calcium chloride concentration (%): 5 ▪ Pectin concentration (%) : 2%	▪ Encapsulation efficiency (%) : 45.12 – 52.91	▪ α-tocopherol concentration (%): 1% ▪ Sodium alginate concentration (%): 1.5 ▪ Calcium chloride concentration (%): 5 ▪ Pectin concentration (%): 2% ▪ Encapsulation efficiency : 59.91%

1.3 Research Gaps

A brief account of the prior art findings, delineated upon several pertinent subjective and objective lacunae. These have been summarized in the following sections.

1.3.1 RSM based Ultrasound Assisted Extraction and Optimization of Bioactives from Guava Leaves

Till date, limited prior art devotes towards the recovery of bioactive constituents from the extracts of the guava leaf using both NUAE and pulsed mode of the UAE (PUAE). An early investigation affirmed that during NUAE of guava leaves, water is the best solvent in comparison with ethanol and methanol (Chakraborty et al., 2020).

Seo et al. (2014) carried out HWE of TPC, TFC and AA from guava leaves using different solvents. The authors did not target upon the efficacy of LR and also UAE (normal and pulse mode). Similarly, Kong et al. (2015) addressed the extraction of polysaccharide compounds from guava leaves using NUAE. However, the authors did not elaborate upon the efficacy of the process towards extraction of bioactives such as TPC, TFC and AA. Also, the authors did not target the efficacy of PUAE and criticality of LR on the response variables.

In this regard, it is important to consider the criticality of LR variable with the given fact that while lower LR dilutes the extract, higher LR may not indicate adequate liquid content for extraction (Campoli et al., 2018). Thus, no investigation considered this as a critical variable towards the maximum recovery of bioactive compounds from the guava leaf extract. Secondly, all mentioned literature reported drying at 40 °C for 2 – 5 h as an essential pre-treatment step and this is bound to detrimentally influence the maximum yield of bioactive components into the aqueous system. Thus, alternate procedures need to be adopted for the better extraction of the bioactives from leafy systems. Thirdly, the reported prior art focussed on either one or two responses but not all conventional bioactive constituent responses in due course of the UAE. Precisely, Zeng et al. (2020) considered only TPC and Li et al. (2019) and Liu et al. (2014) considered only TFC as a response variable and omitted other desired responses such as AA and VITC. Thus, no literature exists that addressed the response characteristics of all four commonly

considered response variables such as TPC, AA, VITC and TFC. However, the consideration of RSM based UAE has been excellent in few mentioned prior art as the methodology facilitates improved insights into the highly non-linear variation of the responses in conjunction with the choice of the process parameters. Further, no literature targeted the comparative performance of PUAE in conjunction with the NUAE as the former is well known to better enable necessary equilibrium conditions for the bioactive components at the leaf-water interface.

Considering the above subjective lacunae, this work targeted the face centered design (FCD) based RSM approach for the NUAE and PUAE of guava leaf extracts. Thereby, the considered degrees of freedom include guava leafy powder to water ratio (LR), ET and ST on the commonly desired bioactive constituent characteristics such as TPC, TFC, AA and VITC. Water has been considered as the solvent due to its non-expensive, non-toxicity and environmentally begign nature (Moorthy et al., 2015). Critical novelty of the conducted investigations in comparison with the reported prior art are (a) evaluation of the criticality of LR in addition to ST and ET (b) degree of non-linear influence of any two process parameters on all measured responses. Such insights have not been indicated in the prior art due to considering LR as a parameter but not as a critical independent variable and (c) sensitivity of the PUAE in conjunction with the NUAE for the achievement of highest possible yields of desired responses.

1.3.2 RSM based Ultrasound-Assisted Extraction and Optimization of Bioactives from Papaya Leaves

Till date, little research has been conducted on the recovery of bioactive components from papaya leaf extracts using both conventional HWE and UAE methods in terms of process parametric and response optimization. Vuong et al. (2013) reported TPC and AA as a response and omitted TFC as a critical response. The research group did not focus upon the optimization of process parameters such as ET, ST, and LR. Similarly, Vuong et al. (2015) reported HWE of antioxidant and anticancer capacity of saponin- enriched papaya leaf extracts using either water or ethanol as an extracting solvent. However, the authors did not elaborate upon the criticality of higher ET and lower LR which could be detrimental

to the bioactives. Also, often sought response variables such as TPC and TFC were also ignored by the authors.

Soib et al. (2020) evaluated the extraction efficacy of UAE, room temperature reflux and agitation methods towards the preparation of methanol based papaya extract. However, the authors did not address the HWE and the optimisation of the process parameters associated with either HWE or UAE or both. Further, the authors adopted fixed choice of process parameters such as LR, and moderate ST. Also, the authors investigated the extraction efficacy in terms of the phytochemical constitutions, antioxidant capacity and wound healing activities.

On the other hand, Abdel-Halim et al. (2021) targeted extraction of polyphenols, flavonoids and anti-hyper-lipidemic activity of papaya leaves. The authors adopted fixed choice of process parameters such as higher LR, moderate ST and moderate ET. In due course of their investigations, the authors did not emphasize upon the optimization of the process parameters with respect to the maximum retention of desirable response variables. Thus, the available prior art affirms that no literature exists with respect to the comparative extraction efficacy studies of sonication (normal and pulsed mode) and HWE based papaya leaf extraction with water as a solvent. Further, no published work exists in the precise field of process optimisation of UAE based bioactive components extraction from papaya leaves.

Considering the cited lacunae, the thesis targets the optimisation of UAE process for papaya leafy system using water as a solvent. This central objective of the thesis has three major sub objectives. Firstly, the optimal papaya leaf extract conditions in the UAE are to be investigated from an integrated perspective involving maximum extraction of TPC, AA and TFC. Secondly, the sensitivity of pulse mode with respect to continuous mode of sonication shall be evaluated for the papaya leaf extract. Thirdly, the best process characteristics are to be assessed with respect to the HWE process. Considering the complexity of the process parametric and response combinations, RSM with FCD can be adopted to evaluate upon the associated sensitivities, relationships and optimalities of the process parameters and responses. Thereby, the targeted degrees of freedom refer to ET, ST and LR for the desired responses as TPC, TFC, and AA of the aqueous papaya extract.

1.3.3 RSM based Ultrasound-Assisted Extraction and Optimization of Bioactives from Papaya Pulp and Papaya Peel

Till date, the RSM based UAE of PPU and PPE has not been investigated and the available niche data indicates poor quality of extraction from the perspective of the UAE. According to the reported literature, no studies exist with the respect to the extraction of bioactive components from the PPU and PPE using bath type ultrasound extraction system and water as a solvent. Uribe et al. (2015) considered the influence of alternate extraction techniques such as high hydrostatic pressure extraction (HHPE), UAE, AE and their combinations on the recovery of bioactives (TPC, AA and VITC) from PPU/PPE mixture system. The authors did not address upon the influence of ET, variations in LR and ST on the response variables. Also, the authors did not consider the efficacy of water as an extracting solvent for the maximization of extraction yield of the bioactives. Precisely, the investigation did not emphasize upon the UAE (normal and pulsed mode) optimization of the process parameters to achieve optimal combinations of response variables. Similarly, Vallejo-Castillo et al. (2020) considered MAE for the extraction of bioactives (TPC, TFC and AA) from PPU/PPE mixture system. The authors addressed variations in MAE power, LR, solvent percentage and did not target upon the critical influence of ST and ET on the response variables. Also, water as a green solvent was not considered by the authors.

On the other hand, Lara-Abia et al. (2022) addressed the application of different levels of pressure, time and temperature in HHPAE processes for the extraction of carotenoids from PPU and PPE using soy bean oil as an extracting solvent. The authors targeted variation in time, temperature and pressure using CCD based RSM. However, they did not emphasize upon the critically of LR on the response variables. In addition, the authors did not consider other indices such as TPC, TFC, AA and VITC as the desired set of response variables.

In summary, it can be concluded that the prior art did not devote towards the aqueous extraction of bioactives from PPU and PPE. Precisely, the available prior art has the following lacunae. Firstly, no investigation targeted the extraction of bioactives from PPU and PPE using bath type sonication system. Secondly, the influence of process parameters of UAE (LR, ST and ET) on bioactive compounds

recovery from PPU and PPE extract was not studied. Thirdly, RSM based optimisation of process parameters of UAE was not targeted. Fourthly, water as an extraction media or green solvent was not considered in the prior art to maximize extraction of the bioactive constituents from PPU and PPE. Thus, the objective of the thesis needs to address aqueous extraction of PPE and PPU by employing the RSM with FCD methodology to evaluate the (1) optimization of process parameters such as ST, ET, and LR to achieve maximum constitution of response variables namely the TFC, TPC, AA, and VITC in PPU and PPE based aqueous extracts and (2) comparison of yield based efficacies of the UAE and HWE for aqueous bioactives extract yield from PPU and PPE.

1.3.4 RSM based Ultrasound-Assisted Extraction and Optimization of Pectin from Pomelo Fruit Peel

The available prior art affirms that several literatures did address the extraction of pectin from pomelo peel using RSM based optimization. A variation in pH, ST and deployment of alternate acids have been considered to converge upon their influence upon the extraction efficiency of pectin. The precise lacunae of relevant literature have been follows:

Methacanon et al. (2014) addressed HWE of pectin using nitric acid from pomelo peel evaluated using single factor and CCD based RSM. The authors varied ET, ST and concentration of calcium chloride solution. However, pH was not considered as a degree of freedom. In addition, the authors did not consider the effect of other acids such citric acid, hydrochloric acid etc. on the extraction yield. Further, the authors did not investigate upon the techno-functional properties (pectin solubility, water holding capacity, oil holding capacity etc.) of the extracted pectin.

Liew et al. (2016) addressed the optimization of PY from pomelo peel using BBD based RSM. The authors targeted the influence of UMAE method with citric acid on the response variables. During their investigation, the authors considered the effect of variations in pH, ST, microwave power and irradiation time on the response variables. However, the authors did not elaborate upon effect of different acids on the PY and did consider other UAE process parameters (ET and LR) on the optimality of response variables.

Chen et al. (2016) evaluated the efficacy of HSME for the extraction of pectin from pomelo peel at a fixed LR. For optimisation purpose, the authors applied BBD based RSM approach. The authors varied microwave power, ST and HCl based pH value for the optimisation of the PY. However, the authors did not consider influence of alternate acids on the DE and the PY. Furthers, the authors did not elaborate upon the techno-functional properties of the extracted pectin.

In another study, Liew et al. (2019) targeted the extraction efficiencies of conventional heating extraction method with acid (CE), UMAE and dynamic sub-critical water extractive technique (SWE) for the extraction of pectin from pomelo peels. The authors chose previous mentioned conditions in their investigation. However, the authors did not elaborate upon the effect of alternate acids on the PY and did not consider the influence of UAE process parameters on the response variables. In addition, the authors did not elaborate upon the optimization of process parameters with respect to PY.

Van Hung et al. (2021) addressed the effect of different acids (citric acid, acetic acid and lactic acid) on extraction yield, chemical structure and antioxidant capacities of high methoxyl pectin's extracted from three alternate varieties of pomelo peels. However, the authors did not target upon the influence of extraction time and pH variation on the PY. Further, the authors have not elaborated upon important techno-functional properties (pectin solubility, water holding capacity, oil holding capacity etc.) of the extracted pectin.

In summary, it can be concluded that the prior art did not devote towards the aqueous extraction of pectin using citric acid from pomelo peel. Precisely, the available prior art has the following lacunae. Firstly, no investigation targeted the citric acid aided extraction of pectin from pomelo peel using bath type sonication system. Secondly, the influence of process parameters such as ST and pH on the recovery from pomelo peel was not studied. Thirdly, the effect of NUAE and PUAE on the pectin recovery was not studied. Fourthly, the optimised results of PUAE of pectin were not compared with the acidic HWE (AHWE). Fifthly, RSM based optimisation of process parameters of UAE was not targeted. Sixthly, the functional properties of the extracted pectin from PPU were not considered. Thus, the objective of the thesis is to extract pectin from pomelo peel by employing the RSM based FCD methodology, thereby to evaluate the (1) optimization of process parameters such as ST and pH to

achieve maximum yield of response variables namely the PY and DE, (2) compare and contrast upon the efficacies of the NUAE, PUAE and AHWE in terms of PY and (3) to characterize the functional properties of the extracted pectin.

1.3.5 RSM based Encapsulation of Papaya Leaf Extract using Ion Gelation method

Till date, very limited literature exists for the optimality of encapsulation of bioactives from papaya leaf. For this purpose, RSM can be used. Additionally, RSM constitutes a set of statistical and mathematical methods that successfully facilitate useful insights into the influences of multiple process variables and subsequent process optimization (Putri et al., 2019). The RSM effectively integrates the combined influences of the three levels of each experimental parameters and develops a functional representation of the response variable as a linear function of experimental parameters, factorial interactions, error, and quadratic effects (Kusuma et al., 2018). Further, the RSM is a time-saving optimization technique due to its ability to evaluate the influence of several parameters and associated interactions with fewer experimental trials (Putri et al., 2019). Accordingly, RSM functions as an efficient tool for the designing, modifying and optimizing processes through the evaluation of responses and numerous process variables (Kusuma et al., 2021).

In this regard, it should be noted that most prior art reports common strategies being adopted for the encapsulation of the bioactives along with the consideration of parametric variations that critically influence encapsulation efficiency. In this regard, the prior art has the following lacunae:

In a study, Pasukamonset et al. (2016) carried out microencapsulation of phenolic extracts of *Clitoria ternatea* (CT) petal flower through extrusion method using sodium alginate and $CaCl_2$. The authors varied CT concentration, sodium alginate, and $CaCl_2$ concentrations. However, the authors did not address the criticality of needle size and flow rate on the encapsulation efficiency and also did not account upon the RSM based sensitivity analysis and optimization of critical independent and dependent variables.

Singh et al. (2018) addressed the optimization conditions of α-tocopherol beads using a combination of sodium alginate as primary wall material and pectin as a filler. However, the authors did not investigate

upon the effect of $CaCl_2$ solution concentration on the encapsulation efficiency. Further, the authors did not consider the effect of syringe pump flow rate needle size on the encapsulation efficiency. Besides, the authors did not consider the RSM based optimisation of process parameters for better encapsulation efficiency. On the other hand, Sampaio et al. (2019) evaluated the influence of type of polymer (sodium alginate and pectin) material on the encapsulation efficiency of watermelon concentrate and drying stability of lycopene-rich beads obtained through ionic gelation using $CaCl_2$. However, the authors did not vary the concentration of sodium alginate and $CaCl_2$ solution. Also, the rate at which the syringe needle extrudes the liquid droplet was not considered for its criticality towards the encapsulation efficiency. Further, the optimization of process parameters using RSM were not targeted by the authors.

Vallejo-Castillo et al. (2020) targeted the development of alginate/pectin microcapsules for the encapsulation of papaya leaf extract. The authors altered sodium alginate to pectin ratio in their investigations. However, authors performed the experiment by using fixed nozzle size of the sterile syringe needle (29G), fixed droplet falling distance (10 cm) and fixed $CaCl_2$ solution concentration (5%). Also, the authors did not vary the concentration of sodium alginate solution. The authors also did not consider the criticality of the liquid droplet extrusion rate from the needle. Furthermore, the authors did not target RSM based process parametric optimization.

Using ion gelation method, Zhang et al. (2021) addressed the encapsulation of MPE in calcium alginate beads. The authors did not consider the effect of variations in concentration of sodium alginate and $CaCl_2$ on the encapsulation efficiency. Besides, the investigations did not deliberate upon the effectiveness of needle size to improve the encapsulation efficiency. Also, the authors did not consider the optimisation of process parameters to obtain optimal data in terms of encapsulation efficiency.

In summary, the RSM based encapsulation of bioactives from papaya leaf extract using sodium alginate and pectin as wall material has not been investigated till date. Considering the cited lacunae, the final thesis objective targets the optimisation of process parameters for the encapsulation of bioactives being obtained from papaya leaf extract. This central objective of the thesis has three major sub objectives. Firstly, the optimum conditions of process parameters for encapsulation of bioactives from papaya leaf extract need to be investigated from an integrated perspective involving maximum encapsulation

efficiency. Secondly, the effect of needle size variation is to be understood in terms of the encapsulation efficiency. Thirdly, the storage and release kinetics also are to be evaluated for the bio-actives in the encapsulated beads. Fourthly, the effect on the encapsulation efficiency in due course of extrusion process with $CaCl_2$ solution needs to be addressed for the papaya leaf extract. Considering RSM based encapsulation of papaya leaf extract with sodium alginate and pectin as a wall material and $CaCl_2$ as a hardening solution, the objective targets the evaluation of the effect of control variables (such as concentration of sodium alginate, $CaCl_2$ and flow rate) on the efficiency of encapsulation. FCD based RSM can be deployed to design the experiments for model development and optimization of process variables.

1.4 Objectives of the Ph.D. Thesis

Two broader research domains have been targeted in the Ph.D. thesis. Among these, the first was to identify the best few leafy and non-leafy resource with maximum extraction of bioactives using ultrasound assisted extraction (UAE) technique. The second was to encapsulate the bioactives obtained in aqueous media in sodium alginate-pectin system using ion gelation method. Accordingly, considering these broad themes, the Ph.D. thesis targets the following objectives in the field of UAE of bioactives and subsequent encapsulation of the bioactives using ion gelation method:

a) RSM based ultrasound-assisted extraction of bioactive compounds from guava leaves for the evaluation of the criticality of loading ratio, extraction time and temperature and associated degree of non-linear influence of the process parameters on the response variables.

b) Comparative efficacy of hot water and ultrasound-assisted extraction of bioactives from papaya leaf system for the evaluation of associated sensitivities, relationships and optimalities of the parameters and responses of the process system.

c) Recovery of bioactive compounds from papaya pulp and papaya peel using ultrasound assisted extraction and thereby optimize process performance in conjunction with the hot water extraction.

d) RSM based continuous and pulsed ultrasound-assisted extraction of pectin from pomelo fruit peel using citric acid and subsequent evaluation of pectin product characteristics and optimal process parameters.

e) Encapsulation of optimally extracted papaya leaf extract (PLE) using ion gelation method for the evaluation of release kinetics and storage properties of PLE encapsulated calcium alginate-pectin beads.

1.5 Organization of the Thesis

The Ph.D. thesis content has been organized in eight chapters. A brief account of the following eight chapters is follows:

Chapter 1 addresses the relevance of customized research towards the extraction of bioactives from leafy and non-leafy sources using various extraction techniques and their encapsulation using alginate-pectin based systems.

Chapter 2 summarizes materials and methods being adopted to carry out the Ph.D. thesis work. These refer to (a) materials required to carry out the thesis work, (b) procedures adopted for the extraction of bioactives from leafy and non-leafy sources and comparison with hot water extraction (c) analysis techniques being deployed for the optimization of the data obtained using RSM, (d) procedures and methodologies associated for optimal pectin extraction from pomelo peel (e) adopted analytical methods for the determination of the physicochemical and nutritional properties of extracted pectin and (f) optimality of encapsulation process being deployed for the encapsulation of UAE based papaya leaf aqueous extract (PLE) in pectin-calcium alginate system using ion gelation method and associated product characterization.

Chapter 3 addresses the FCD model based RSM design methodology approach for the normal and pulsed UAE of guava leaf extract. Thereby, influence of considered degrees of freedom such as dried guava leafy powder to water ratio (loading ratio), extraction temperature (ET) and sonication time (ST) on the desired bioactive constituent characteristics such as TPC, TFC, AA and VITC have been elaborated. Further, a comparative study has also been addressed to evaluate the sensitivity of the pulsed

UAE in conjunction with the normal UAE for the achievement of highest possible yields of the considered responses.

Chapter 4 addresses the comparative efficacy of hot water and optimally performing UAE process for the bioactives extraction from the papaya leaf (PL) system. The RSM based FCD has been deployed to evaluate upon the associated sensitivities, relationships and optimalities of the process parameters and responses. Thereby, extraction temperature, sonication time and loading ratio have been considered as degrees of freedom for the maximum extraction of TPC, TFC, and AA in aqueous the papaya extract. Further, the sensitivity of pulse mode with respect to normal mode of sonication was also evaluated for the papaya leaf – UAE system. Additionally, the best UAE process characteristics have been assessed with respect to those of HWE system.

Chapter 5 reports upon the findings associated to normal UAE and pulsed UAE of bioactive compounds from papaya pulp (PPU) and papaya peel (PPE), respectively, with a bath-type pulsed sonication method. The impact of pulsed UAE mode process parameters (loading ratio, sonication time, and extraction temperature) on the recovery of bioactive compounds (TFC, TPC, VITC and AA) from PPU and PPE extract have been addressed by adopting RSM-based FCD optimization. For this purpose, water was used as an extraction medium to enhance bioactive compounds extraction from PPU and PPE system. Further, a comparative efficacy of pulsed UAE with respect to the HWE for the evaluated responses namely, TFC, TPC, VITC and AA have been reported.

Chapter 6 presents the finding associated to the UAE of pectin from pomelo peel for a fixed choice of loading ratio (1:15 g/mL) and extraction temperature (65ºC). For comparative purposes, two modes of sonication, namely, normal and pulsed mode have been considered and the response variables have been evaluated as a function of extraction time and pH using FCD based RSM design of experiments. The extracted pectin product was analyzed for chemical, thermal, morphological, techno-functional and physiochemical properties.

Chapter 7 elaborates upon the findings of the papaya leaf extract (PLE) encapsulation with sodium alginate – pectin system using ion gelation method. The optimized PLE with best choice of UAE system process parameters in this study have been considered. RSM based FCD design was applied for the statistical design of experiments. These experiments were conducted by considering sodium alginate

concentration, calcium chloride concentration and flow rate of syringe pump as independent variables and encapsulation efficiency as dependent variables. The obtained encapsulated calcium alginate-pectin PLE beads were reported in terms of release kinetics, storage studies (room temperature and refrigerated temperature), influence of alternate needle sizes on encapsulation efficiency and subsequent chemical, compositional and morphological data of the product.

While the first section of **chapter 8** highlight upon significant research based conclusions of the Ph.D. thesis, the following section outlines on prospective future research directions in the domains targeted in the work.

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Chapter 2:

Materials and Methods

Chapter 2

Materials and Methods

In this chapter, methodologies being followed for the bioactives extraction from selected leafy and non-leafy vegetable sources and subsequent encapsulation with ion-gelation method for the papaya leaf extract have been delineated along with relevant materials. Section 2.1 summarizes all materials being used for the dissertation works. Section 2.2, 2.3, 2.4, 2.5 and 2.6 respectively elaborate on the methods followed for the UAE and HWE of guava leaves, papaya leaves, papaya pulp and pomelo peel for bioactives and pectin extraction, respectively. In these sections, various subsections highlight the adopted procedures, extract analysis, design of experiments, analytical methods for physicochemical and morphological properties, and other characterization methods (FTIR, TGA, DSC and XRD). In section 2.6, pectin-alginate based encapsulation of papaya extract has been delineated.

2.1 Materials and Methods

2.1.1 Raw Materials

Various leafy and non-leafy sources being investigated in this thesis refers to guava leaves, papaya leaves, papaya pulp, papaya peel and pomelo peel. Among these, the guava leaves and papaya leaves were procured indigenously in the IIT Guwahati campus. Papaya and pomelo fruit were procured from a local market, IIT Guwahati, India. Prior to their usage, the samples were kept in air tight samples bags in a refrigerator.

2.1.2 Chemicals and Reagents

Merck, India, provided aluminium chloride ($AlCl_3$) (98% pure), tri-sodium citrate di-hydrate ($C_6H_9Na_3O_9$) (99%), sodium nitrite ($NaNO_2$) (98% pure), sodium hydroxide (NaOH) (97% pure), sodium bicarbonate ($NAHCO_3$) (99.5% pure), sodium carbonate (Na_2CO_3) (99.5% pure), L-ascorbic acid (C_6H8O_6) (99%

pure), oxalic acid di-hydrate ($C_2H_6O_6$) (99.5% pure), citric acid monohydrate ($C_6H8O_7.H_2O$) (99.5%), nitric acid (HNO_3) (69%), hydrochloric acid (HCl) (37%) and acetic acid (CH_3COOH) (99.5 %). Sodium alginate (91%) was provided by Qualikems Fine Chem Private Limited, Vadodara, India. Gallic acid (98% pure) and Quercetin (99% pure) were obtained from Sigma-Aldrich, Bangalore, India. M/s. Sisco Research Laboratories Private Limited, India, provided phenolphthalein indicator, Folin Ciocalteu reagent (FCR), 2,6-Dichlorophenol indophenol sodium salt (DCPIP) (98% pure), soy oil and 2,2-diphenyl-1-picreylhydrazyl (DPPH) (98% pure). M Tedia, USA supplied ethanol (C_2H_5OH) (95% pure). Distilled water (DI) was obtained from a commercial laboratory scale unit (Model: Elix-3, Milli-Q; Make: M/s Millipore, USA).

2.2 Ultrasound-Assisted Extraction (UAE) of Bioactive Constituents from Guava Leaves

2.2.1 Sample Preparation

Fresh middle aged guava leaves were procured from IIT Guwahati campus (elevation range: 45 –1960 m, 21 ± 1 ℃ temperature, 51 ± 1 % humidity and 55mm precipitation) during a sunny and in day light environment in the month of February from a middle aged guava plant bearing few fruit buds. The green coloured guava leaves were handpicked and weighed about 0.58 ± 0.32 g. The average length and breadth of the leaves were 9.2 ± 0.08 and 2.8 ± 0.12 cm respectively. To minimise contamination and natural degradation of bioactive compounds, fresh guava leaves were handpicked prior to all UAE experiments. Thereafter, the leaves were washed with DI water to remove adhering impurities and contaminants. Subsequently, using a sterile knife, the leaves were cut manually into small pieces (Tran et al., 2021) and were shade dried in the ambient environment conditions (21 ± 1 °C temperature and 51 ± 1 % humidity) for 2 – 3 h. The shade dried guava leaves are difficult to grind for the realization of a uniform particle size. This is due to their lesser density and lower sample size. Hence, the shade dried guava leaves were cut into small pieces and a paste was prepared by adding a precise quantity of water to the shade dried

leaves. The paste was then subjected to sonication. The UAE process parameters have been confirmed by the FCD based RSM design approach.

2.2.2 Ultrasound-Assisted Aqueous Extraction

Ultrasonic bath (Make: Elma, Model: Elmasonic S 30H, Germany, 37 Khz and 280 Watt) was used to carry out the aqueous extraction of the bioactive compounds from the cut guava leaf pieces in a controlled environment. The experiments were conducted with a 100 mL beaker. For all UAE experiments, the solid sample mass and solvent (water) mass was chosen as per the specifications obtained from the FCD based RSM design. For this purpose, the design data set for the process parameters namely extraction time, extraction temperature, and guava leaf to solvent (water) ratio (loading ratio) were obtained from Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA). For this purpose, based on few trials, the lower (0.02 g/mL, 40 °C and 5 min) and higher range (0.2 g/mL,70 °C and 20 min) specifications of the process parameters were provided to the software. Thereafter, sonication was conducted using either continuous or pulsed sonication (the number of pulses per second are 3) methods. This was achieved through a bath type sonication bath (Make: Elma, Model: Elmasonic S 30H, Germany)(Chakraborty et al., 2020). In due course of the sonication process, it was ensured that the bath was always kept at constant temperature. To maintain the desired temperature in the sonication bath, a copper pipe was placed inside the sonication bath and was used to circulate water to the adjacent water bath. Since experimental investigations did not exceed 20 min, such manual control strategies were appropriate. Following extraction, the crude aqueous extract was filtered using 0.45 mm filter paper (Whatman #1, GE Healthcare Life Sciences, India) and was subsequently centrifuged at 7000 rpm for 15 min. The collected supernatant was stored at 4°C for the systematic evaluation of bioactive constituents (Sengar et al., 2020).

2.2.3 Experimental Design

Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA) was used to facilitate the FCD based RSM for the selection of desired combinations of independent variables. Three independent variables namely A_1 (extraction temperature), A_2 (extraction time), and A_3 (guava leaf to water ratio or loading ratio) were adopted with three respective coded levels (−1, 0, and 1). Four dependent or response variables namely TPC, TFC, % AA and VITC have been evaluated for the extract samples being obtained from the pulse or normal mode of the UAE. The selected design facilitated 20 experimental runs with 6 central replicates. The upper (0.2 g/mL,70 ^{o}C and 20 min) and lower (0.02 g/mL, 40 ^{o}C and 5 min) limits of the degrees of freedom (guava leaf to water ratio, temperature and time) were selected as per preliminary experiments. Thereby, reasonable boundaries have been set to achieve optimal process parameters that indicate reduced cost of operation in terms of lowest combinations of extraction time and extraction temperature and highest value of loading ratio. Using the following second-order polynomial mathematical expression, independent variables have been correlated with the responses (Zeng et al., 2020).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i \mathrm{X}_i + \sum_{i=1}^{n} \beta_{ii} \mathrm{X}^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i \beta_i X_j \qquad (2.1)$$

where Y is the desired response; β_0, β_i, β_{ii}, and β_{ij} are the coefficients of intercept and terms of linear, squared, and their interaction in the regression, respectively; X_i and X_j are the distinct independent variables ($i \neq j$).

Analysis of variance (ANOVA) was carried out to analyse the fitness of regression coefficients of linear, interaction and quadratic terms with the experimental data sets. The statistical significance of all terms were ascertained through the F-test and likelihood (p) values. The appropriate fitness of the polynomial models was further ascertained using the combinations of the determination coefficient (R^2), adjusted

determination coefficient (R^2 adj), coefficient of variance (% CV), and adequate precision of the respective model (Li et al., 2019).

2.2.4 Hot Water Extraction (HWE)

The HWE of the guava leaves was conducted only to serve as a control sample purpose. For this case, the best possible degrees of freedom based on the optimal values obtained from normal mode of sonication of guava leaf extract were chosen. This is also due to the reason that a relevant prior art confirmed that the HWE has been inferior to ultrasound aided extraction in terms of extraction efficacy (Soib et al., 2020). As a result, the best degrees of freedom of the UAE process were chosen for the HWE process efficacy in terms of TPC, TFC, % AA and VITC. These were obtained from the numerical optimization tool of the Design expert software.

2.2.5 Bioactive Analysis of the Extract

The guava leaf extract was investigated in terms of the TPC, TFC, % AA and VITC content in the guava leaf extract sample. The TPC was estimated using the FCR method (Tharasena & Lawan, 2014). Compared to the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric ion reducing antioxidant power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) methods, the deployed DPPH method was chosen due to its simplicity, rigorous procedure, lower cost and elimination of complex instrumentation and specialized training. Comparatively, FRAP and ORAC require expertise and relies on a more complex reaction mechanism (Rumpf et al., 2023). The antioxidants also need to be evaluated. However, due to limited availability of resources, such studies have not been considered to realize the targeted objectives of the Ph.D. thesis. However, they can be addressed in the near future.

The evaluation of antioxidant activity is not sufficient for the subjective evaluation of the competence of the extracted bioactives in the application domain. The anti-inflammatory and anti-diabetic activity of the antioxidants also need to be evaluated. However, due to limited availability of resources, such studies

have not been considered as the targeted objectives of the Ph.D. thesis. However, such studies can be considered in the near future.

Using a calibration curve being prepared with gallic acid, the TPC content of the sample was expressed in terms of mg of gallic acid equivalents (GAE)/g fresh weight of guava leaf (mg/g sample). $AlCl_3$ method was adopted to determine the TFC of guava leaf extract (Tharasena & Lawan, 2014). The TFC of the sample was determined in terms of mg of QE/g fresh weight of guava leaf. The AA of guava leaf extract was evaluated by using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay method (Sutanto et al., 2015). The samples were analysed for their absorbance at 510 nm. Thereby, the % AA was determined using Eq. (2.2):

$$A.A\,(\%) = \frac{A_C - A_S}{A_C} \times 100 \tag{2.2}$$

where A_c and A_s correspond to the absorbance of control and sample respectively.

For the VITC evaluation**,** 2,6-Dichlorophenol indophenol (DCPIP) titration assay was deployed to determine the ascorbic acid content of the guava leaf extract (Anjali et al., 2012). Using titration volumes V_1 and V_2, the VITC content was determined using Eq. (2.3):

$$VITC\,(mg/100\ g) = \frac{0.5 \times V_2 \times 20 \times 100}{V_1 \times 5 \times W_S} \tag{2.3}$$

where V_1 and V_2 are the volumes of dye consumed by standard and sample, respectively, and W_s is the sample mass.

2.2.6 Proximate Analysis

The moisture, fat and ash content of the guava leaf was evaluated using AOAC 2010 method (AOAC, 2010). Relevant expressions for the estimation of moisture content, fat and ash content have been presented as follows:

$$Moisture\ \ content\ \ (\%) = \frac{W_s - W_d}{W_s} \times 100 \tag{2.4}$$

where W_d and W_s denote the weights of the sample obtained and dried sample (105 °C), respectively.

$$Fat\ \ (\%) = \frac{W_f}{W_S} \times 100 \tag{2.5}$$

where $W_{f\ and}$ W_s are the weight of treated and sample taken respectively.

$$Ash\ \ content\ \ (\%) = \frac{W_a}{W_S} \times 100 \tag{2.6}$$

where W_a and W_s are the weight of treated and sample taken respectively.

2.2.7 Chlorophyll Content

The total chlorophyll (To-Chl), chlorophyll a (Chl-a), and chlorophyll b (Chl-b) content of guava leaf samples were quantified with a spectrophotometer (Model No.: UV-2600, Make: Shimadzu, Singapore) (Olatunde et al., 2018). The absorbance of collected supernatant of the UAE extract was evaluated at 663 and 645 nm. Chlorophyll a, chlorophyll b and total chlorophyll contents were estimated using the following expressions:

$$Chlorophyl\ l\ a\ (mg/g) = 12.7\ (A_{663}) - 2.69\ (A_{645}) \tag{2.7}$$

$$Chlorophyl\ l\ b\ (mg/g) = 22.9\ (A_{645}) - 4.68\ (A_{663}) \tag{2.8}$$

$$Total\ chlorophyl\ l\ (mg/g) = 20.2\ (A_{645}) - 8.02\ (A_{663}) \tag{2.9}$$

where A_{663} and A_{645} correspond to absorbance measured at 663 and 645 nm respectively.

2.2.8 Statistical Analysis

Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA) was deployed to examine the optimality of extraction conditions. Thus, the software tool was used to design and conduct experiments, analyse data, and develop mathematical models for the subsequent optimization of the UAE processes. All investigations were carried out in triplicate, and the findings were reported as the mean ± standard deviation ($p < 0.05$ was considered to be significant).

2.3 Ultrasound Assisted Extraction of Bioactives from Papaya Leaves

2.3.1 Raw Materials and Chemicals

The raw materials and chemicals deployed for the studies have been summarized in sections 2.1.1 and 2.1.2 of the Ph.D. thesis.

2.3.2 Sample Preparation

Fresh papaya leaves were acquired from IIT Guwahati's campus. Subsequently, the leaves were rinsed with water to eliminate any residual pollutants and were allowed to dry at room temperature. Thereafter, the leaves were cut into tiny bits and were eventually crushed into powder. Finally, in a beaker (100 mL capacity), UAE was performed by dispersing the papaya leaf powder in a sufficient quantity of DI water (Vuong et al., 2013).

2.3.3 Ultrasound-Assisted Extraction (UAE) of Papaya Leaf Extract

The procedure for the UAE of the papaya leaf system was similar to that being delineated in section 2.2.2 of the Ph.D. thesis.

2.3.4 Experimental Design

The experimental design methodology being deployed for the RSM study has been similar to that presented in sections 2.2.3 of the Ph.D. thesis

2.3.5 Hot Water Extraction (HWE)

The HWE of papaya leaf system was conducted as per the outlined procedure in section 2.2.4 of the Ph.D. thesis.

2.3.6 Extract Analysis

The TPC, TFC and AA of the prepared aqueous extract of papaya leaf was conducted as per the procedure outlined in section 2.2.5 of the Ph.D. thesis.

2.3.7 Statistical Analysis

Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA) was used to examine the optimality of extract conditions. All investigations were carried out in triplicate, and the findings were reported as the mean ± standard deviation ($p < 0.05$ was considered to be significant).

2.4 Ultrasound-Assisted Extraction of Bioactives from Papaya Pulp and Peel

2.4.1 Raw Materials and Chemicals

The raw materials and chemicals deployed for the studies have been summarized in sections 2.1.1 and 2.1.2 of the Ph.D. thesis.

2.4.2 Papaya Pulp (PP) and Papaya Peel (PPE) Extract Preparation

Fresh unripe green papaya fruit was acquired from a local supplier at IIT Guwahati campus. The papaya fruit was washed thoroughly with DI water to remove adhering contaminants and other impurities.

Thereby, natural degradation was minimized and contamination of bioactive ingredients was duly addressed. The papaya was then manually peeled with a sterile knife to obtain pulp and peel separately. After removing the seeds and mucilage, the papaya pulp (PP) and papaya peel (PPEE) were diced into small pieces (5 mm) (Vallejo-Castillo et al., 2020). The UAE (continuous and pulsed) was then performed by mixing a precise quantity of the PP and PPE chunks (5 mm) with the appropriate amount of water in a 100 mL beaker. These quantities were specified as per the FCD-RSM approach.

2.4.3 UAE Procedure for the Extraction of Bioactives from Papaya Pulp (PPU) and Peel (PPE)

The aqueous UAE of PPU and PPE (both continuous and pulsed mode) chunk samples was performed as per the method outlined in section 2.2.2 of the Ph.D. thesis. An ultrasonic bath (Make: Elma, Model: Elmasonic S 30H, Germany) equipped with digital time and temperature was used. The experiments were carried out in a 100 mL beaker in a controlled environment. The design based data set for process parameters (sonication time, extraction temperature, and loading ratio) was generated using the Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA). The software was provided with a set of lower (0.2 g/mL, 40 ^{o}C, 5 min) and upper (0.5 g/mL, 70 ^{o}C, 20 min) boundary data of the process parameters and these were based on a few experimental trial runs. Thereafter, pulsed sonication was performed using a bath type sonication system which was maintained at a constant extraction temperature for each combination of sonication time and loading ratio in due course of the extraction process. After extraction, the aqueous extract was centrifuged (Heraeus, Multifuge X3R, Thermo Fisher scientific) for 15 min at 8,382 g. The resultant supernatant was collected and kept at 4°C for the subsequent analysis of the bioactive constitution of the aqueous extract (Wani & Uppaluri, 2022a).

2.4.4 Experimental Design

Design Expert software (Version 13.0, State-Ease Inc., MN, USA) was used to optimize the UAE performance for bioactives extraction from PP and PPEE. For the selection of appropriate combinations

of independent process parameters, FCD based RSM was employed. For the process optimization purpose, the statistical design of experiments involved 20 experimental runs with six replicates at the centre point in a three-factored and three-level FCD approach. For each run, triplicate experiments were performed and the average values along with relevant deviation have been reported. The higher (0.5 g/mL, 70 ^{o}C, 20 min) and lower (0.2 g/mL, 40 ^{o}C, 5 min) limits of the independent variables (loading ratio, extraction time, and sonication time) were chosen based on preliminary investigations (Wani & Uppaluri, 2022c). The best fit model was determined based on a statistical analysis of the responses using a second order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i \mathrm{X}_i + \sum_{i=1}^{n} \beta_{ii} \mathrm{X}^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i \beta_i X_j \quad (2.10)$$

where β_0, β_i, β_{ii}, and β_{ij} ($i \neq j$) are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are the independent variables.

2.4.5 Hot Water Extraction (HWE) of PPU and PPE

The HWE of PP and PPEE was performed by using hot water bath (Make: EQUITRON, Model 7514). Briefly, in two separate 100 mL beakers containing a specific amount of water, PP and PPE pieces were placed and extraction was done in a hot water bath equipped with a temperature controller. The extraction conditions were determined using the best and most relevant data from the UAE of the PP and PPEE systems. After extraction, the solutions were filtrated using filter paper (Whatman #1, GE Healthcare Life Sciences, India) and thereby, the supernatant was collected and analysed for TPC, TFC, AA and VITC (Wani & Uppaluri, 2022b).

2.4.6 Analysis of Extract

The TPC, TFC, AA, VITC and moisture content analysis have been evaluated by following procedures outlined in sections 2.2.5 of the Ph.D. thesis.

2.4.7 Electric Consumption and CO_2 Emission

The electrical consumption and CO_2 emission for UAE and HWE respectively, were calculated using the following equations (Kusuma & Mahfud, 2017: Kusuma et al., 2019):

$$E_C = \frac{P \times t}{1000} \tag{2.11}$$

where E_C is electric consumption (kW h), P is power consumption (W) and t is time (h).

$$E_{CO_2} = \frac{E_C \times 800}{1000} \tag{2.12}$$

where E_{CO2} is CO_2 emission (kg) and E_c is the electric consumption (kW h).

2.4.8 Statistical Analysis

Design Expert software (Version 13.0, State-Ease Inc., MN, USA) was used to analyse the process extraction conditions. Pareto charts were prepared using Design Expert software (Version 13.0, State-Ease Inc., MN, USA). In addition, statistical parameters such as mean absolute error (MAE), root mean squared error (RMSE), sum of squared error (SSE) and mean squared error (MSE) were also calculated.

2.5 Ultrasound-Assisted Extraction of Pectin from Pomelo Peel

2.5.1 Raw Materials and Chemicals

The deployed raw materials and chemicals have been summarized in sections 2.1.1 and 2.1.2 of the Ph.D. thesis.

2.5.2 Sample Preparation

Pomelo fruit was purchased from local market (Amingoan, North Guwahati, India). Thereafter, it was thoroughly washed with DI water to remove any dirt and other macroscopic contaminants. Eventually, flavedo (greenish yellow peel) was removed manually using a sterile knife. Subsequently the albedos (spongy white peel) were separated from the pomelo fruit pulp. Thereafter, both flavedo and albedo were

cut into small pieces and dried in an oven (Oven: Forced Hot Air oven; Make: Reico equipment and instrument; Model: ROV/DC) at 40 °C for 12 h. The dried pieces were grinded (Grinder: Blade type; Make: Philips; Model: Classic) and sieved with a mesh (250 – 355 m sieve) (Methacanon et al., 2014). The peel powder was stored in an air tight container and was kept in a desiccator until further use. For a specified pH condition, the UAE extraction was then carried out by placing a precise amount of peel powder in a 200 mL beaker along with the appropriate amount of water. These have been set based on the FCD-RSM approach.

2.5.3 Pectin Extraction and Recovery

Two alternate extraction methods were deployed for pectin recovery from pomelo peels. These refer to the conventional acidic hot water extraction (AHWE), and advanced UAE technology. The efficacy of these two processes under their ideal process conditions was compared to evaluate upon the efficacy of the processes for pectin recovery from pomelo peels.

2.5.4 Procedure of Pectin Extraction from Pomelo Peel using UAE

The RSM with a FCD was deployed to investigate the influence and optimality of the UAE process conditions for the pectin recovery from pomelo peel. The procedure was similar to that of Shivamathi et al. (2019). Using an ultrasonic bath (Make: Elma, Model: Elmasonic S30 H, 37 kHz frequency), the aqueous extraction of pectin from pomelo peel was conducted using citric acid (CA) for both continuous ultrasounds assisted extraction (NUAE) and pulsed ultrasound assisted extraction (PUAE). Based on prior studies, the best choice of loading ratio (1:15 g/mL) and extraction temperature of 65°C were chosen for the UAE experiments (Wang et al., 2016). The UAE extraction process was performed for the variant combinations of pH and sonication time and these were obtained from the FCD based RSM design. For this purpose, the pH and sonication time were varied from 1.5 – 3.5 and 5 – 30 min respectively. These

variable ranges were chosen based on preliminary research. During sonication experiment, the operating temperature of the sonication bath was regulated through the controlled circulation of cold water.

The UAE experiment for a case was conducted as follows: Precisely, 1g of pomelo peel powder was dispersed in 15 mL of DI water and in a 100mL beaker. The mixture was then stirred for 10 min on a magnetic stirrer. Thereafter, the resultant mixture was subjected to sonication for various combinations of pH and sonication time provided by the software. After sonication, the aqueous solution was centrifuged (Heraeus, Multifuge X3R, Thermo Fisher scientific) at 8382 g for 15 min and the supernatant was collected and filtered using filter paper (Whatman #1, GE Healthcare Life Sciences, India). The concentrated supernatant was then added to cold ethanol (3:1 volume ratio of ethanol to solution). Thereafter, the mixture was left overnight for the mature of realization of the pectin precipitate. Subsequently, the precipitate was separated and was washed three times with ethanol and was finally dried at 50°C for 12 h (Methacanon et al., 2014). Prior to characterization, each experiment data set was based on a triplicate experimental approach.

2.5.5 Design of Experiments

The design of experiments, analysis of the response data and numerical optimization was performed using the Design Expert statistical software (version 13.0, Stat-Ease Inc., Minneapolis, USA). The design consisted 13 experimental runs and 5 replicates at the centre point. Triplicate experiments were carried out for each run, and the average values and pertinent deviation have been reported. The pectin extraction parameters were varied according to the FCD involving two factors and three levels (-1,0,1). The influence of design variables including pH and extraction time on responses such as yield and degree of esterification were studied. Thereby, parameters such as extraction temperature and loading ratio were kept fixed. The experimental data were fitted to a second-order polynomial equation to establish the relationship between independent variables and the responses (Wang et al., 2016). The generalised form of the equation is:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i \beta_i X_j \quad (2.13)$$

where β_0, β_i, β_{ii}, and β_{ij} ($i \neq j$) are the regression coefficients for intercept, linear, quadratic, and interaction terms, respectively, and X_i and X_j are the independent variables.

2.5.6 Acidic Hot Water Extraction (AHWE) of Pectin

AHWE was conducted as per the extraction procedures mentioned in a relevant prior art by Methacanon et al. (2014), and with few modifications. The AHWE based pectin extraction was based on the optimal process variable data set of the pulsed UAE system. After AHWE experiment, the aqueous solution was cooled and centrifuged (Heraeus, Multifuge X3R, Thermo Fisher scientific) at 8382 g for 15 min. Thereafter, subsequently followed procedure was similar to that mentioned in the section 2.5.4 of the Ph.D. thesis.

2.5.7 Pectin Characterisations

2.5.7.1 Proximate Analysis

The moisture, fat and ash of the extracted pectin was conducted as per the procedure mentioned in section 2.2.6 of the Ph.D. thesis. Pectin characterization in terms of its gelation ability with sugar and acid is an important parameter in assessing upon its quality for functional applications. Thus, the mentioned pectin characterization can be considered in future investigations.

2.5.7.2 Pectin Yield (PY)

The extracted pectin was weighed and its yield was determined on dry basis and with the following expression (Van Hung et al., 2021):

$$Yield\ (\%) = \frac{M_0}{M} \times 100 \quad (2.14)$$

where M_0 and M are the weights of extracted dried pectin and dried pomelo peel powder respectively.

2.5.7.3 Degree of Esterification (DE)

Titration method was deployed to estimate the degree of esterification (DE) of extracted pectin and with few modifications to the prior art method (Shivamathi et al., 2019). Precisely, 100 mg of extracted pectin was moistened with 1 mL of ethanol and thereby dispersed in 25 mL of DI water. The mixture was sonicated for 10 min to achieve complete dissolution. Thereafter, two drops of phenolphthalein were added to the mixture and the mixture was titrated with 0.1 M NaOH until pale pink colour appeared in the solution. The volume of NaOH consumed after this titration step was noted as V_1. Eventually 10 mL of 0.1 M HCl was added to the titrated sample and a colourless solution was obtained. After stirring for 10 min, the solution was once again titrated with 0.1 M NaOH until pink colour appeared in the solution. Thereby, the NaOH consumed for the second titration was noted as V_2.

Using V_1 and V_2, the DE was evaluated using the expression:

$$D.E\ (\%) = \frac{V_2}{V_1 + V_2} \times 100 \qquad (2.15)$$

2.5.7.4 Nutritional Analysis of Extracted Pectin

The TPC, TFC and AA of the extracted pectin was analysed as per the procedures summarized in section 2.2.5 of the Ph.D. thesis. The evaluated characteristics of the extract such as TPC, TFC and AA do not have highest subjective insights with respect to the constituents of the extract. Such studies require LCMS analysis and compounds quantification with mass spectra. This is beyond the scope of the objectives set for the Ph.D. thesis.

2.5.7.5 Fourier transform Infrared Spectroscopy (FTIR)

The FTIR spectra of extracted pectin samples were measured with a FTIR spectrophotometer (Shimadzu, Japan) and as per the procedure mentioned in a relevant prior art (Van Hung et al., 2021). For this

purpose, the powdered pectin was mixed with potassium bromide (KBr) and was compressed into a pellet, and the spectrum was recorded in transparent mode in the wavelength range of 4000 to 400 cm^{-1} and with 4 cm resolution.

2.5.7.6 Thermal Analysis (TGA and DSC)

The TGA (Thermogravimetric Analysis) profile of extracted pectin (6 – 7mg) was investigated with a thermogravimetric analyser (TGA-4000, PerkinElmer, U.S.A.) (Wang et al., 2016). The analysis was conducted in a controlled N_2 environment and at a heating rate of 10 °C/min in the temperature range of 25 - 700 °C. DSC analysis was performed using Netzsch DSC (204 F1 Phoenix, USA). For this purpose, 5 mg dried and finely ground extracted pectin sample was added into a standard aluminium crucible and immediately sealed. The crucible was heated from 30 °C to 400 °C at a heating rate of 10 °C/min in dynamic inert nitrogen atmosphere (50 mL/min). Simultaneously, an empty standard aluminium crucible was used as reference (Wang et al., 2014).

2.5.7.7 Morphological Analysis

The morphology of extracted pectin was investigated using FESEM instrument (field-emission scanning electron microscopy, Make Sigma 300: Zeiss, model: Germany) (Kusrini et al., 2018). For this purpose, standard double-sided adhesive tape was used to mount the dried pectin onto a metal stub. Further, to ensure upon the conductivity of the samples, they were coated with gold (Sputter coater 7620, Quorum) and were examined at an accelerating voltage between (2–5kV range)

2.5.7.8 X-ray Diffraction (XRD) Analysis

An X-ray diffractometer (D8 Advance, Bruker, Germany) equipped with Cu K-α radiation was used to conduct the XRD study (Tran et al., 2021). The extracted pectin powder was scanned in the diffraction angle range of 5° – 50° (step size: 0.02; time: 2 s/step).

2.5.8 Techno-Functional Properties

Since viscosity is an important techno-functional property of the pectin for its industrial utility, the same can be considered in the future work as an important characterization parameter. Such studies may involve its optimization or fine tuning or appropriate considerations after optimization of other desired responses.

2.5.8.1 Pectin Solubility (PS)

The solubility of dried extracted pectin powder was determined as per the procedure mentioned by Hosseini et al. (2019) and with few modifications. For this purpose, pectin samples (50 mg) were dispersed in 40 mL DI water and the mixture was stirred at room temperature for 20 min. The dispersion was thereafter centrifuged at 4000 rpm for 20 min. The undissolved portion of the mixture was then collected and dried in an oven at 50°C until constant weight of the sample was achieved. The % solubility was calculated based on the pectin dissolved in the water. The solubility of the pectin was determined in terms of the percentage of the total soluble matter. For this, the following expression was used:

$$PS\,(\%) = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \qquad (2.16)$$

2.5.8.2 Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

WHC and OHC of the pectin samples were determined using a method mentioned in the relevant prior art (Kazemi et al., 2019). The procedure is as follows. Precisely, 1 g of extracted pectin powder was placed into a 50 mL tube and 10 mL of distilled water or soy oil were added. Thereafter, the mixture was vortexed for 1 min and centrifuged at 4000 g for 20 min. Subsequently, the supernatant was discarded and the remnant was weighed. Based on the measured weights, the WHC and OHC were expressed as grams of water or oil held per gram of pectin.

2.5.8.3 Emulsification Properties

The emulsifying properties such as the emulsifying capacity (EC) and emulsion stability emulsion (ES) were evaluated as per the procedure mentioned by Hosseini et al. (2019). For this purpose, 10 mL of pectin solution (1% w/v concentration) was mixed with 5 mL of sunflower oil. The mixtures were then vortexed for 3 min. Thereafter, the emulsions were centrifuged at 1000 g for 5 min. The volumes were used to evaluate the EC as follows:

$$EC\,(\%) = \frac{V_F}{V_I} \times 100 \tag{2.17}$$

where V_F and V_I are the emulsion layer and total volume of the system respectively. Also, the emulsion was incubated for 30 min at 80 °C and was centrifuged at 948g for 5 min. Thereby, with the measured volumes, the emulsion stability (ES) was determined using the expression:

$$ES\,(\%) = \frac{V_R}{V_I} \times 100 \tag{2.18}$$

where V_R and V_I are the remained emulsion layer and initial volume of the system respectively.

2.5.9 Statistical Analysis

Design Expert (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA) was used to examine the optimality of extraction process conditions. All investigations were conducted in triplicate, and the findings were reported as the mean ± standard deviation ($p < 0.05$ was considered to be significant).

2.6 Encapsulation of Papaya Leaf Extract (PLE) by Ion Gelation Method

The PLE encapsulation experiments were conducted in two phases. In the first phase, the PLE extract was prepared at optimised UAE process conditions. In the second phase, the optimised PL extract was

encapsulated using ion gelation based extrusion technology with sodium alginate and pectin as the wall material.

2.6.1 Raw Materials and Chemicals

The deployed raw materials and chemicals for the investigations have been summarized in sections 2.1.1 and 2.1.2 of the Ph.D. thesis. The deployed sodium alginate possessed a molecular weight of 216.121 g/ml and a ratio of guluronic acid to mannuronic acid as 1:1.018.

2.6.2 Preparation of Papaya Leaf Extract (PLE)

The PLE was prepared as per the optimised data set obtained in due course of investigations addressed in 2.3.2 of the Ph.D. thesis. For this, a known quantity of papaya leaf powder was dispersed in a known quantity of DI water. Thereafter, the mixture was stirred on a magnetic stirrer for few minutes. Thereafter, the mixture was subjected to pulsed mode of sonication as per the optimum conditions obtained for the case. Eventually, the mixture was filtered with a filter paper (Whatman #1, GE Healthcare Life Sciences, India) and the supernatant was collected and stored in refrigerator for TPC and AA analysis.

2.6.3 Encapsulation of Papaya Leaf Extract (PLE)

The encapsulation of PLE was conducted with the ion gelation method. A two-step procedure delineated by Zhang et al. (2021) was followed along with minor modifications. Thus, calcium alginate-pectin beads were prepared by adding mixture of sodium alginate and pectin to the 100 mL of the optimized PLE. The process variables for the case were based on the design indicated by FCD based RSM. The pectin used corresponded to that obtained with the best process conditions reported in section 2.5.4 of the Ph.D. thesis. This mixture was heated at 50 ºC on a magnetic stirrer for 5 min at 600 rpm to achieve a uniform mixture. Thereafter, the mixture was kept aside for 20 min to allow the removal of air bubbles in the solution. Thereby, using a syringe pump (22G stainless steel needle, 0.5 – 3.5 mL/min flow rate), the solution was added drop wise to (3 – 9 % w/v) $CaCl_2$ solution. The height between the needle and the collecting solution surface was fixed at 10 cm. The beads subsequently obtained were allowed to solidify

in a stirred gelling bath (3 – 9 % w/v, $CaCl_2$) for 30 minutes. Thereafter, the system was filtered with a filter paper (Whatman #1, GE Healthcare Life Sciences, India) and the beads were washed thrice with DI water. Finally, the obtained solidified wet beads were divided in two portions. One portion of the beads was dried at 40 ºC for 24 h and were termed as dry beads. They were stored in a desiccator until further use. The other portion of the wet beads were stored in amber flask with and without PLE extract and were denoted as wet beads. In addition, the calcium alginate pectin beads were also prepared by dissolving sodium alginate (optimised constitution) and pectin in a mixture of $CaCl_2$ solution (optimised constitution) and PLE extract and the beads obtained by these two methods were compared with respect to their encapsulation efficiency.

2.6.4 Experimental Design for Encapsulation

FCD-RSM design was applied for the statistical design of experiments. These experiments were conducted by considering sodium alginate concentration, $CaCl_2$ concentration and syringe pump flow rate as independent variables and encapsulation efficiency as a dependent variable.

2.6.5 Encapsulation Efficiency (EE)

The encapsulation efficiency of the calcium alginate pectin beads was determined using the following expression (Singh et al., 2018).

$$EE\ (\%) = \frac{T_e}{T_i} \times 100 \qquad (2.19)$$

where T_e and T_i refer to the encapsulated TPC in beads and PLE respectively.

2.6.6 Effect of Pectin on Encapsulation Efficiency

To evaluate the influence of pectin on the encapsulation efficiency, calcium alginate beads were prepared without pectin and encapsulation efficiencies were compared with the calcium alginate pectin beads. The procedure involved the addition of either sodium alginate (optimized constitution) to the PLE and the extrusion of the mixture in $CaCl_2$ solution) or by extruding sodium alginate solution (optimized

constitution) in a mixture of PLE and $CaCl_2$ solution. For all cases, the encapsulation efficiency of beads prepared with and without pectin were compared.

2.6.7 Effect of Different Needle Size on Encapsulation Efficiency

To evaluate the influence of alternate needle sizes on the encapsulation efficiency, needles with 18G, 21 G, 23G and 24G size were deployed. The encapsulation efficiency was determined as per the procedure outlined in section 2.6.5 of the Ph.D. thesis.

2.6.8 Characterization of Papaya Leaf Extract (PLE)

The TPC and AA of the prepared PLE extracts were determined as per the procedure outlined in section 2.2.5 of the Ph.D. thesis.

2.6.9 Characterization of Calcium Alginate Pectin Beads

2.6.9.1 Total Polyphenolic Content and Antioxidant Activity

For the estimation of TPC content of encapsulated beads, firstly, 1g encapsulated beads were dispersed into 20 mL (5% w/v) tri-sodium citrate di-hydrate solution. The solution was kept under stirring condition for 15 min until complete dispersion occurs for the encapsulated beads. Thereafter, the mixture was filtered with Whatman #1 paper and the resultant solution was subjected for TPC analysis (Sampaio et al., 2019). The TPC of encapsulated beads was estimated using FCR method (section 2.2.5 of the Ph.D. thesis). Eventually, the TPC was expressed as mg GAE/g sample. For AA analysis, the procedure outlined in section 2.2.5 of the Ph.D. thesis was followed.

2.6.10 Release Kinetics of Phenolic Compounds in Water

The total phenolic release kinetics studies from calcium alginate pectin beads was carried out as per the procedure reported by Zhang et al. (2021) and with minor modifications. To summarize, at room temperature (about 25 °C), firstly, 1.0 g of calcium alginate beads were dissolved in 20 mL DI water and the mixture was stirred continuously for 30 min. Thereafter, at predetermined intervals (5 – 180 min), 0.5

mL of the supernatant was taken out from the beaker for the TPC analysis conducted as per the procedure outlined in section 2.2.5 of the Ph.D. thesis and an equivalent volume of DI water was then added to the beaker to maintain constant volume of the mixture. Thereby, the phenolic compounds release rate was determined with expression:

$$\text{Re}lease\ (\%) = \frac{M_t}{M_\alpha} \times 100 \tag{2.20}$$

where M_t and M_∞ represents the mass fraction of polyphenols released at time t, and infinite time ($t = \infty$) respectively.

Alternate kinetics models expressions were used to predict the polyphenolic constituents release rates from the calcium alginate pectin beads. These include zero-order, Higuchi, Korsmeyer-Peppas and Peppas-Sahlin models, respectively. These have been represented as presented as (Zhang et al., 2021):

$$\frac{M_t}{M_\alpha} = k_0 t \tag{2.21}$$

$$\frac{M_t}{M_\alpha} = k_H t^{1/2} \tag{2.22}$$

$$\frac{M_t}{M_\alpha} = k_p t^n \tag{2.23}$$

$$\frac{M_t}{M_\alpha} = k_d t^m + k_r t^{2m} \tag{2.24}$$

where M_t/M_α denotes the mass fraction of polyphenols released at time t (min); k_0, k_H and k_p respectively denotes the zero-order release, Higuchi rate and Korsmeyer kinetic constants; n denotes the release exponent; k_d and k_r denotes the Fickian and relaxation kinetic constants, and m denotes the Fickian diffusion exponent.

2.6.11 Stability of Total Phenolic Compounds during Storage

The stability characteristics of calcium alginate pectin beads under different storage temperatures were assessed. To do so, firstly, a portion of the calcium alginate pectin beads were stored at room temperature. The other portion of beads were stored in the refrigerator at 4 °C for 30 days respectively (Ćorković et al., 2021). At predetermined time intervals of 5 days, the TPC of encapsulated beads was determined by following FCR method (presented in section 2.2.5 of the Ph.D. thesis). Thereby, the results were expressed as mg GAE/g sample.

2.6.12 DSC, FTIR and FESEM analysis of Calcium Alginate Pectin Beads

The FTIR, DSC and FESEM analysis of calcium alginate pectin beads have been evaluated by following respective procedures summarized in sections 2.5.7.4, 2.5.7.5, and 2.5.7.6 respectively of the Ph.D. thesis.

2.6.13 Particle Size of the Beads

The particle size distribution of the dried beads was measured using Delsa Nano C particle size analyser (Make: Beckman Coulter) (Joni et al., 2020).

2.6.14 Statistical Analysis

Design Expert software (Version 13.0.5.0, 64-bit, 18 May 2021, State-Ease Inc., MN, USA) was used to examine the optimality of process conditions. All investigations were carried out in triplicate, and the findings were reported as the mean ± standard deviation ($p < 0.05$ was considered to be significant).

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Chapter 3:

RSM based Ultrasound-Assisted Extraction of Bioactive Constituents from Guava Leaves

Chapter 3

RSM based Ultrasound-Assisted Extraction of Bioactive Constituents from Guava Leaves

This chapter elucidates on the parametric optimality of ultrasound-assisted extraction (UAE) of bioactive constituents from guava leaves. Response surface methodology (RSM) was adopted to design and optimize the associated process parameters. Section 3.1 presents a brief overview of the area of research. In the following section, experimental design details based on RSM modelling approach have been presented. Along with the relevant discussion, sections 3.3 and 3.4, respectively elaborate upon the model fitting and ANOVA summary for Normal and Pulsed mode of UAE respectively. Thereafter, section 3.5 focuses on the response surface characteristics of Normal UAE (NUAE) and Pulsed UAE (PUAE) system. The following section provides a comparative assessment of both modes of the UAE and associated hypothesis. Section 3.7 presents a discussion with respect to best available literature data. Finally, section 3.8 presents an overall summary of the research findings in the context of best UAE process parameters and future scope.

Overview

In this chapter, the ultrasound-assisted extraction (UAE) of bioactive compounds (total flavonoid content (TFC). antioxidant activity (AA), total polyphenolic content (TPC) and vitamin C (VITC) as response variables) has been addressed for guava leaves (Psidii guajava folium). For comparative purposes, two sonication modes, namely NUAE and PUAE mode have been considered and the response variables have been addressed as a function of extraction temperature (ET), sonication time (ST) and guava leaf to water ratio (loading ratio or LR) by targeting the design of experiments using response surface methodology

(RSM). Literature comparison of best data indicates promising performance of the UAE in comparison with conventional extraction process.

3.1 Introduction

This chapter discusses RSM-based UAE of guava leaves using water as an extractant. The purpose of the conducted investigations was to scope and optimize the efficacy of UAE in conjunction with hot water extraction (HWE). The critical influence of degrees of freedom such as LR, ET and ST on the commonly desired bioactive constituent characteristics such as TPC, TFC, AA and VITC have been targeted in experimental investigations that were augmented with RSM-based ANOVA analysis. Face centred design (FCD) based RSM was deployed to design the experiments for subsequent model development and optimization of process variables. Pertinent observations have been documented in terms of comparative criticality of the degrees of freedom, best fit model and efficacy of the PUAE mode.

3.2 Experimental Data Summary during Normal UAE and Pulsed UAE of Bioactives from Guava Leaf Extract

From proximate analysis, the (% w/w) moisture, (% w/w) ash and (% w/w) fat content of the guava leaf were obtained as 80.2 ± 0.43 %, 4.2 ± 0.57 % and 0.84 ± 0.61 % respectively. Chlorophyll a, chlorophyll b and total chlorophyll contents of guava leaf extracts were obtained as 6.2 ± 0.04 mg/g, 4.2 ± 0.12 mg/g and 10.4 ± 0.09 mg/g respectively.

The maturity of the guava leaves does influence its characteristics such as the content of parameters like total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA). With maturity, it is very likely that these parameters reach a saturation and subsequent reduction due to aging phenomena. As leaves mature, their bioactive compounds are very likely to alter in their concentration and bio-availability. The obtained UAE characteristics of bioactives extraction from guava leaves indicated that among the chosen degrees of freedom, the LR was the most important followed by ET and ST. Such an insight is not available in the literature due to the consideration of the fixed choice of the

LR. Table 3.1 conveys that for a variation in LR from 0.02 to 0.2 g/mL, the TPC, TFC, VITC and % AA yield varied significantly during NUAE process and as 15.49 – 67.95 mg GA/g sample, 44.73 – 264.47 mg QE/g sample, 5.75 – 21.56 mg/100g, and 50.19 – 77.50 %, respectively. For the PUAE case, these value ranges enhanced marginally and varied as 19.49 – 72.62 mg GA/g sample, 28.08 – 288.13 mg QE/g sample, 6.15 – 18.83 mg/100g, and 30.08 – 86.07 % respectively (Table 3.2). The best experimental data corresponds to the highest combinations of 0.2 g/mL LR, 70 °C ET and 20 min ST for both NAUE and PUAE. Prior art conveys that phenolic content in fresh guava leaves alters as 13 - 26 mg (GAE)/ gram of dried leaves (Li et al., 2014). After UAE extraction, the TPC altered as 15 to 75 mg GAE/g of sample. This affirms that UAE achieved about 34 – 86 % TPC extraction efficiency in the aqueous extract. From an engineering perspective, about 95% of left behind biomass needs appropriate utilization strategies. Some of these refer to application as a feedstock for the production of value added products such as compost, biofuels etc., Alternatively, the incineration of biomass can facilitate a waste to energy scheme for electrical power and heat generation.

Process scale-up requires the utilization of the optimized extraction parameters after their validation. Accordingly, the UAE can serve as an efficient downstream processing technique. Similar strategies are to be considered for waste to energy and waste to value conversion schemes. Also the cost effectiveness based feasibility of the bioactive compounds extraction (UAE process) and their application needs to be addressed along with the environmental sustainability studies.

Incidentally, Zeng et al. (2020) reported TPC variation from 53.77 – 59.63 mg GA/g sample for a variation in ET from 50 – 70 °C and ST from 25 – 45 min but for fixed choice of the LR (0.025 g/mL). Similarly, Li et al. (2019) reported TFC of the extract to vary from 4.16 – 5.1 mg QE/g sample for a variation in ET from 60 – 80 °C and ST from 25 – 45 min and fixed LR (0.025g/mL). Thus, potentially improved bioactive constituent characteristics have been confirmed in the findings of this work in comparison with the literature. Thereby, these findings instil confidence to consider UAE of guava leaves with water solvent as a viable low cost extraction method for bio-active constituents.

Table 3.1: Parametric and response data summary of RSM based normal UAE of guava leaf-water system.

S.No	Temperature (°C)	Time (min)	Guava leaf / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)	Vitamin C (mg/100g)
1.	40	5	0.02	15.49 ± 0.80	44.73 ± 0.99	50.19 ± 0.41	5.75 ± 0.93
2.	70	20	0.2	65.57 ± 0.94	289.77 ± 0.79	80.30 ± 0.84	14.16 ± 1.06
3.	55	5	0.11	55.23 ± .06	185.67 ± 0.82	67.51 ± 0.24	14.53 ± 0.10
4.	40	5	0.2	58.81 ± .08	220.3 ± 0.7	71.86 ± 0.11	10.04 ± 1.40
5.	70	12.5	0.11	61.58 ± 0.41	225.22 ± 0.25	73.03 ± 0.37	12.33 ± 0.86
6.	55	12.5	0.11	60.17 ± 0.78	205.66 ± 0.55	69.47 ± 0.73	18.04 ± 1.25
7.	70	5	0.02	27.27 ± 0.51	53.33 ± 0.47	55.10 ± 0.25	8.77 ± 0.50
8.	70	20	0.02	30.49 ± 0.45	103.2 ± 0.34	62.68 ± 0.12	6.38 ± 0.94
9.	55	12.5	0.02	27.89 ± 0.43	60.86 ± 0.12	60.68 ± 0.25	15.13 ± 0.34
10.	55	12.5	0.11	60.22 ± 0.57	206.55 ± 0.64	69.86 ± 0.35	17.04 ± 1.25
11.	55	12.5	0.11	60.32 ± 0.51	207.55 ± 0.82	69.62 ± 0.85	18.8 ± 0.22
12.	55	12.5	0.11	59.25 ± 0.54	206.34 ± 0.84	69.27 ± 0.82	20.85 ± 1.01
13.	55	12.5	0.11	58.55 ± 0.22	208.77 ± 0.82	70.92 ± 0.64	18.9 ± 0.41
14.	40	12.5	0.11	54.45 ± 0.39	195.22 ± 0.60	70.27 ± 0.47	14.23 ± 0.48
15.	40	20	0.2	63.66 ± 0.39	245.03 ± 0.47	74.91 ± 0.74	19.08 ± 0.38
16.	55	12.5	0.2	63.17 ± 0.75	251.04 ± 0.94	75.69 ± 0.45	25.71 ± 0.78
17.	40	20	0.02	23.57 ± 0.8	50.6 ± 0.41	61.89 ± 0.47	9.6 ± 0.19
18.	55	12.5	0.11	58.78 ± 0.59	207.89 ± 0.25	71.86 ± 0.59	19.59 ± 0.28
19.	55	20	0.11	62.31 ± 0.30	218.33 ± 0.63	72.62 ± 0.55	20.04 ± 0.49
20.	70	5	0.2	62.9 ± 0.74	235.66 ± 0.70	74.38 ± 0.27	11.54 ± 0.82

Table 3.2: Parametric and response data summary of RSM based pulsed UAE of guava leaf-water system.

S.No	Temperature (°C)	Time (min)	Guava leaf / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)	Vitamin C (mg/100g)
1.	40	5	0.02	19.49 ± 0.21	28.08 ± 0.45	30.08 ± 0.04	6.15 ± 0.18
2.	70	20	0.2	75.57 ± 0.13	325.11 ± 0.94	76.55 ± 0.02	13.64 ± 0.25
3.	55	5	0.11	58.23 ± 0.01	190.25 ± 0.89	69.54 ± 0.05	14.59 ± 0.04
4.	40	5	0.2	53.66 ± 0.03	230.32 ± 0.05	70.19 ± 0.36	10.32 ± 0.46
5.	70	12.5	0.11	64.58 ± 0.33	222.44 ± 0.23	75.88 ± 0.17	12.2 ± 0.09
6.	55	12.5	0.11	62.82 ± 0.48	192.55 ± 0.87	80.24 ± 0.09	19.89 ± 0.17
7.	70	5	0.02	29.27 ± 0.24	38 ± 0.82	45.78 ± 0.47	9.04 ± 0.04
8.	70	20	0.02	31.49 ± 0.17	47.8 ± 0.38	45.51 ± 0.16	7.38 ± 0.22
9.	55	12.5	0.02	28.89 ± 0.04	26.6 ± 0.09	58.41 ± 0.15	17.67 ± 0.26
10.	55	12.5	0.11	62.32 ± 0.05	191.66 ± 0.03	80.29 ± 0.33	21.04 ± 0.32
11.	55	12.5	0.11	62.25 ± 0.22	190.33 ± 0.28	82.82 ± 0.29	20.8 ± 0.19
12.	55	12.5	0.11	62.55 ± 0.13	193.11 ± 0.41	80.3 ± 0.19	20.54 ± 0.02
13.	55	12.5	0.11	62.67 ± 0.04	189.77 ± 0.12	81.06 ± 0.10	19.58 ± 0.10
14.	40	12.5	0.11	58.45 ± 0.02	190 ± 0.82	68.6 ± 0.31	14.78 ± 0.34
15.	40	20	0.2	66.66 ± 0.11	267.33 ± 0.01	75.59 ± 0.09	21.76 ± 0.07
16.	55	12.5	0.2	68.17 ± 0.09	264.38 ± 0.05	88.77 ± 0.06	24.98 ± 0.08
17.	40	20	0.02	27.57 ± 0.09	48.32 ± 0.55	38.09 ± 0.22	9.03 ± 0.42
18.	55	12.5	0.11	61.17 ± 0.08	199 ± 0.73	81.7 ± 0.14	20.9 ± 0.05
19.	55	20	0.11	65.31 ± 0.15	221.89 ± 0.12	75.79 ± 0.29	20.34 ± 0.03
20.	70	5	0.2	64.9 ± 0.42	300.05 ± 0.31	74.38 ± 0.20	11.78 ± 0.10

3.3. Model fitness and ANOVA findings for Normal UAE of Guava Leaf Extract

The analysis of variance (ANOVA) data for TPC, TFC, VITC, and AA in due course of NUAE process have been summarised in Table 3.3. For all responses, the R^2, adj R^2, C.V (%) and adequate precision for the quadratic model imply that the model is best fit model. Also, the lack of fit was insignificant for all cases. All these demonstrate best fitness of the quadratic model. For the quadratic model representing TPC case, the linear terms (A, B, C), interaction terms (AB, AC) and quadratic terms (A^2, B^2 and C^2) had been significant along with higher F and lower p values. The ANOVA parameters associated with the best fit quadratic model representing total TFC as a function of ET, ST, and LR indicated similar pattern. With higher F and lower p values, the linear terms (A, B, C), interaction terms (AB, BC), and quadratic terms (A^2, B^2 and C^2) had been significant. The quadratic model fitness characteristics for AA case affirmed similar inferences. For the case, p values ($p < 0.0001$) have been convincing towards best fitness of quadratic model. For the VITC case, linear (B, C), interaction terms (AB, BC) and square terms (A^2, B^2), have been significant with higher F and lower p ($p < 0.0001$) values. Henceforth, even for the case, the quadratic model is also the best fit model.

The best fit quadratic model expressions for the responses namely TPC, TFC, AA and VITC are respectively presented using Eqs. (10), (11), (12), and (13) and as follows:

$$TPC = 59.88 + 3.18A + 2.59B + 19.54C - 0.88AB - 1.59AC - 0.47BC - 2.35A^2 - 1.60B^2 - 12.34C^2 \quad (3.1)$$

$$TFC = 206.33 + 15.13A + 16.72B + 92.91C + 9.17AB - 0.14AC + 2.89BC + 3.83A^2 - 4.39B^2 - 50.44C^2 \quad (3.2)$$

$$A.A(\%) = 70.63 + 1.64A + 3.34B + 8.66C - 0.16AB + 0.28AC - 1.29BC + 0.33A^2 - 1.25B^2 - 3.13C^2 \quad (3.3)$$

$$VITC = 19.25 - 0.55A + 1.86B + 3.49C - 1.58AB - 0.40AC + 1.28BC - 6.53A^2 - 2.52B^2 + 0.61C^2 \quad (3.4)$$

Table 3.3: Best fit model parameters and ANOVA data summary of alternate responses during normal UAE of guava leaf-water system.

	Total Polyphenol		**Total Flavonoids**		**Antioxidants Activity**		**Vitamin C**	
Components	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**
Model	562.42 (Quadratic)	< 0.0001 (Quadratic)	1616.42 (Quadratic)	< 0.0001 (Quadratic)	52.52 (Quadratic)	< 0.0001 (Quadratic)	25.71 (Quadratic)	< 0.0001 (Quadratic)
A	100.72	< 0.0001	316.86	< 0.0001	13.02	0.0048	1.39	0.2652
B	66.69	< 0.0001	387.15	< 0.0001	54.04	< 0.0001	15.87	0.0026
C	3757.06	< 0.0001	11948.14	< 0.0001	363.85	< 0.0001	55.68	< 0.0001
AB	6.16	0.0324	93.17	< 0.0001	0.095	0.7647	9.16	0.0128
AC	20.04	0.0012	0.021	0.8878	0.29	0.5990	0.59	0.4593
BC	1.78	0.2123	9.23	0.0125	6.46	0.0293	5.94	0.0350
A^2	15.11	0.0030	5.59	0.0396	0.15	0.7068	53.57	< 0.0001
B^2	6.96	0.0248	7.32	0.0221	2.08	0.1800	8.00	0.0179
C^2	416.04	< 0.0001	968.31	< 0.0001	13.06	0.0047	0.47	0.5083
Lack of Fit	2.24	0.1990	1.18	0.4307	3.04	0.1242	1.58	0.3142
R squared		0.9980		0.9993		0.9793		0.9586
Adequate Precision		72.675		131.288		26.864		17.261

3.4. Model fitness and ANOVA findings for Pulsed UAE of Guava Leaf Extract

For all responses, Table 3.4 summarises the ANOVA results. The adequacy of the models for all responses were ensured through the lack of fit, fitted R^2, adjusted R^2 and adequacy precision values. A non-significant ($p > 0.05$) lack of fit, higher combinations of R^2, adjusted R^2, C.V (%) and adequacy precision (> 4), affirmed that the quadratic model is adequate to predict the response. For the TPC case, A, B, C, AB, BC, A^2 and C^2 have been significant terms. All these possessed high F values and $p < 0.0001$. Hence, they reasonably confirm good fitness of the quadratic model. Similarly, for the TFC case, the model possessed a high F value (1191.45) and a lower p value ($p < 0.0001$). All terms including terms (A, B, C), interaction terms (BC, AC), and quadratic terms (A^2, B^2 and C^2) possessed higher F and lower p values for the quadratic model representing TFC. Similarly, for the AA case, all terms have been significant terms with high F values and $p < 0.0001$ for the best fit quadratic model.

The best fit quadratic model for the VITC response can be confirmed through A, B, C, AB, AC, BC, A^2 and B^2 significant terms. Further, they all possessed high F values and $p < 0.0001$.In summary, for all cases, quadratic models have been the best fit models.

$$TPC = 62.46 + 4.00A + 4.10B + 19.23C - 1.02AB + 0.81AC + 1.67BC - 1.20A^2 - 0.94B^2 - 14.18C^2 \quad (3.5)$$

$$TFC = 194.44 + 16.93A + 12.37B + 119.84C - 2.80AB + 14.76AC + 4.00BC + 9.24A^2 + 9.09B^2 - 51.49C^2 \quad (3.6)$$

$$A.A = 80.97 + 3.55A + 2.16B + 16.76C - 1.44AB - 2.25AC - 0.021BC - 8.59A^2 - 8.16B^2 - 7.24C^2 \quad (3.7)$$

$$VITC = 20.49 - 0.80A + 2.03B + 3.32C - 1.76AB - 0.99AC + 1.51BC - 7.05A^2 - 3.08B^2 + 0.78C^2 \quad (3.8)$$

Table 3.4: Best fit model parameters and ANOVA data summary of alternate responses during pulsed UAE of guava leaf-water system.

Components	Total Polyphenol		Total Flavonoids		Antioxidants Activity		Vitamin C	
	F value	**P value**	**F value**	**P value**	**F value**	**P value**	**F value**	**P value**
Model	803.34 (Quadratic)	< 0.0001 (Quadratic)	1191.45 (Quadratic)	< 0.0001 (Quadratic)	329.98 (Quadratic)	< 0.0001 (Quadratic)	85.98 (Quadratic)	< 0.0001 (Quadratic)
A	219.43	< 0.0001	193.55	< 0.0001	72.87	< 0.0001	8.39	0.0159
B	231.33	< 0.0001	103.35	< 0.0001	26.80	0.0004	53.88	< 0.0001
C	5073.80	< 0.0001	9692.21	< 0.0001	1619.79	< 0.0001	144.63	< 0.0001
AB	11.51	0.0069	4.23	0.0668	9.55	0.0114	32.68	0.0002
AC	7.14	0.0234	117.68	< 0.0001	23.27	0.0007	10.23	0.0095
BC	30.67	0.0002	8.65	0.0147	2.083E-003	0.9645	23.92	0.0006
A^2	5.42	0.0422	15.83	0.0026	116.92	< 0.0001	179.26	< 0.0001
B^2	3.36	0.0968	15.32	0.0029	105.64	< 0.0001	34.11	0.0002
C^2	759.42	< 0.0001	492.12	< 0.0001	83.05	< 0.0001	2.22	0.1671
Lack of Fit	3.16	0.1160	1.69	0.2901	2.24	0.1981	3.35	0.1051
R squared		0.9986		0.9991		0.9966		0.9872
Adequate Precision		90.563		111.422		64.097		31.155

3.5. Response Surface Characteristics of Bioactives during Normal UAE of Guava Leaf Extract

3.5.1. Effect of Normal UAE Process Parameters on Total Polyphenols

The Design Expert software enabled greater insights into the binary interaction of any two process parameters on the desired response. Fig. 3.1(a – c) depicts the influence of process parameters on the NUAE of TPC characteristics of the guava leaf extract. For a fixed choice of LR of 0.11 g/mL and for a variation in ST from 8.75 – 16.25 min and ET from 47.5 – 62.5°C, the TPC response surface plot indicated a variation from 50 – 59.75 mg GAE/g with maximum value being obtained as 65.57 ± 0.94 mg GAE/g at LR of 0.2 g/mL, 20 min of ST and a ET of 70 °C (Fig. (3.1a)). The TPC can be analysed to vary significantly as a non-linear increasing function with ST and ET and thereby confirmed its sensitivity towards heat and prolonged sonication. From the 3D surface plot, it is evident that there has been an initial enhancement in the TPC yield up to a combination of 16.25 min ST and 62.5 °C ET and thereafter the yield achieves a steady plateau.

Comparatively, Zeng et al. (2020) conveyed that for the considered system, the TPC varied from 53.77 – 59.63 mg GA/g sample for a wider variation in ET 50 –70°C and ST 25 – 45 min but for fixed choice of the LR (0.025 g/mL). Hence, it is apparent that higher LR was not investigated by the authors and hence the TPC variation has not been significant. Similar inference can be deduced from the findings of Liu et al. (2014) who reported lower TPC range 12.7 – 26.1 mg GA/g sample for a mixed variation in ET 20 – 80°C and ST 5 – 10 min but for fixed choice of the LR (0.083 g/mL). Thus, lower LR did not enhance TPC yield and hence LR is a critical and important degree of freedom to influence TPC characteristics.

The TPC response characteristics with respect to LR and ET also affirms a non-linear trend. For the fixed choice of ST of 12.5 min, the TPC varied 15 – 65 mg GA/mL for a variation in LR from 0.07 – 0.18 g/mL and ET from 47.5 – 62.5°C. Among both parameters, the LR has been significant to influence the pertinent TPC characteristics. The obtained profile indicated reaching a plateau based on marginal non-

linear variations. However, the TPC sensitivity has not been as significant as that of ET and ST. For comparison purpose, no data exists in the mentioned prior art and hence this work is anticipated to serve a useful bench mark for further research in the chosen theme.

Similarly, for fixed choice of ET (55 °C), the TPC yield varied as 22 – 58 mg GA/mL for a variation in ST and LR as 8.75 – 16.25 min and 0.07 – 0.16 g/mL respectively. The graph (Fig. (3.1c)) affirmed that the TPC enhanced and reached a plateau for the variation in ST and LR. Among both process parameters, the influence of concentration has been dominant. No relevant literature is available to obtain comparative analysis for the same. Hence, this work can serve as a useful benchmark data for such analyses.

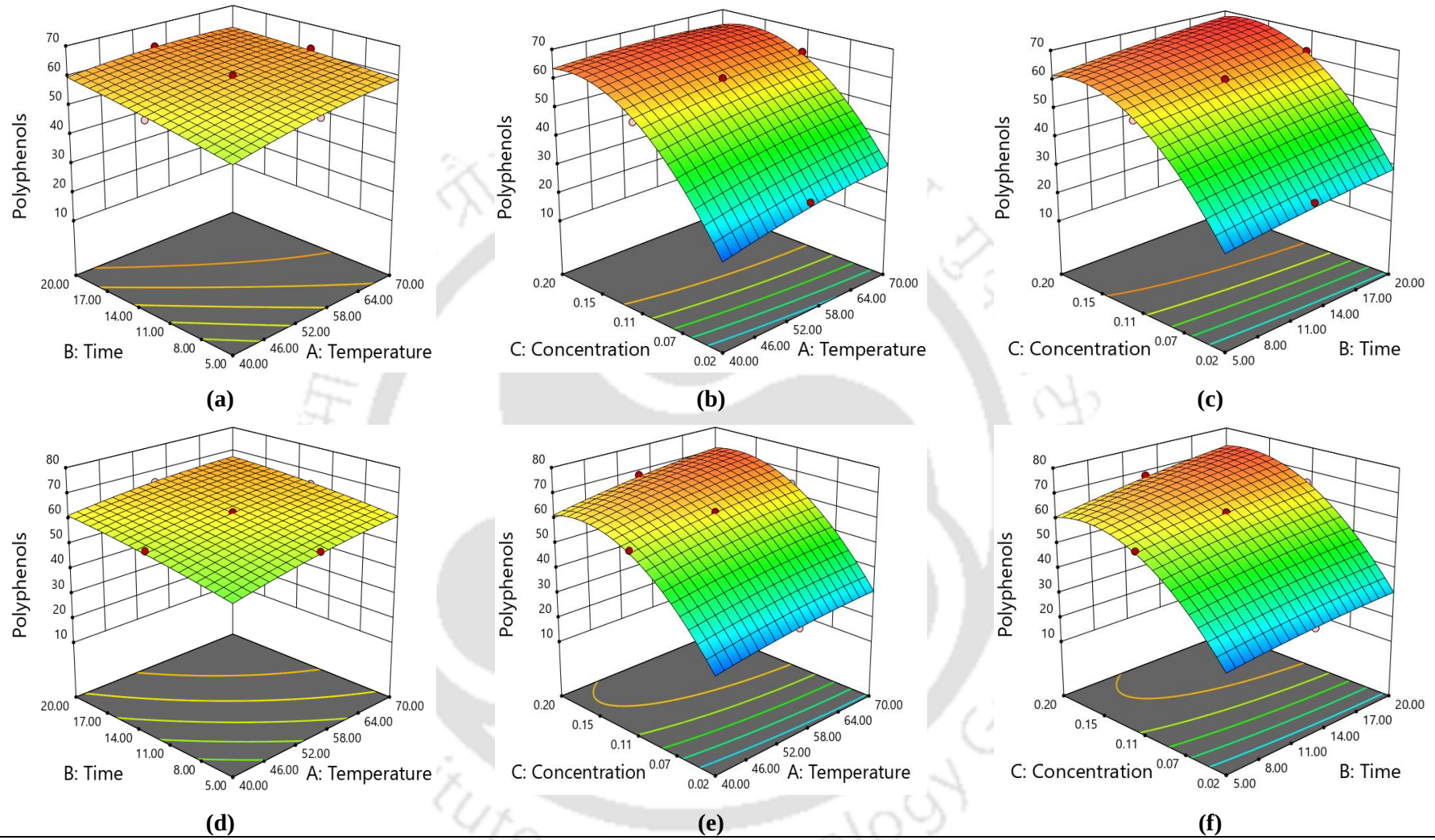

Figure. 3.1 : Response surface plots of total polyphenols during normal UAE (a – c) and pulsed UAE (d – f) of guava leaf system.

3.5.2. Effect of Normal UAE Process Parameters on Total Flavonoids

The impact of process factors on the NUAE of TFC characteristics of guava leaf extract has been illustrated in Fig. 3.2 (a – c). The TFC response surface plot indicated a variation from 198.25 – 230.75 mg QE/g for a fixed choice of LR of 0.11 g/mL and a variation in ST from 8.75 – 16.25 min and ET from 47.5 – 62.5°C with a highest amount of TFC 289.77 ± 0.79 mg QE/g being obtained for the case of LR of 0.2 g/mL at 70 °C ET and 20 min ST. The 3D surface plot shows that the TFC yield increases initially and upto a combination of 12.5 min ST and 55 °C ET. Thereafter, the TFC yield reaches a steady plateau. The TFC can be seen to vary with ST and ET and as a marginally increasing non-linear function. This confirms limited susceptibility to heat and extended sonication. In comparison, Li et al. (2019) reported that for the NUAE, the TFC ranged varied marginally from 4.16 – 5.1 mg QE/g sample with a variation in ET 60 – 80°C and ST 25 – 45 min but for lower chosen choice of LR (0.025 g/mL) for the studied system. As a result, it is clear that the authors did not consider a higher LR. For this reason, the TFC variation was not significant.

A non-linear trend is also apparent in the TFC response characteristics with respect to LR and ET. The TFC enhanced from 50 – 215 mg QE/mL for a LR variation from 0.07 – 0.16 g/mL and ET variation from 47.5 – 62.5°C and for a fixed ST of 12.5 min. Once again the LR did considerably the influence the TFC. Based on marginal nonlinear variations, the deduced profile suggested hitting a plateau. The TFC sensitivity was not as significant as that being obtained for ET and LR case. Since no data exists in the relevant prior art for comparison, the conducted investigation in this work have been expected to serve as a valuable basis for future research in this domain.

Similarly, the TFC yield enhanced from 40 – 212.5 mg QE/mL for enhancement in ST and LR from 8.75 – 16.25 and 0.75 – 0.16 g/mL, respectively for a fixed choice of ET 55 °C. The TFC improved and achieved a plateau for ST and LR variations, as shown in Fig. 3.2(c). From the 3D plot, it can be seen that LR but not ST influencing TFC significantly. Due to no relevant literature, a comparative evaluation

could not be addressed. However, the research may be used as a reference data for further research in the chosen theme.

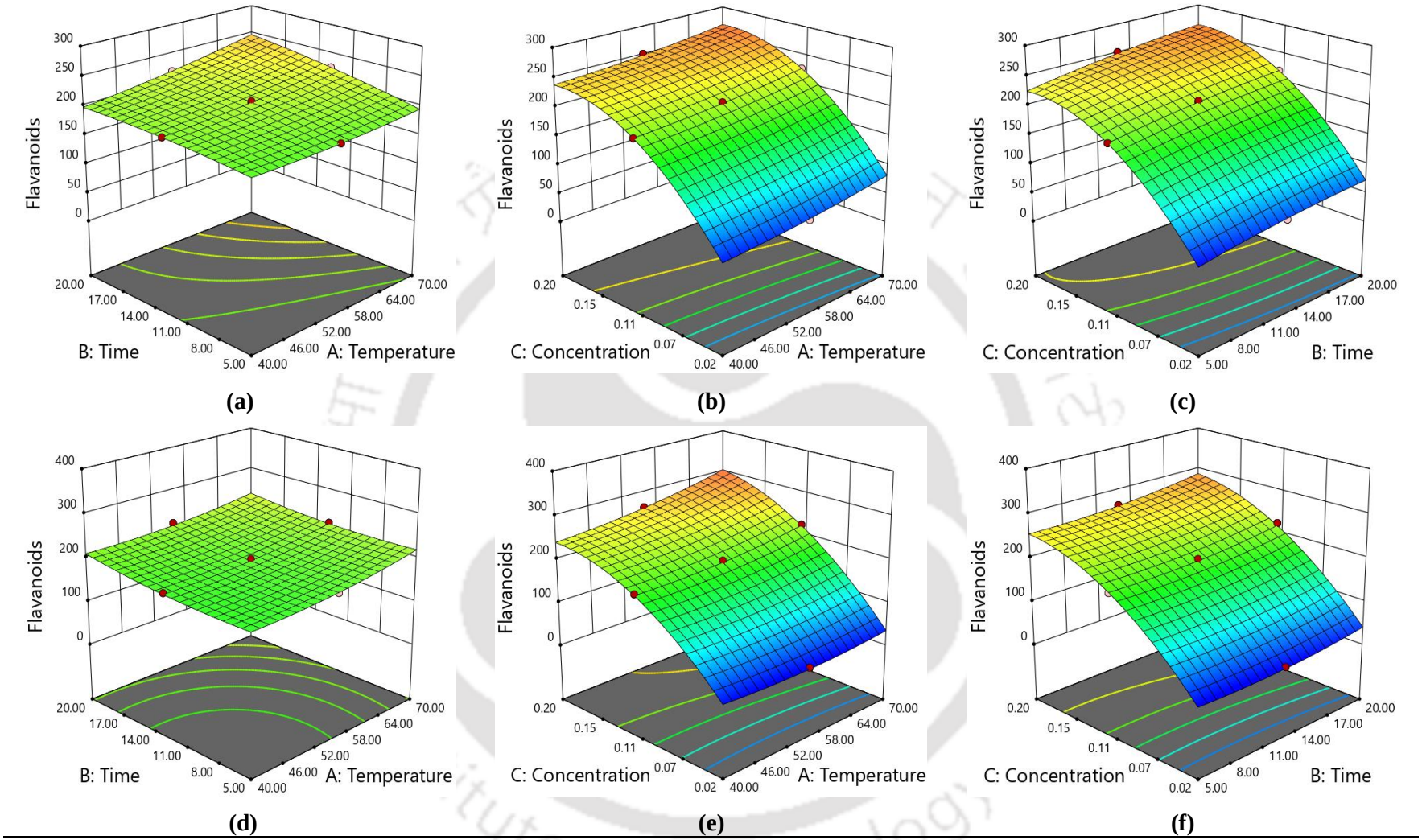

Figure. 3.2: Response surface plots of total flavonoids during normal UAE (a – c) and pulsed UAE (d – f) of guava leaf system.

3.5.3. Effect of Normal UAE Process Parameters on Antioxidant Activity

Fig. 3.3 (a – c) depicts the effect of process parameters on the AA characteristics of NUAE of guava leaf aqueous extract. The AA response plot affirmed the AA value range of 64 – 70 % for a fixed LR of 0.11 g/mL and ST variation from 8.75 – 16.25 min and ET variation from 47.5 – 62.5°C. The highest and the lowest AA was recorded as 80.30 ± 0.84% at a LR of 0.2 g/mL and 50.19 ± 0.41% at a LR of 0.02 g/mL respectively. The 3D surface plot demonstrates that the AA yield initially enhances up to a combination of 12.5 ST and 55 °C ET. Thereafter, it reaches and settles into a stable plateau. The AA varied considerably with ST and ET and as a fairly enhancing non-linear function. This affirms that the AA is vulnerable to heat and sustained sonication. Due to the non-availability of similar data in the literature, the obtained data trends can serve as a useful resource for further investigations.

Additionally, a non-linear trend has been apparent in the AA response characteristics associated to LR and ET combination. The percentage AA varied as 57 –74.5 % for a LR of 0.07 – 0.16 g/mL and an ET of 47.5 – 62.5°C and for 12.5 min fixed ST. Among both parameters, the LR has a significant effect on the AA trends. The computed profile confirms a plateau and hence marginal non-linear trend. On the other hand, the AA trend has not been as sensitive as that obtained for ET and LR. Due to absence of comparable data in the prior art, the obtained trends can be used for reference based analyses.

Similarly, for a given choice of ET 55 °C, the % AA yield varied from 52 –71.5 % for an alteration in ST and LR from 8.75 – 16.25 min and 0.07 – 0.16 g/mL, respectively. As demonstrated in Fig. (3c), AA improved and reached a plateau for binary modification in ST and LR. The 3D plot conveys that LR had the most critical influence among both process parameters. Due to the non-availability of comparable data in the prior art, the indicated trends can be used as a basis for further investigations targeting finer characterisation.

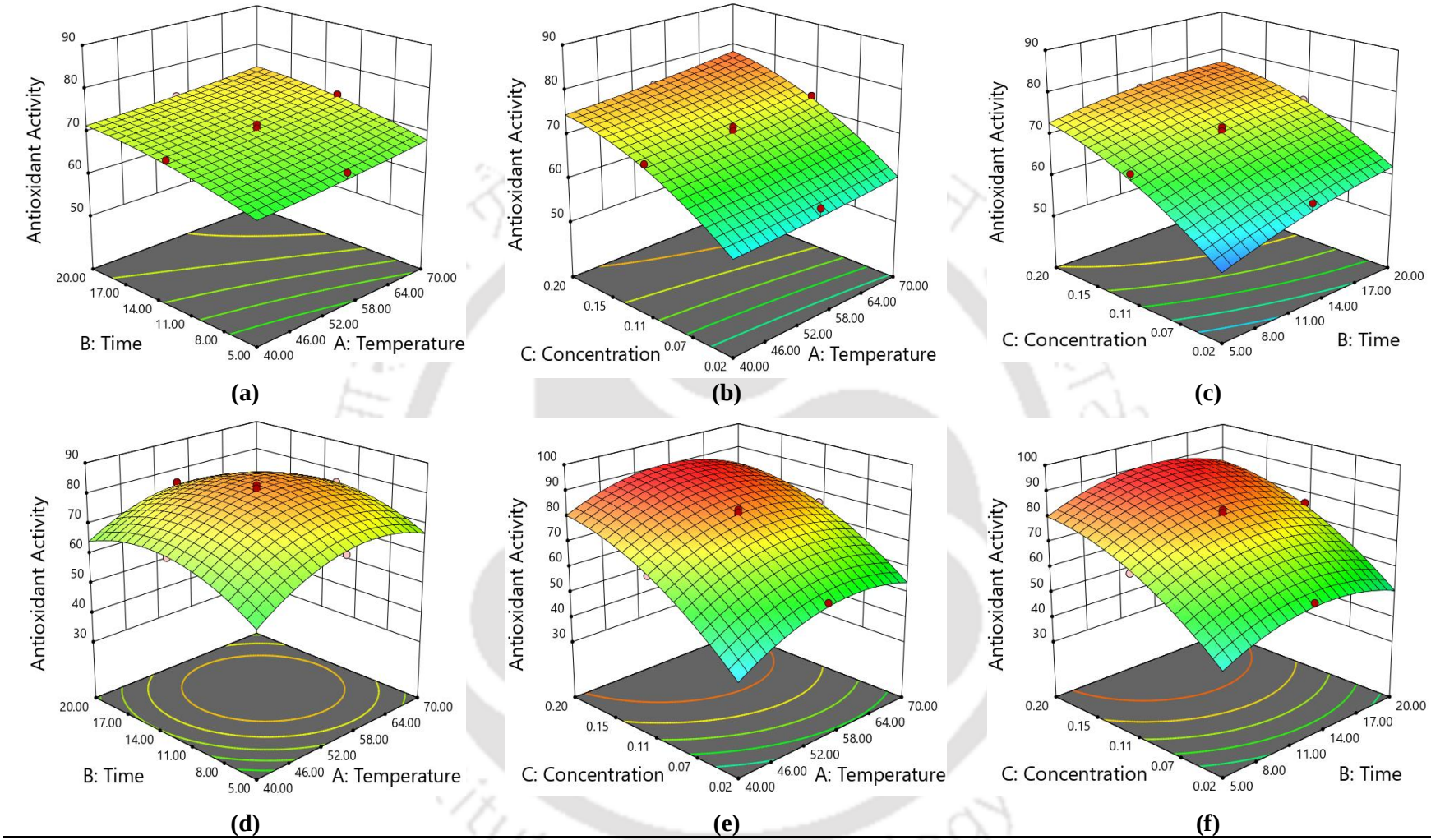

Figure. 3.3: Response surface plots of total antioxidant activity during normal UAE (a – c) and pulsed UAE (d – f) of guava leaf system.

3.5.4. Effect of Normal UAE Process Parameters on Vitamin C

The influence of process factors on the VITC characteristics of guava leaf extract has been shown in Fig. 3.4 (a – c). For a LR of 0.11 g/mL and an alteration of ST from 8.75 – 16.25 min and ET from 47.5 – 62.5°C, the VITC response surface plot confirmed a wider range of VITC content (7 – 14 mg/100g). The maximum VITC (25.71 ± 0.78 mg/100g) has been obtained at a LR of 0.2 g/mL, ST of 12.5 min and ET of 55 °C. With respect to ET, the VITC yield first increases upto a combination of 12.5 min of ST and 55 °C of ET. Thereafter, the VITC followed a declined trend. The VITC changed significantly with ET as a non-linear function to infer upon its sensitivity with heat and prolong sonication. However, the VITC profile indicated non-linear increase with ST. The obtained data can provide useful insights into further characterisation due to non-availability of such data in the literature.

Similarly, with respect to combination of LR and ET, the VITC response characteristics exhibited a non-linear pattern. For a LR variation from 0.07 – 0.16 g/mL and ET variation from 47.5 – 62.5°C for a fixed ST of 12.5 min, the VITC varied from 9 – 18 mg/100g. Among the chosen parameters, the LR had a significant influence on the VITC trend. The computed profile affirm a plateau based on minor non-linear trends with respect to LR but a significant non-linear trend with respect to ET. On the other hand, the sensitivity of the VITC was lower than that obtained for ET and LR. No data has been provided in the literature for comparative analysis.

Similarly, the VITC yield ranged from 13 – 16.25 mg/100g with an enhancement in ST and LR of 8.75 – 16.25 min and 0.07 – 0.16 g/mL, respectively, and for a fixed choice of ET 55 °C. The 3D graph (Fig. (3.4c)) confirmed that the VITC improved and achieved a plateau for ST and but for LR variations, there has been an initial increase in the yield of VITC and marginal reduction thereafter. Among both parameters, LR has the significant influence. No data has been provided in the literature for comparative analysis.

Similar response surface plot trends have been analysed for the PUAE cases and have not been further explained. For the NUAE case, the optimised value for TPC, TFC, % AA and VITC have been 67.95 mg GAE/g, 264.47 mg QE/g, 77.50 % and 21.56 mg/100g respectively and the ideal process-product characteristics refer to ultrasonic ET of 62.19 °C, ST of 14.94 min, and loading proportion of 0.19 g/mL. For the PUAE case, TPC, TFC, % AA, and VITC had maximum values of 72.62 mg GAE/g, 288.13 mg QE/g, 86.07 %, and 18.83 mg/100g, respectively for the optimal choice UAE process-product characteristics as ultrasonic ET of 66.21 °C, ST of 14.31 min, and loading of 0.18 g/mL.

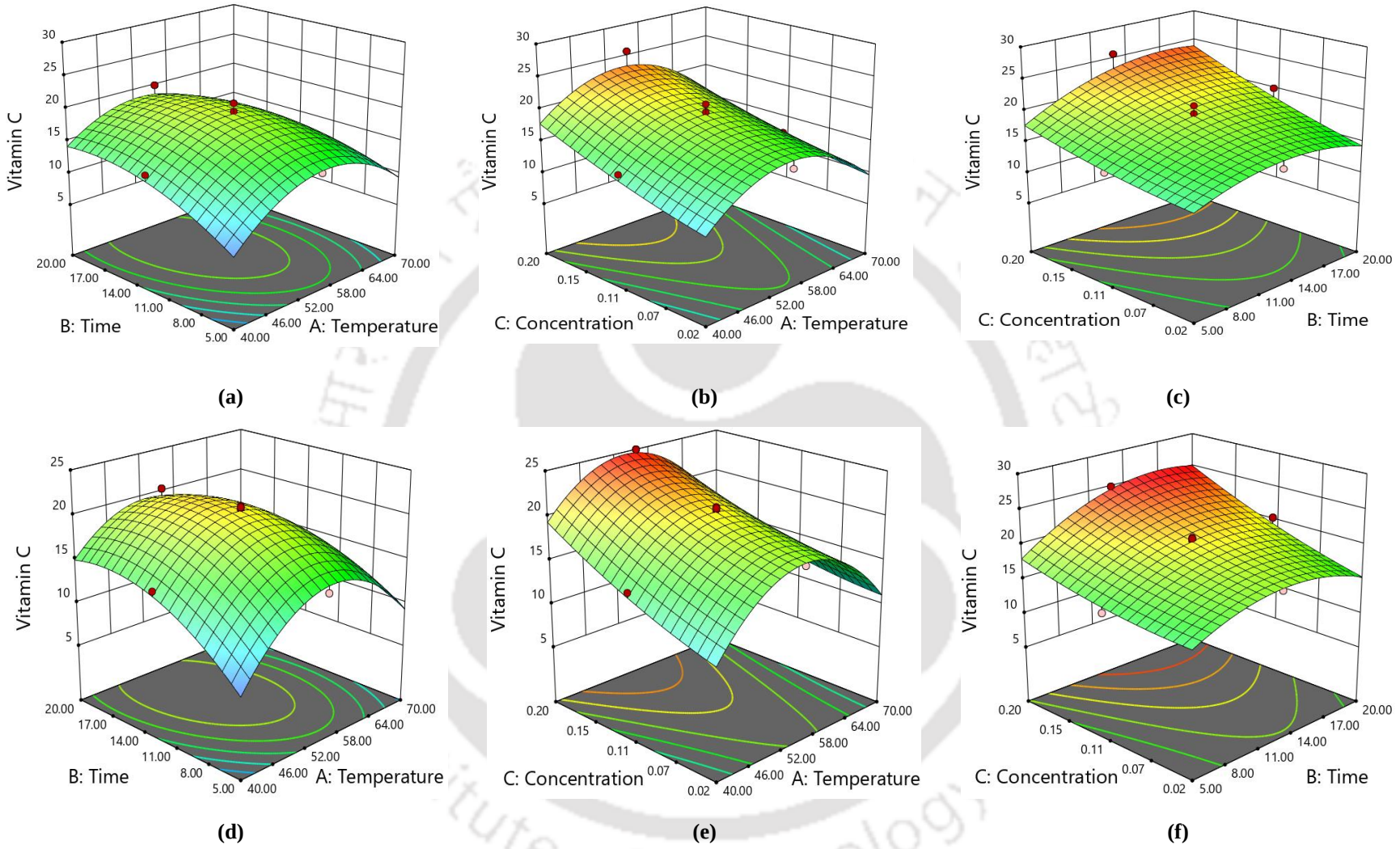

Figure. 3.4: Response surface plots of vitamin C during normal UAE (a – c) and pulsed UAE (d – f) of guava leaf system.

3.6. Comparative Assessment of Normal UAE and Pulsed UAE of Bioactives from Guava Leaf System

A critical comparison of the best findings of both NUAE and PUAE affirmed that significant variation did not exist in the optimal values. However, excepting VITC content in the guava leaf extract, the pulse mode enabled the maximum content of other bioactive constituents. This is possibly due to shorter ST duration of ultrasonic waves in the medium and hence better agitation (Kumar et al., 2021). Further, a lag in the diffusion of components during the lapse phase may also have its relevance in both modes and the order of the effect of degrees of freedom was: LR > ET > ST. Further, for both modes, ET had relatively less influence upon the extraction of VITC in the guava leaf extract. Thus, cavitation produced during PUAE mode of UAE has been highly effective (Lavilla & Bendicho, 2017).

For both UAE modes, it was found that while other bioactive constituents enhanced with ET enhancement from 40 to 70°C, it was contrary for the VITC case. Similar trends have been reported in the literature (Sun et al., 2011). For the bio-constituents, the enhancement has been due to their greater solubility and diffusion and improved penetration of the solvent into the solid matrix at higher ETs (Christou et al., 2021). The detrimental influence of ET on VITC was due to the loss of relevant thermosensitive bioactive compounds. Similarly, for an increase in ST from 5 – 20 min, all mentioned bioactive components enhanced for both modes of UAE. This was not the case for VITC for which the maximum extraction was obtained at 12.5 min of ST for both modes of UAE. Also, LR has been a critical factor to substantially influence upon the extraction of bioactive compounds. A large quantity of solvent would allow better release of bioactive constituents from the solid matrix. This is due to better and easier access to the matrix (Patience et al., 2021). For both modes of the UAE, the highest amount of extraction was observed for a LR of 0.2 g/mL. Thus, in summary PUAE provided marginally best performance of the UAE guava leaf extraction process.

3.7 Efficacy of Critical Findings of Bioactives Extraction from Guava Leaf with respect to Prior Art

Table 3.5 summarize the relative comparison of the work conducted till now along with the optimised findings of this work in the field of UAE of the guava leaves. The table affirms that the prior art did not emphasize upon the critical influence of LR. It shall be noted that the composition of various plant sources does get altered with various factors such as agro-climatic zones, soil conditions, maturity of leaves, harvesting methods, etc. However, the thesis work primarily focused on the process optimality for bioactives extraction. These factors are beyond the scope of the targeted perspectives. In other words, nonetheless, such studies must be considered and the subjective depth of the reported findings shall be assessed for an alteration of these factors in the near future. In the present work, it can be concluded that the LR played a very important role in the extraction of the bioactive compounds followed by other two process parameters, viz., ET and ST. Comparatively, the UAE conducted by Zeng et al. (2020) indicated 17.6 % lower concentrations of TPC, and 86.1 % lower LR value with respect to those being obtained for the optimised PUAE process. In addition, Li et al. (2019) performed ultrasound assisted water extraction of flavonoids using BBD based on the RSM from guava leaves and found 82.2 % reduced TFC and 86.1 lower LR in comparison with the best optimal data set reported in this work. These variations can also be accounted to the variations in the sample preparation prior to the UAE. In addition, compared with the best optimal findings reported in this work (PUAE), the HWE indicated lower bioactives constitution by 40 %, 36.03 %, 32.7 % and 56.7 % for the TPC, TFC, AA and VITC respectively. Also, with respect to extraction efficiency of NUAE and PUAE, it can be deduced that this study provided better findings than those indicated in the previous studies. This work emphasised upon room ET of drying for 2 – 3 h as a pre-treatment step. This may not be the case in few literatures that adopted prolonged drying ST at 40 °C. Thus, it can be elucidated that the pulse mode of extraction did comparatively better in terms of TPC, TFC, and AA.

Table 3.5: Optimal data summary of process and product parameters of UAE-guava leaf system.

Extraction Process	Temperature (°C)	Time (min)	Loading ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE /g)	Antioxidant Activity (%)	Vitamin C (mg/100g)	Literature
UAE	63.23	38.38	0.025	59.82	-	-	-	Zeng et al. (2020)
UAE	72.69	35.15	0.025	-	5.12	-	-	Li et al. (2019)
UAE	59.8	5.1	12.1	26.1	-	-	-	Liu et al. (2014)
HWE	62.19	14.94	0.19	43.7	184.3	57.87	8.15	This work
NUAE	62.19	14.94	0.19	67.95	264.47	77.50	21.56	This work
PUAE	66.21	14.31	0.18	72.62	288.13	86.07	18.83	This work

3.8 Summary

Several important findings can be deduced from the findings of this work for the UAE of guava leaves. These are as follows:

- Firstly, the UAE has been demonstrated to be beneficial and promising low cost environmentally friendly technology for the effective extraction of bioactive constituents from guava leaves.
- Secondly, except for the VITC content, the PUAE performed better in comparison with the NUAE.
- Thirdly, the optimal process-product characteristics were ultrasonic ET of 62.19 °C, ST of 14.94 min, and LR of 0.19 g/mL.
- Fourthly, these findings are comparatively marginal for the NUAE with TPC, TFC, AA and VITC as 72.62 mg GAE/g, 288.13 mg QE/g, 86.07 % and 18.83 mg/100g respectively.
- Fifthly and most importantly, the non-linear effect of the LR was comparatively higher than the ET and ST in achieving the maximum extraction of the mentioned bioactive component characteristics. Non-linearities associated to the binary process parametric interaction can be concluded from the response surface plots to follow the rank as LR – ET > LR – ST > ET – ST.

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Chapter 4:

Comparative Efficacy of Hot Water and Ultrasound-Assisted Extraction of Bioactives from Papaya Leaf System

Chapter 4

Comparative Efficacy of Hot Water and Ultrasound-Assisted Extraction of Bioactives from Papaya Leaf System

This chapter elaborates upon the optimal process-product characteristics of a bath type sonication and hot water system based aqueous extraction of papaya leaves. Response surface methodology (RSM) was adopted to design and optimize the associated process parameters. Section 4.1 presents a brief overview of the area of research. Following this, in section 4.2 experimental details along with RSM modelling based design of experiments approach have been presented. Along with the relevant discussions, sections 4.3 and 4.4 respectively elaborate upon the model fitting and ANOVA summary for NUAE and PUAE respectively. Thereafter, in section 4.5, the response surface characteristics of NUAE and PUAE process have been delineated. In the following sections, the optimal process-product characteristics of both modes of UAE have been summarized. Thereafter, section 4.7 provides a comparative assessment of both modes of the UAE and associated hypothesis. Subsequently, section 4.8 presents a discussion of the best findings with respect to best available literature data. Finally, section 4.9 presents an overall summary of the research findings in the context of best UAE process parameters and future scope.

Overview

In this chapter, UAE (NUAE and PUAE) of papaya leaves was conducted by considering three independent variables (ST, ET and LR) and three responses or dependent variables (TPC, TFC and AA). The experiments were designed with the Design Expert software (Version 13.0.) with FCD approach to customize studies that targeted the combined influence of the mentioned independent variables on the desired responses. The software generated 20 alternate combinations of the chosen degrees of

freedom. Thereby, experiments were conducted in triplicate to evaluate their influence on the chosen responses. Further, HWE was targeted as a control case for which design variables have been specified as the optimal value set being obtained from numerical optimization of the ANOVA based best fit model. The UAE was conducted with both continuous and pulsed modes of sonication (NUAE and PUAE). For comparative purposes, normal and pulsed mode have been considered and the response variables have been addressed as a function of ET, ST and papaya leaf to water ratio (loading ratio or LR) by targeting the design of experiments using RSM. Literature comparison of best data confirmed promising performance of the UAE in comparison with conventional extraction process.

4.1. Introduction

In this this chapter, the RSM based optimisation of UAE aqueous process for papaya leafy system has been delineated. The purpose of the conducted investigations was to scope and optimize the efficacy of UAE in conjunction with HWE. The RSM with FCD has been adopted to evaluate upon the associated sensitivities, relationships and optimalities of the process parameters and responses. Such an approach considered ET, ST and LR as degrees of freedom and TPC, TFC, and % AA as the desired responses of the aqueous papaya extract. Pertinent observations have been documented in terms of comparative criticality of the degrees of freedom, best fit model and efficacy of the pulsed mode.

4.2. Experimental Details

Among all process parameters, LR followed by ET and ST played a vital role in the UAE of bioactives from the papaya leaves. Table 4.1 and 4.2 affirm upon the direct influence of LR (0.02 to 0.2 g/mL) on the TPC, TFC and % AA values of the aqueous extract system. For the NUAE, the extraction yield of TPC, TFC and % AA varied from 32.83 – 148.15 mg GA/g sample, 47.9 – 531.5 mg QE/g sample and 18.65 – 86.3% respectively. For the PUAE case, corresponding values varied as 52.92 – 158.88 mg GA/g sample, 56.18 – 547.41mg QE/g sample and 26.82 – 89.24 % respectively (Table 4.2). Among all cases, the best value set has been obtained for the case of 0.2 g/mL LR, 70 ^{o}C ET and 20 ST.

Table 4.1: FCD-RSM based parametric and response variable data summary of bioactives extraction from papaya leaf through normal UAE.

S.No	Temperature (°C)	Time (min)	Papaya leaf / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)
1.	40	5	0.02	32.83 ± 0.79	47.9 ± 0.82	18.65 ± 0.73
2.	70	20	0.2	148.15 ± 0.98	531.5 ± 0.24	86.38 ± 0.42
3.	55	5	0.11	119.79 ± 0.39	291 ± 0.47	68.16 ± 0.82
4.	40	5	0.2	129.67 ± 0.42	435.82 ± 0.82	73.49 ± 0.37
5.	70	12.5	0.11	131.41 ± 0.47	299.04 ± 0.46	74.91 ± 0.45
6.	55	12.5	0.11	123.43 ± 0.56	301.5 ± 0.85	71.51 ± 0.19
7.	70	5	0.02	60.0 ± 0.82	81.26 ± 0.88	40.27 ± 0.03
8.	70	20	0.02	81.13 ± 0.56	96.51 ± 0.83	45.13 ± 0.39
9.	55	12.5	0.02	50.57 ± 0.27	79.33 ± 0.42	32.24 ± 0.11
10.	55	12.5	0.11	124.88 ± 0.04	304.3 ± 0.47	72.45 ± 0.70
11.	55	12.5	0.11	121.03 ± 0.13	303.21 ± 0.94	70.72 ± 0.29
12.	55	12.5	0.11	125.43 ± 0.59	293.87 ± 0.85	71.84 ± 0.33
13.	55	12.5	0.11	123.5 ± 0.24	302.67 ± 0.32	69.91 ± 0.78
14.	40	12.5	0.11	110.43 ± 0.28	274.38 ± 0.22	61.08 ± 0.40
15.	40	20	0.2	141.81 ± 0.38	506.57 ± 0.29	80.97 ± 0.16
16.	55	12.5	0.2	135.59 ± 0.15	505.27 ± 0.20	81.01 ± 0.47
17.	40	20	0.02	53.27 ± 0.22	100.27 ± 0.24	26.29 ± 0.39
18.	55	12.5	0.11	123.68 ± 0.85	300.56 ± 0.28	70.56 ± 0.22
19.	55	20	0.11	139.59 ± 0.42	320.54 ± 0.22	75.03 ± 0.29
20.	70	5	0.2	134.4 ± 0.37	511.43 ± 0.18	82.46 ± 0.25

Table 4.2: FCD-RSM based parametric and response variable data summary of bioactives extraction from papaya leaf through pulsed UAE.

S.No	Temperature (°C)	Time (min)	Papaya leaf / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)
1.	40	5	0.02	52.92 ± 0.36	56.18 ± 0.36	26.82 ± 0.74
2.	70	20	0.2	158.88 ± 0.74	547.41 ± 0.14	89.24 ± 0.16
3.	55	5	0.11	109.48 ± 0.33	284.7 ± 0.83	68.7 ± 0.42
4.	40	5	0.2	118.78 ± 0.36	420.49 ± 0.22	79.08 ± 0.36
5.	70	12.5	0.11	131.28 ± 0.34	338.01 ± 0.47	77.65 ± 0.74
6.	55	12.5	0.11	116.45 ± 0.41	309 ± 0.82	73.51 ± 0.22
7.	70	5	0.02	59.92 ± 0.36	87.16 ± 0.26	39.97 ± 0.39
8.	70	20	0.02	66.61 ± 0.26	113.41 ± 0.17	56.97 ± 0.11
9.	55	12.5	0.02	48.95 ± 0.20	79.9 ± 0.42	38.5 ± 0.29
10.	55	12.5	0.11	119.16 ± 0.25	303.4 ± 0.45	71.6 ± 0.34
11.	55	12.5	0.11	118.86 ± 0.16	305.78 ± 0.36	74.5 ± 0.41
12.	55	12.5	0.11	120.27 ± 0.33	299.7 ± 0.82	72.89 ± 0.40
13.	55	12.5	0.11	121.97 ± 0.78	298 ± 0.33	72.6 ± 0.09
14.	40	12.5	0.11	115 ± 0.36	289.33 ± 0.82	68.44 ± 0.08
15.	40	20	0.2	151.55 ± 0.24	482.3 ± 0.70	83.54 ± 0.19
16.	55	12.5	0.2	137.71 ± 0.70	498.6 ± 0.84	82.45 ± 0.84
17.	40	20	0.02	65.58 ± 0.13	97.28 ± 0.28	34.1 ± 0.33
18.	55	12.5	0.11	118 ± 0.42	298.6 ± 0.41	72 ± 0.50
19.	55	20	0.11	128.65 ± 0.24	316.14 ± 0.24	78.1 ± 0.37
20.	70	5	0.2	139.13 ± 0.79	510.15 ± 0.62	82.2 ± 0.25

4.3. Model fitting and ANOVA for Normal UAE

For the NUAE case, Table 4.3 presents the analysis of variance (ANOVA) data for TPC, TFC, and AA. Among all alternate models, the best fit model refers to a second order quadratic model for all cases. Corresponding fitness of the best model has been affirmed with very high values of regression coefficients (R^2 values of 0.9988, 0.9995 and 0.9989 respectively), higher F values (892.10, 2198.87 and 995.88 respectively), acceptable values for adequate precision (98.664, 145.102 and 105.604 respectively). Further, for all cases, the overall model p-value was low (< 0.0001).

The lack of fit for the best fit quadratic model refers to the values of 1.43, 2.21 and 0.91 for TPC, TFC and AA respectively. Thereby, the lower F values for the lack of fit affirmed that the lack of fit was not significant. Further, higher p values have been obtained for TPC, TFC and AA. These observations affirmed very good model significance for each case.

Among all best fit quadratic models, the F-values of various terms representing TPC indicated all linear, all bi-linear but AB term and all quadratic terms except A^2 term to be significant. Similarly, for the TFC case, the significance corresponds to all linear, all bilinear except interaction AC term and lower significance of quadratic terms. Also, for the AA case, all linear, interaction term BC and quadratic term C^2 terms have been significant to influence critically the measured response variable. Further, corresponding p-values have been lower than 0.0001 and thereby assured upon the quadratic model's best fitness. Accordingly, the best fit quadratic model for all responses have been specified as follows:

$$TPC = 123.47 + 8.71A + 8.73B + 41.18C + 0..29AB - 5.49AC - 1.96BC - 2.27A^2 + 6.50B^2 - 30.11C^2 \quad (4.1)$$

$$TFC = 300.12 + 15.48A + 18.80B + 208.53C - 10.97AB + 8.87AC + 2..90BC - 12.07A^2 + 6.99B^2 - 6.48C^2 \quad (4.2)$$

$$A.A = 70.75 + 6.87A + 3.08B + 24.17C - 0.79AB - 3.26AC - 0.14BC - 2.14A^2 + 1.46B^2 - 13.51C^2 \quad (4.3)$$

Table 4.3: ANOVA data of best fit models and their parameters representing normal UAE of papaya leaf-water system.

Components	Total Polyphenol		Total Flavonoids		Antioxidants Activity	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	892.10 (Quadratic)	< 0.0001 (Quadratic)	2198.87 (Quadratic)	< 0.0001 (Quadratic)	995.88 (Quadratic)	< 0.0001 (Quadratic)
A	268.56	< 0.0001	106.97	< 0.0001	564.76	< 0.0001
B	269.67	< 0.0001	157.74	< 0.0001	113.39	< 0.0001
C	6006.38	< 0.0001	19412.00	< 0.0001	6998.29	< 0.0001
AB	0.23	0.6389	43.02	< 0.0001	6.02	0.0341
AC	85.55	< 0.0001	28.08	0.0003	101.83	< 0.0001
BC	10.88	0.0080	3.00	0.1138	0.18	0.6794
A^2	5.02	0.0489	17.87	0.0017	15.15	0.0030
B^2	41.13	< 0.0001	6.00	0.0342	6.98	0.0247
C^2	883.06	< 0.0001	5.15	0.0466	601.54	< 0.0001
Lack of Fit	1.43	0.3520	2.21	0.2026	0.91	0.5386
R squared		0.9988		0.9995		0.9989
Adequate Precision		98.664		145.102		105.604

4.4. Model fitting and ANOVA for Pulsed UAE

The ANOVA data of the best fit quadratic model have been presented in Table 4.4. The trends have been similar to those being elaborated for the NUAE case. The evaluated R^2 (0.9965 for TPC, 0.9994 for TFC and 0.9979 for AA), adequate precision (58.56 for TPC, 133.43 for TFC and 78.50 for AA), high F value (316.82 for TPC, 1726.47 for TFC, 518.05 for AA) and low p value (< 0.0001) for all cases affirmed upon the model adequacy. The non-significant lack of fit of 2.97, 1.82 and 1.40 for TPC, TFC and AA affirmed a good fitness of the quadratic model. For the TPC case, all linear terms and quadratic term C^2 term have been significant to influence upon the response variable. Similarly, for the TFC case, all linear terms, BC and C^2 term have been highly prominent to influence upon the variations of the response variable. Also, for the AA case, all linear terms, interaction term BC term and quadratic term C^2 term had a predominant influence on the response variable. These dominant influences have been assured by the higher F value and lower p value for the mentioned variables. Thereby, the best fit quadratic models to represent TPC, TFC and AA characteristics of the PUAE of papaya leaves extraction process can be summarized as:

$$TPC = 118.26 + 5.20A + 9.10B + 40.91C - 2.37AB + 2.46AC + 4.15BC + 6.16A^2 + 2.08B^2 - 25.15C^2 \quad (4.4)$$

$$TFC = 303.85 + 24.54A + 19.27B + 203.02C - 5.58AB + 14.11AC + 4.62BC + 7.67A^2 - 5.58B^2 - 16.75C^2 \quad (4.5)$$

$$A.A = 72.81 + 5.39A + 4.50B + 22.03C + 1.52AB - 3.38AC - 1.57BC + 0.29A^2 + 0.64B^2 - 12.28C^2 \quad (4.6)$$

Table 4.4: ANOVA data of best fit models and their parameters representing pulsed UAE of papaya leaf-water system.

Components	Total Polyphenols		Total Flavonoids		Antioxidant Activity	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	316.82 (Quadratic)	< 0.0001 (Quadratic)	1726.47 (Quadratic)	< 0.0001 (Quadratic)	518.05 (Quadratic)	< 0.0001 (Quadratic)
A	38.02	0.0001	219.94	< 0.0001	219.41	< 0.0001
B	116.58	< 0.0001	135.61	< 0.0001	153.10	< 0.0001
C	2353.73	< 0.0001	15058.53	< 0.0001	3670.35	< 0.0001
AB	6.34	0.0305	9.08	< 0.0001	13.88	0.0039
AC	6.79	0.0262	58.17	0.0130	69.00	< 0.0001
BC	19.34	0.0013	6.22	< 0.0001	15.00	0.0031
A^2	14.66	0.0033	5.91	0.0317	0.17	0.6898
B^2	1.68	0.2244	3.13	0.0354	0.85	0.3778
C^2	244.72	< 0.0001	28.19	0.1073	313.79	< 0.0001
Lack of Fit	2.97	0.1285	1.82	0.2635	1.40	0.3600
R squared		0.9965		0.9994		0.9979
Adequate Precision		58.56		133.43		78.50

4.5. Response Surface Characteristics of Bioactives during Normal UAE of Papaya Leaf Extract

4.5.1 Influence of Normal UAE Process Parameters on Total Polyphenols

For the NUAE, Fig. 4.1(a-c) depict the influence of any two process parameters on the response surface characteristics of TPC in the aqueous papaya extract. The response surface graph clearly depicts that for any two combinations of ST, ET, and LR, the TPC yield increases. For an ET of 70°C, the TPC increased from 60 – 148.15 mg GAE /g for a variation in ST and LR from 5 – 20 min and 0.02 – 2 g/mL respectively. Similarly, for a LR of 0.2 mg/mL, the TPC varied from 129.67 – 148.15 mg GAE/g for a variation in ST from 5 – 20 min and ET from 40 – 70°C. Also, for a fixed choice of 20 min ST, the TPC varied from 53.27 – 148.15 mg GAE/g for a variation in process parameters as 0.02 – 0.2 g/mL LR and 40 – 70 °C ET. Thus, highest TPC yield (148 mg GAE/g) was achieved for 70°C, 20 min and 0.2 g/mL case. The depicted response surfaces affirmed non-linear and quadratic variation in the TPC with respect to any two process parameters. Among all chosen degrees of freedom, the TPC yield was analyzed to be sensitive as per the order of LR > ST > ET.

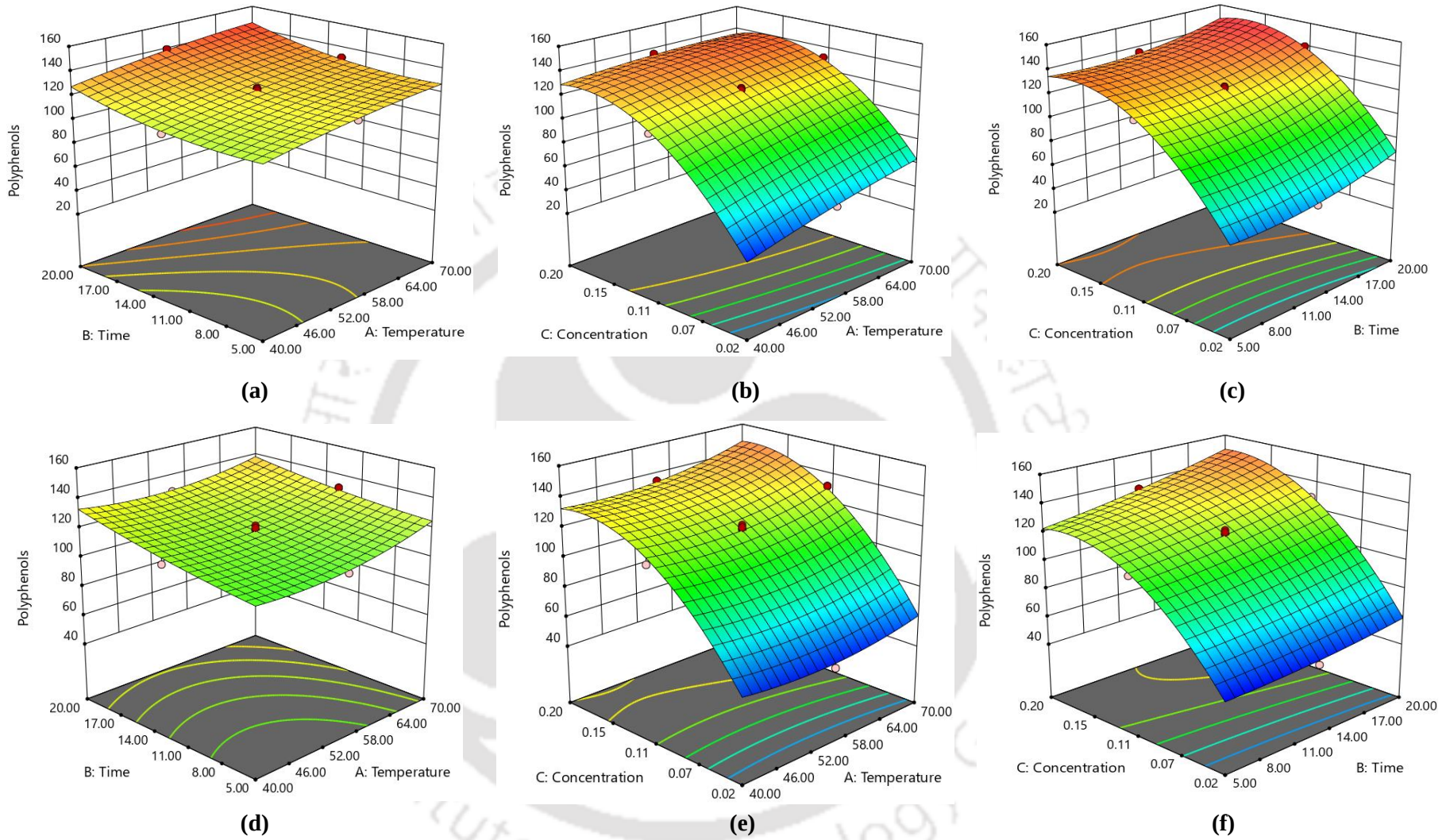

Figure 4.1: Response surface plots of total polyphenols for normal UAE (a - c) and pulsed UAE (d-f) of papaya leaf system.

4.5.2. Influence of Normal UAE Process Parameters on Total Flavonoids

The influence of NUAE process parameters on TFC characteristics of papaya leaf extract has been illustrated in Fig. 4.2 (a – c). In comparison with ST and ET, the LR had a profound influence on the TFC. For the case that involved either the binary interaction of ST and LR or ET and LR, almost parallel curve to the axis existed for the response variable with respect to the variation in either ST (Fig. 4.2b) or ET (Fig. 4.2c). However, for the LR, the TFC profile indicated a raising trend. For the 70 ^{o}C ET case, the TFC increased significantly from 81.26 – 531.5 mg QE/g for an increase in ST from 5 – 20 min and LR of 0.02 – 2 g/mL. However, the TFC did not vary much and varied only between 435.82 – 531.5 mg QE/g for a variation in ST and ET from 5 – 20 min and 40 – 70 ^{o}C respectively and for a fixed choice of 0.2 g/mL LR. Further, the binary combination of ET and ST varied TFC in the lower range of 60 – 129.67 mg QE/g for a fixed choice 20 min ST. Among all cases, the optimal process parametric combination referred to 0.2 g /mL LR, 70 ^{o}C ET and 20 min ST at which the highest TFC yield of 531.5 mg QE/g was achieved. The TFC extraction has been analyzed to be significantly influenced as per the order of LR followed with ET and ST.

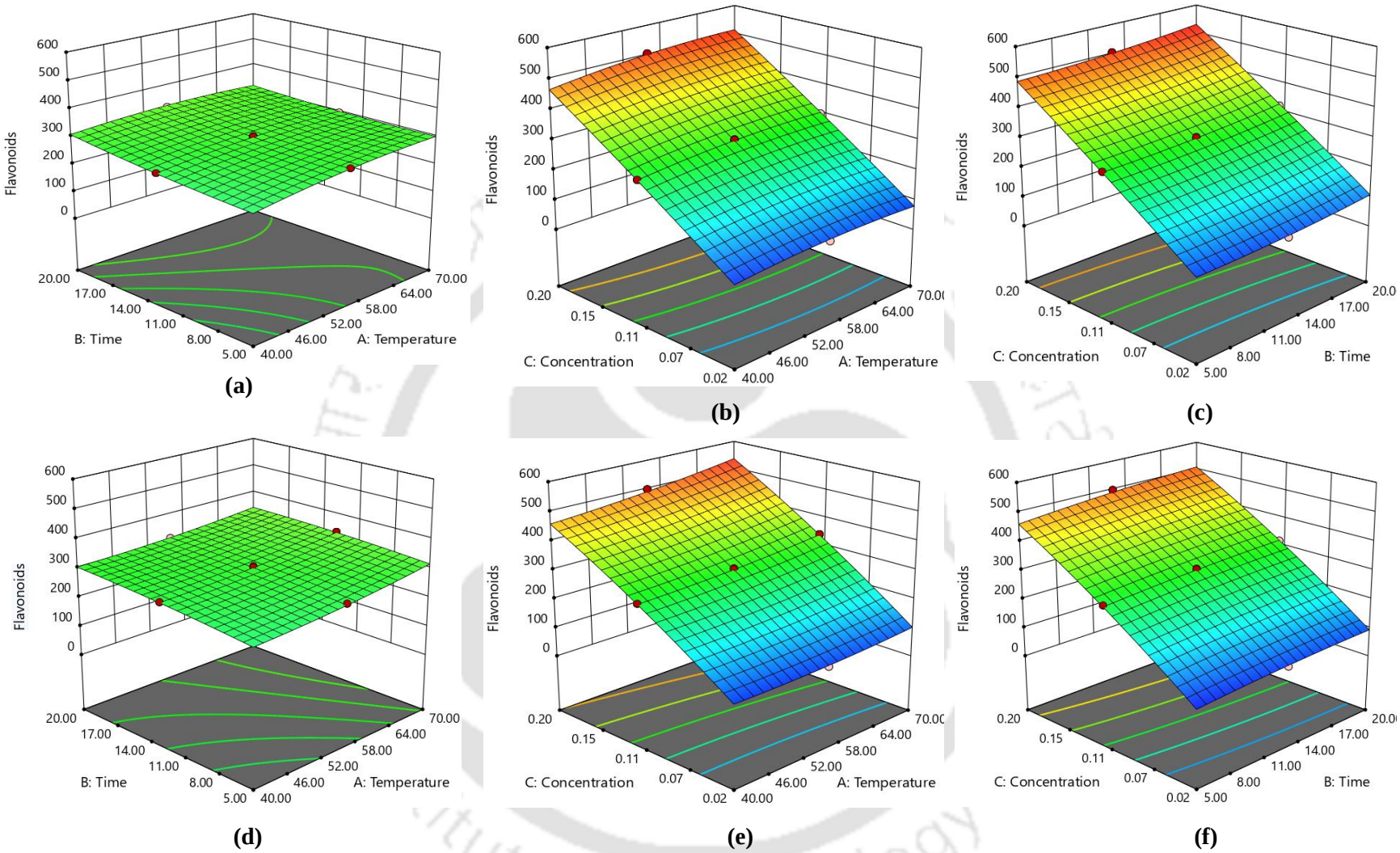

Figure 4.2: Response surface plots of total flavonoids for normal UAE (a - c) and pulsed UAE (d - f) of papaya leaf system.

4.5.3. Influence of Normal UAE Process Parameters on Antioxidant Activity

Fig. 4.3(a – c) depict the influence of NUAE process parameters on the AA characteristics of papaya leaf aqueous extract. The response surface characteristics of AA affirmed that loading followed with ET and ST influenced the response variable profiles. For a fixed choice of LR, the 3D plot indicated enhanced AA for any combination of ST and ET. Also, the AA increased significantly from 40.27 – 86.38% for a variation in ST and LR from 5 – 20 min and 0.02 – 2 g/mL respectively and for a fixed ET (70 ^{o}C). Similarly, for fixed choice of 0.2 g/mL LR, the AA varied relatively insignificant (73.49 – 86.38%) for respective variations in ST and ET as 5 – 20 min and 40 – 70°C respectively. For a fixed choice of 20 min ST, the AA varied considerably from 26.29 – 86.38% for a variation in LR and ET from 0.02 – 0.2 g/mL and 40 to 70 °C respectively. Among all cases, the best combination of process parameters such as 0.2 g/mL LR, 70 ^{o}C ET and 20 min ST indicated maximum antioxidant activity of 86.38%. Similar response surface plot trends did exist for the PUAE cases and have not been therefore delineated.

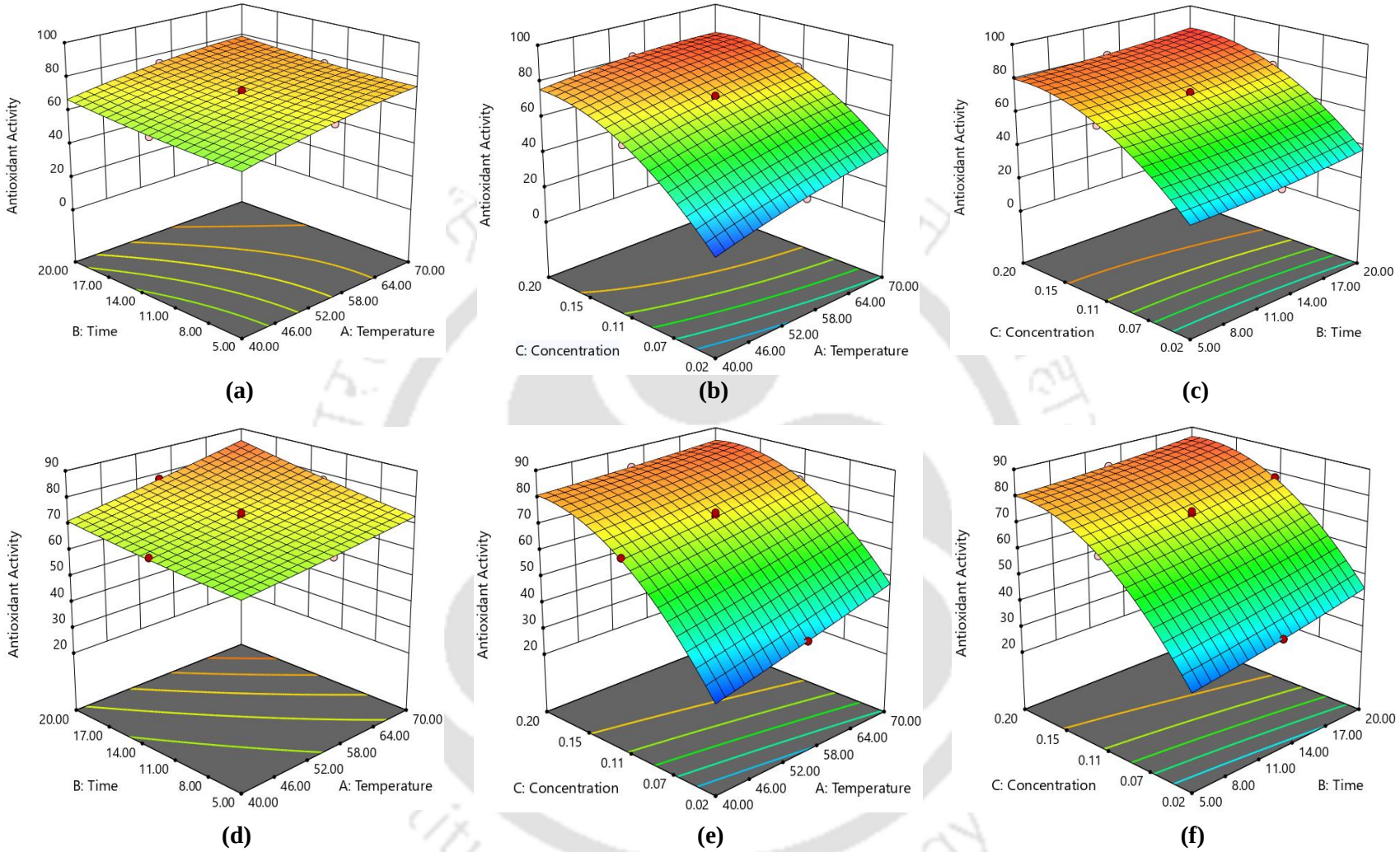

Figure 4.3: Response surface plots of antioxidant activity for normal UAE (a - c) and pulsed UAE (d - f) of papaya leaf system.

4.6. Optimal Process-Product Characteristics

Based on the best fit response variable models, the Design Expert software was deployed for the numerical optimization by considering the chosen parametric choice range as upper and lower bounds and maximization of TPC, TFC and % AA as the alternate desired objective functions. Thereby, optimal process parametric and response variable values have been obtained.

For the NUAE process, the highest TPC, TFC and % AA have been obtained as 149.12 mg GAE/g, 533.46 mg QE /g, and 86.71 % respectively for an optimal process parametric choice of 62.84 ºC ET, 19.98 min ST and 0.2 g/mL LR. For the PUAE case, the corresponding optimal values were 160.54 mg GAE/g TPC, 548.32 mg QE /g TFC, and 89.87 % AA respectively for an optimal process parametric choice of 69.92 ºC ET, 19.92 min ST, and 0.2 g/mL LR.

Due to pertinent combinations of cavitation, mechanical agitation and thermal effect, both PUAE and NUAE indicated the order of the influence of the process parameters as LR > ET > ST. The optimal ST choice has been 20 minutes which is reasonable to accommodate in large scale processing systems for commercial product development. Thereby, ultrasound is very likely to accelerate the leaching of the targeted compounds from the leaf cell wall and reach the extraction solvent (Zou et al., 2014). However, longer ST with ultrasound treatment might induce a reduction in bio-active constituents in the aqueous extract and their markers. The obtained results are in agreement with relevant prior art (Rostagno et al., 2007). The authors clearly indicated that 20 min of ST was sufficient enough to extract phenolic compounds from soy beverages. Additionally, the LR has a substantial effect on bioactive chemical extraction. A high quantity of solvent would assist in the release of bioactive components from the solid matrix. This is due to improved and more convenient access of the solvent to reach solid matrix. In general, a higher solvent volume can leach targeted compounds more effectively and result in better extraction yield. Zou et al. (2014) affirmed LR as the primary factor to recover higher yield of mangiferin from mango leaves. In the

conducted investigations, a LR of 0.2 g/mL indicated highest amount of extraction for both modes of UAE.

4.7. Comparative Assessment of Normal UAE and Pulsed UAE

A comparison of the best results obtained for both PUAE and NUAE processes was considered. Such an evaluation inferred that the PUAE performed comparatively better but not significantly in comparison to the NUAE mode of operation. Except for AA, the optimum levels for TPC and TFC enhanced marginally. The process parametric optimality indicated significant enhancement in the optimal ET but not ST and LR. This may be due to the fact that the AA is more sensitive to elevated ET (Vuong et al., 2015) and this was the case for the PUAE with about 7 ^{o}C higher optimal ET. During the PUAE process, bio-active compounds have been transported more efficiently from the core of the sample to the ruptured walls. The possible reason for this has been passage of the cavitational waves through the medium in short ST intervals and thereby facilitating better agitation and thermal effects (Al-Dhabi et al., 2017; Azmir et al., 2013). Also, the optimal ET also increased significantly for the PUAE case in comparison with the NUAE. This would have as well enhanced the bioactive constituent marker yield. The critical influence of the ET has been obvious with the fact that higher ETs facilitate higher numbers of cavitation nuclei and thereby enhance threshold cavitation that fosters acoustic cavitation (Maran et al., 2017). Thus, higher yield of TPC, TFC and AA has been achieved for higher ETs for both cases.

The PUAE has been considered based on the justification that the process reduces extraction ST, enhances thermolabile compound extraction and reduces possible deterioration in the final extract in terms of the oxidized products (Turrini et al., 2019). Further, the intermittent ST lag during pulsed sonication effectively customizes the efficient transport of bio-active compounds from the sample core to its ruptured walls. This is possibly due to significant cavitation effect in terms of the passage of the cavitational waves through the medium in shorter ST intervals and henceforth better agitation and thermal effects (Al-Dhabi et al., 2017; Azmir et al., 2013). Also, for the PUAE, the optimal ET increased significantly in comparison with that being obtained for the NUAE mode of operation. The same is due to the enhanced bioactive constituent marker yield. The critical influence of the ET has been obvious with the fact that higher ETs facilitate higher

numbers of cavitation nuclei and thereby enhances threshold cavitation that fosters acoustic cavitation (Yerena-Prieto et al., 2022). Also, the RSM analysis involving competent model identification through the ANOVA and subsequent numerical optimization was targeted for both NUAE and PUAE cases.

4.8. Literature Comparison

Table 4.5 presents a summary of the best findings obtained in this work for PUAE and NUAE in conjunction with those being reported in the literature. Further, few experimental investigations have also been considered to supplement these findings with HWE by adopting conditions being reported optimal in this work and in the literature. Such efforts aim towards consolidating few insights which are very important from the perspective of processing associated to papaya leaf systems. Several important inferences can be deduced from the table. Notably, the best findings correspond to PUAE of papaya leaf systems that enabled the realization of 160.54 mg GAE/mg TPC, 548.32 mg QE/mg TFC and 89.87% AA in the extract for the optimal process parametric choice of 69.92 ^{o}C ET, 19.92 min ST and 0.2 g/mL LR. Comparatively, there has been an increase in the optimal value of ET by 11.2 % for PUAE. However, the ST of extraction and LR remained almost unchanged. The output responses namely TPC, TFC and AA for the PUAE were enhanced by 7.11%, 2.21% and 3.51% respectively in comparison with those being evaluated for the NUAE process.

Also, the study conducted by Vuong et al. (2013) affirmed that the HWE involved 82.17 % and 98.47 % lower concentrations of TPC and TFC respectively, along with 62.5 % reduction in LR in comparison with the best optimal findings obtained in this work. This might be due to significantly lower LR of 0.025 g/mL being deployed in the literature and also due to the inefficient HWE process (Vuong et al., 2013). Three major reasons can be indicated for such significant reduction namely the significant role of cavitation during ultrasound, lower LR in the literature and dried powder based extraction (Luo et al., 2018). Among these, the last reason has been confirmed by the findings reported by our research group for HWE. For example, the HWE being conducted in the thesis for 0.075 g/mL LR and similar other conditions being reported in

the mentioned literature indicated significantly higher TFC and TPC in comparison with those mentioned in the literature by the authors (Vuong et al., 2013).

Further, with respect to the best findings reported in the literature for the UAE based papaya leaf extraction process, it is apparent that significantly lower TPC and TFC have been obtained by Abdel-Halim et al. (2021). However, these are comparatively better than those reported by Vuong et al. (2013) for the HWE. Thus, a 9-fold increase in the LR did not proportionally enhance the TPC and TFC values being obtained in our work. Compared to our findings, the authors reported 72.92 % lower TPC and 97.55% lower TFC for 87.5% lower LR. The associated reasons have been poor LR and drying of leaves at 30 ^{o}C. However, nonlinear dependence of TPC and TFC are also evident.

Considering all these, it is here with concluded that UAE with raw papaya leaf systems is the best to achieve highest extraction efficacy in terms of desired bioactive constituents such as TPC, TFC and AA. Thereby, the relevance of the thesis work and the associated novelty of the obtained data is justified. The table affirms that the prior art did not emphasize upon the RSM based UAE extraction of the bioactive components from the papaya leaves. Also the influence of the process parameters on the yield of bioactive compounds have not been explored. In general, it has been observed that the PUAE process provided better combinations of optimal variable data set in comparison with the conventional NUAE and HWE (control) process. For the case, the TPC, TFC and AA enhanced 23.68 %, 44.37% and 4.9% respectively in comparison with the values being obtained for the control sample (HWE).

Table 4.5: Literature and reported optimal data of bioactives extraction from papaya leaf extraction with hot water extraction and UAE processes.

Extraction Process	Temperature (ºC)	Time (min)	Loading ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)	Literature
HWE	70	20	0.075	28.61	8.36	NA	Vuong et al. (2013)
UAE	NA	NA	0.025	43.46	13.40	NA	Abdel-Halim et al. (2021)
Maceration	NA	NA	NA	NA	NA	78.62	Musa et al. (2009)
HWE	70	20	0.2	122.52	305	85.5	This work
HWE	70	60	0.2	110.90	228	81.50	This work
HWE	70	20	0.075	107.17	218.66	71.5	This work
NUAE	62.84	19.98	0.2	149.12	533.16	86.71	This work
PUAE	69.92	19.92	0.2	160.54	548.32	89.87	This work

4.9 Summary

The following critical conclusions have been deduced from the relevant reported findings of the thesis work.

- Firstly, fresh papaya leafy samples need to be utilized to achieve maximum bioactive compounds extraction from the leafy samples using either HWE or UAE.
- Secondly, among HWE, NUAE and PUAE, the PUAE provided the best response and parametric choice of 160.54 mg GAE/g of TPC, 548.32 mg QE /g of TFC, and 89.87 % of AA, 69.92°C ET, 19.92 min ST, and 0.2 g/mL LR.
- Thirdly, compared to the NUAE, the PUAE mode performed marginally better in terms of response and process conditions. Further, it can be concluded that the nonlinear effect of LR was the dominant factor followed by the ET and ST in the extraction of the targeted bioactive components.
- Finally, compared to the HWE, the UAE based process accounts for 31.03%, 91.58% and 5.1% enhancement in TPC, TFC and AA respectively, which indicate its promising efficacy.

In summary, all these findings affirm upon the possible scope for the UAE based papaya leafy extraction process to support ongoing emphasis on the development and utility of commercial herbal dry supplements deduced with the nonconventional leafy system based extracts.

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Chapter 5:

Ultrasound-Assisted Extraction of Bioactive Constituents from Papaya Pulp and Papaya Peel

Chapter 5

Ultrasound-Assisted Extraction of Bioactive Constituents from Papaya Pulp and Papaya Peel

This chapter elaborates upon the findings and discussion related to the performance of NUAE, PUAE and HWE processes for the extraction of bioactive compounds from papaya pulp (PPU) and papaya peel (PPE) system. Thereby the efficacy of PUAE has been compared with respect to NUAE and HWE processes and in terms of the evaluated responses namely AA, TFC, VITC and TPC. After presenting a brief introduction in section 8.1, the section 8.2 of the chapter devotes towards experimental data summary obtained through RSM modelling based design of experiments approach. Thereafter, sections 8.3 delineates upon the comparative assessment of electrical consumption and CO_2 emission of PUAE and HWE process. Sections 8.4 and 8.5, respectively elaborate upon the obtained ANOVA results and eventual identification of best fit model during PUAE of PPU and PPE. Response surface characteristics based relation between the process variables and optimisation of process parameters for PPU and PPE system have been elaborated respectively in sections 8.6 and 8.7 of the chapter. A comparative analysis of NUAE and PUAE for the PPU and PPE systems have been delineated in section 8.8 of the chapter. Thereafter, comparison with the best literature data has been conveniently presented in section 8.9. Finally, section 8.10 outlines the key findings for the targeted thesis objectives in the chapter.

Overview

The NUAE and PUAE of PPU and PPE samples have been conducted in an ultrasonic bath. The effect of extraction parameters such as LR, ST, and ET on the recovery of bioactive compounds from PPU and PPE systems to achieve a maximum yield of TFC, TPC, AA, and VITC for PPU and PPE based aqueous extracts using FCD based RSM has been assessed. A comparative efficacy of NUAE and PUAE with respect to HWE process for the evaluated responses has been evaluated. Also, the criticality of LR as an additional degree of freedom for the evaluation of its efficacy in addition to ST and ET and

introspection into the associated non-linearities of the influence of any two degrees of freedom on all measured responses have been thereafter addressed. The findings suggest that both NUAE and PUAE processes has greater potential than the traditional extraction method (such as the HWE) for the production of PPU and PPE aqueous extracts for medicinal and functional food applications.

5.1 Introduction

This article focusses on the ultrasound-assisted extraction (NUAE and PUAE) of nutritionally significant bioactive components from PPU and PPE systems. The optimization of process parameters has been achieved with FCD based RSM. The influence of independent process variables such as ST (5 – 20 min), ET (40 – 70 °C), and LR (0.2 – 0.5 g/mL) on the desired response variables such as TFC, AA, TPC, and VITC of the PPU and PPE aqueous extract system have been evaluated. For comparative analysis, findings from the HWE have been compared with the optimal finds of NUAE and PUAE for both PPU and PPE system. The study indicated that the PPU extract contained the highest level of bioactives in comparison with the PPE system.

5.2 Experimental Data Summary

The findings affirmed that the fresh PPU and PPE samples constituted a moisture content of 90.4 ± 0.43 % and 82.2 ± 0.17% (w/w), respectively. The NUAE and PUAE extraction process parameters for the extraction of bioactive compounds from the PPU and PPE systems were chosen based on a few experimental trials. For both processes, the ST, ET and LR were varied from 5 – 20 min, 40 – 70 °C and 0.2 – 0.5g/mL respectively. The bioactive yield has been significantly affected with LR and the yield increased with the LR. This can be explained with the mass transfer principle (Sengar et al., 2020). Similar findings were reported by authors who performed antioxidant extraction from pomegranate peel and black chokeberry peel. Similar interferences were evident for ST and ET (Daoutidou et al., 2021). For both processes, the upper levels of process parameters for ST and ET were selected as 20 min and 70°C respectively. This has been due to the reason that most bioactive components get degraded for process conditions of higher ET and long-time duration (Wani & Uppaluri, 2022). However, in this

study, it was identified that at higher LR (0.5g/mL), the scope of the yield enhancement narrowed. Similar observations have been observed for the recovery of antioxidants from black chokeberry peel and pomegranate peel (Daoutidou et al., 2021). The TPC, VITC, TFC and % AA yield for PPU and PPE case varied significantly during NUAE and PUAE processes. These have been respectively summarized in Tables 5.1, 5.2, 5.3 and 5.4. For the NUAE case (Table 5.1), for a variation in LR from 0.2 - 0.5 g/mL, ST from 5 - 20 min, and ET from 40 - 70 °C, the TPC, VITC, and % AA yield respectively varied as 53.35 - 145.21 mg GAE/g sample, 16.44 - 139.5 mg/100g, and 35.25 - 85.83%, respectively. For the PUAE case (Table 5.3), for an alteration in LR from 0.2 – 0.5 g/mL, ST from 5 – 20 min and ET from 40 – 70 °C, the TPC, VITC and % AA yield for PPU altered considerably as 80.87 – 169.16 mg GAE/g, 28.52 – 164.94 mg/100g, and 31.29 – 92.9 %, respectively. Similar results have been found for the PPE of both NUAE and PUAE processes (Tables 5.2 and 5.4). For the NUAE of PPE (Table 5.2), a variation in LR, ST, and ET from 0.2 - 0.5 g/mL, 5 - 20 min, and 40 - 70 °C respectively resulted in yields of TPC, TFC, and AA ranging from 53.68 - 126.6 mg GAE/g sample, 70.67 - 302 mg QE/g, and 47.67 - 80.36%. Similarly, for the PUAE of PPE (Table 5.4), the yield of TPC, TFC and AA varied from 97.46 – 145.44 GAE/g, 97.36 – 292.2 mg QE/g and 46.51 – 89.17 % for an increment in LR, ST and ET from 0.2 – 0.5 g/mL, 5 – 20 min and 40 – 70 °C respectively. In this context, Uribe et al. (2015) indicated TPC variations of 23.8 – 129.1 mg GAE/100 g sample, AA of 17.6 – 20.6 mM TE/100 g and VITC of 70.6 – 74.1 mg/100 g FW for an variations in ST from 5 – 15 min but for a fixed choice of LR (0.25 g/mL) for the PPU/PPE mixture system.

Similar conclusions were drawn by Vallejo-Castillo et al. (2020) who found that TPC, TFC of the PPU/PPE mixture extract to alter from 600 – 1034.45 mg GAE /100 g sample and 20 – 44.85 mg QE /100g respectively, for an variation in LR (0.024 – 0.0125 g/mL) but for a fixed ST (3 min). Thereby, the findings of this study in comparison with the best reported prior art suggest improved bioactive constituent characteristics. As a result, these findings support the view point that the UAE of PPU/PPE – water system is a promising low-cost extraction approach for the extraction of bioactive components. Considerations for process scalability of the UAE process shall be quantified in terms of capacity, equipment size, sonication power and frequency, solvent choice based on solubility, alterations in

sample morphology due to scale up based alterations in adopted procedures, process and parametric optimality and safety. The optimal set of data reported in the thesis can serve as useful benchmark for this purpose. For both NUAE and PUAE processes and for the PPU, the best experimental data were obtained as 0.35 g/mL LR, 55 °C ET and 12.50 min ST. For the NUAE and PUAE and for the PPE case, the best experimental data were obtained as 0.35 g/mL LR, 70 °C ET, and 12.50 min ST for TPC and TFC. However, for the AA, the best experimental data correspond to 55 °C ET, 12.50 min ST and 0.35 g/mL LR.

Furthermore, the HWE ensured lesser amount of bioactives in comparison to both NUAE and PUAE of PPU and PPE. The HWE was conducted at the optimized condition of NUAE and PUAE processes. During HWE, the TPC, VITC and % AA yield for PPU case were obtained as 105 ± 0.97 mg GAE/g sample, 89.4 ± 0.05 mg/100g, and 76.50 ± 0.68 %, respectively. Similarly, for the PPE, the yield of TPC, TFC and AA were obtained as 88.5 ± 0.33 mg GAE/g sample, 145 ± 0.13 mg QE/g and 71.5 ± 0.11 % respectively.

Additionally, the HWE process based extract exhibited fewer bioactives than that obtained with the PUAE of PPU and PPE. During the HWE, TPC, VITC, and % AA yield for the PPU case were 105 mg GAE/g sample, 89.4 mg/100g, and 76.50 %, respectively. Similarly, for the PPE, the yields of TPC, TFC, and AA were 88.5 mg GAE/g sample, 145 mg QE/g, and 71.5 %, respectively.

The bioactive constituents yield obtained in this study have been greater than those reported in the pertinent prior art for PPU and PPE. This is possibly due to the consideration of lower LR and inclusion of drying as a pre-treatment step in the prior art that prompted to be detrimental to the bioactives constitution in the extract. Furthermore, UAE process conditions have been less severe than those deployed in conventional extraction techniques that deploy organic solvent, acidic pH and higher ET for the extraction of mentioned constituents in the liquid media (Patience et al., 2021). These variations in the prior art data can also be attributed to several other factors such as variety of fruit, harvest time, climatic conditions and ripening stage of the papaya fruit. However, their influence is probably insignificant than those addressed and reported in this work.

Table 5.1: RSM based data summary of parametric and response variables for normal UAE of papaya pulp-water system.

S.No	Temperature (°C)	Time (min)	Papaya pulp / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Vitamin C (mg/100g)	Antioxidant Activity (%)
1.	40.00	5.00	0.20	53.35 ± 0.61	16.44 ± 0.64	35.25 ± 0.82
2.	40.00	20.00	0.20	94.76 ± 0.82	56.22 ± 0.38	50.82 ± 0.25
3.	55.00	12.50	0.20	80.8 ± 0.43	30 ± 0.33	47.26 ± 0.26
4.	70.00	5.00	0.20	89.38 ± 0.33	30.47 ± 0.03	55.19 ± 0.40
5.	70.00	20.00	0.20	115.05 ± 0.62	75.23 ± 0.12	67.67 ± 0.01
6.	40.00	5.00	0.50	118.5 ± 0.82	76.96 ± 0.20	70.63 ± 0.15
7.	40.00	20.00	0.50	140.25 ± 0.14	98.87 ± 0.45	78.13 ± 0.12
8.	55.00	12.50	0.50	129.74 ± 0.14	90.5 ± 0.33	74.7 ± 0.83
9.	70.00	5.00	0.50	138.2 ± 0.47	106 ± 0.82	78.9 ± 0.37
10.	70.00	20.00	0.50	143.92 ± 0.29	134.46 ± 0.25	83.61 ± 0.24
11.	40.00	12.50	0.35	114.8 ± 0.94	101 ± 0.73	66.13 ± 0.39
12.	55.00	5.00	0.35	118.33 ± 0.42	105 ± 0.59	69.66 ± 0.15
13.	55.00	12.50	0.35	124.84 ± 0.68	117.3 ± 0.14	76.12 ± 0.54
14.	55.00	12.50	0.35	125.7 ± 0.70	118.7 ± 0.33	75.1 ± 0.82
15.	55.00	12.50	0.35	124.52 ± 0.21	113.56 ± 0.71	72.78 ± 0.83
16.	55.00	12.50	0.35	124.68 ± 0.12	115.57 ± 0.61	76.12 ± 0.17
17.	55.00	12.50	0.35	122.4 ± 0.82	118.5 ± 0.41	73.55 ± 0.25
18.	55.00	12.50	0.35	125.9 ± 0.45	117.4 ± 0.59	74.5 ± 0.82
19.	55.00	20.00	0.35	145.21 ± 0.31	139.5 ± 0.68	85.83 ± 0.29
20.	70.00	12.50	0.35	137.06 ± 0.34	132.6 ± 0.28	81.1 ± 0.25

Table 5.2 : RSM based data summary of parametric and response variables for normal UAE of papaya peel-water system.

S.No	Temperature (°C)	Time (min)	Papaya peel / water ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)
1.	55.00	20.00	0.35	119.26 ± 0.54	290 ± 0.82	80.36 ± 0.17
2.	40.00	5.00	0.50	117.42 ± 0.19	240.89 ± 0.04	70.62 ± 0.29
3.	55.00	12.50	0.35	116.7 ± 0.83	270 ± 0.94	74.1 ± 0.45
4.	70.00	5.00	0.20	83.5 ± 0.41	135.64 ± 0.10	58.6 ± 0.28
5.	55.00	12.50	0.35	116.4 ± 0.70	269.7 ± 0.33	73.75 ± 0.11
6.	40.00	12.50	0.35	109.79 ± 0.42	250.87 ± 0.17	68.2 ± 0.09
7.	55.00	12.50	0.35	117.1 ± 0.92	272 ± 1.14	75.79 ± 0.12
8.	70.00	5.00	0.50	119.67 ± 0.01	260 ± 0.33	74.92 ± 0.00
9.	40.00	5.00	0.20	53.68 ± 0.06	70.67 ± 0.30	47.67 ± 0.31
10.	70.00	20.00	0.50	123.7 ± 0.82	275.56 ± 0.20	75.3 ± 0.14
11.	70.00	20.00	0.20	102.39 ± 0.04	176.08 ± 0.92	67.85 ± 0.16
12.	55.00	12.50	0.20	81.21 ± 0.40	117.55 ± 0.72	55 ± 0.82
13.	55.00	5.00	0.35	105.93 ± 0.30	253 ± 0.33	72.59 ± 0.17
14.	55.00	12.50	0.50	120.86 ± 0.12	252.03 ± 0.13	72.84 ± 0.56
15.	40.00	20.00	0.50	121.79 ± 0.55	253.11 ± 0.04	74.3 ± 0.29
16.	55.00	12.50	0.35	115.8 ± 0.52	274.4 ± 0.19	75.5 ± 0.00
17.	55.00	12.50	0.35	115.5 ± 0.24	270.2 ± 0.90	75.8 ± 0.38
18.	40.00	20.00	0.20	76.77 ± 0.03	105.2 ± 0.29	55 ± 0.82
19.	55.00	12.50	0.35	116.45 ± 0.22	274 ± 0.42	74.5 ± 0.24
20.	70.00	12.50	0.35	126.6 ± 0.28	302 ± 0.36	77.7 ± 0.17

Table 5.3: RSM based data summary of parametric and response variables for pulsed UAE of papaya pulp-water system.

S.No	Temperature (°C) (A)	Time (min) (B)	Papaya pulp / water ratio (g/mL) (C)	Total Phenols (mg GAE/g)	Antioxidant Activity (%)	Vitamin C (mg/100g)
1.	70.00	5.00	0.20	98.4 ± 0.60	60.6 ± 0.59	59.31 ± 0.95
2.	55.00	12.50	0.50	147.04 ± 0.76	83.42 ± 0.42	136.43 ± 0.17
3.	55.00	12.50	0.35	151.4 ± 0.52	84.2 ± 0.52	140.7 ± 1.27
4.	70.00	20.00	0.20	115.8 ± 0.45	72.76 ± 0.31	96.83 ± 0.14
5.	40.00	5.00	0.50	119.16 ± 0.47	77.17 ± 0.49	124.72 ± 0.77
6.	70.00	20.00	0.50	162.5 ± 0.62	86.88 ± 0.28	153 ± 0.82
7.	40.00	20.00	0.20	97.43 ± 0.22	45.05 ± 0.22	53.93 ± 0.28
8.	55.00	12.50	0.35	151.12 ± 0.54	82.21 ± 0.05	142.9 ± 0.45
9.	55.00	12.50	0.20	105.49 ± 0.17	53.14 ± 0.61	57.57 ± 0.06
10.	55.00	12.50	0.35	150.54 ± 0.05	82.2 ± 0.09	144.5 ± 0.62
11.	70.00	5.00	0.50	141.78 ± 0.04	80.9 ± 0.78	129.08 ± 0.04
12.	40.00	20.00	0.50	140.5 ± 0.93	85.2 ± 0.37	131.49 ± 0.04
13.	55.00	5.00	0.35	143.65 ± 0.26	78.7 ± 0.06	132.85 ± 0.16
14.	40.00	12.50	0.35	126.7 ± 0.24	70.14 ± 0.24	121.85 ± 0.40
15.	40.00	5.00	0.20	80.87 ± 0.59	31.29 ± 0.37	28.52 ± 0.15
16.	55.00	20.00	0.35	169.16 ± 0.61	92.9 ± 0.45	164.94 ± 0.25
17.	70.00	12.50	0.35	150.2 ± 0.52	87.17 ± 0.26	149.87 ± 0.36
18.	55.00	12.50	0.35	153.7 ± 0.41	80.67 ± 0.05	142.85 ± 0.16
19.	55.00	12.50	0.35	152.1 ± 0.22	80.2 ± 0.42	140.85 ± 0.14
20.	55.00	12.50	0.35	150.6 ± 0.21	81.2 ± 0.51	144.8 ± 1.27

Table 5.4: RSM based data summary of parametric and response variables for pulsed UAE of papaya peel-water system.

S.No	Temperature (°C) (A)	Time (min) (B)	Papaya peel / water ratio (g/mL) (C)	Total Phenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)
1.	40.00	5.00	0.20	97.46 ± 0.50	97.36 ± 0.24	46.51 ± 0.24
2.	40.00	20.00	0.50	127.24 ± 0.28	192.54 ± 0.22	75.67 ± 0.03
3.	55.00	12.50	0.35	135.8 ± 0.82	267 ± 0.75	73 ± 0.82
4.	70.00	20.00	0.50	136.39 ± 0.62	257.17 ± 0.28	83.99 ± 0.37
5.	55.00	12.50	0.35	133 ± 0.82	262 ± 0.82	74.67 ± 0.31
6.	70.00	5.00	0.50	130.73 ± 0.19	234.78 ± 0.05	81.35 ± 0.71
7.	55.00	12.50	0.35	132.73 ± 1.18	261 ± 0.71	74.8 ± 0.43
8.	40.00	12.50	0.35	132.27 ± 0.62	228.47 ± 0.29	69.35 ± 0.53
9.	70.00	20.00	0.20	118.32 ± 0.05	178.73 ± 0.49	69.42 ± 0.42
10.	70.00	12.50	0.35	145.44 ± 0.07	292.2 ± 0.12	89.17 ± 0.52
11.	40.00	5.00	0.50	122.73 ± 0.25	173.28 ± 0.43	71.42 ± 0.58
12.	40.00	20.00	0.20	107.15 ± 0.19	131.31 ± 0.04	52.73 ± 0.46
13.	55.00	12.50	0.35	134.1 ± 0.50	261.65 ± 0.24	75.1 ± 0.45
14.	70.00	5.00	0.20	110.64 ± 0.14	139.35 ± 0.22	65.62 ± 0.19
15.	55.00	20.00	0.35	140.03 ± 0.34	278.91 ± 0.78	78.17 ± 0.25
16.	55.00	12.50	0.50	127.12 ± 0.08	218.57 ± 0.65	77.25 ± 0.13
17.	55.00	12.50	0.20	105.78 ± 0.83	137.56 ± 0.37	58.55 ± 0.25
18.	55.00	12.50	0.35	131.7 ± 0.82	265 ± 0.66	75.08 ± 0.46
19.	55.00	12.50	0.35	135.6 ± 0.47	266 ± 0.42	76.89 ± 0.08
20.	55.00	5.00	0.35	129.12 ± 0.08	253.28 ± 0.04	72.51 ± 0.41

5.3 Comparison of Electric Consumption and CO_2 Emission between Pulsed UAE and HWE for Extraction of Bioactives

The electrical consumption requirements towards bioactives extraction from PPU and PPE using the PUAE and HWE methods for 20 min ST were 0.093 and 0.33 kWh, respectively. As a result, bioactive extraction using HWE consumes 3.55 times more electricity than PUAE. Thereby, it can be hypothesised that in comparison with the HWE, the PUAE method requires lower operating cost to extract bioactives from PPU and PPE. Furthermore, PUAE of bioactives produced lesser amount of CO_2 (0.074 kg) in comparison to the HWE (0.26 kg) due to electrical consumption. As a result, the deployment of PUAE method for the extraction of bioactives from PPU and PPE can be inferred to be more sustainable and environment friendly process than the HWE process. Life cycle assessment studies can further supplement and substantiate upon the optimality of acidic conditions based pectin extraction. Due to the demanding nature of such studies as an elaborated objective and limitations of adequate resources, such studies were not targeted in the thesis and can be addressed in the near future.

5.4 ANOVA based Identification of Best Fit Model during Normal UAE and Pulsed UAE of Papaya Pulp

For the PPU case, Table 5.5 and 5.6 elucidates on the analysis of variance (ANOVA) data for TPC, AA, and VITC and for NUAE and PUAE processes respectively. For all conditions, the best fit model is the quadratic model. The suitability of the model's fitness has been confirmed with very high regression coefficients of determination (R^2) (for NUAE process, TPC, AA, and VITC has R^2 values of 0.9983, 0.9907, and 0.9970, respectively. Similarly, for the PUAE process, TPC, AA, and VITC have R^2 values of 0.9973, 0.9958 and 0.9979, respectively), higher F values (646.33, 118.96, and 373.84, respectively for NUAE and 411.12, 263.60, and 539.28, respectively for PUAE) for TPC, AA and VITC, respectively, and higher adequate precision values (99.305, 41.384 and 65.504 respectively for NUAE and 70.496, 60.871 and 78.154, respectively for PUAE of TPC, AA and VITC). Furthermore, the overall model p-value was low (0.0001) for all the cases. The lack of fit values of 1.21, 2.20, and 2.86 for NUAE and 3.34, 0.90, and 2.90 for PUAE of TPC, AA, and VITC, respectively, for the best fit

quadratic model confirms inadequate lack of fitness data. As a result, the lack of fit was not significant and this was confirmed by the lower F values. In addition, TPC, AA, and VITC all had higher p values. For each case, these findings demonstrated that the model was very significant.

With the exception of the A^2 term, all linear (A, B, C), interaction terms (AB, AC, BC), and quadratic terms (B^2, C^2) were significant for all best fit quadratic models for NUAE case. Additionally, all linear terms (A, B, C), interaction terms (AC, BC), and quadratic terms (B^2, C^2) have been demonstrated to have a substantial impact on the measured response variable for the VITC case. Similarly, for the AA case, the significance corresponds to all linear (A, B, C) interaction (AC, BC) but not the AB term and lower significance of quadratic terms. Additionally, the associated p-values were below 0.0001 and thereby confirmed on the quadratic model's best fitness. Consequently, the best fit quadratic model for all responses have been established as follows:

$$TPC = 124.96 + 10.19A + 12.14B + 23.73C - 3.97AB - 4.12AC - 4.95BC + 0.55A^2 + 6..39B^2 - 20.11C^2 \quad (5.1)$$

$$AA = 74.39 + 6.55A + 5.64B + 12.98C - 0.73AB - 2.88AC - 1.98BC + 0.33A^2 + 3.80B^2 - 12.96C^2 \quad (5.1)$$

$$VITC = 115.57 + 12.93A + 16.94B + 29.84C + 1.44AB + 3.95AC - 4.27BC + 3.13A^2 + 8.58B^2 - 53.42C^2 \quad (5.3)$$

For the PUAE case, all linear (A, B, C) and quadratic terms (A^2, B^2, C^2), with the exception of the bi-linear terms (AB, AC, BC), were significant for the best fit quadratic model for the TPC. Similarly, for the AA case, the significance corresponds to all linear (A, B, C) bi-linear (AC, BC) but not the AB term and the quadratic terms (A^2, B^2, C^2). Also, for the VITC case, all linear (A, B, C), bi-linear (AB, AC, BC) and quadratic term (A^2, B^2, C^2) terms have been significant to influence critically the measured response variable. Further, corresponding p-values have been lower than 0.0001 and thereby assured upon the quadratic model's best fitness. Accordingly, the best fit quadratic model for all responses have been summarized as follows:

$$TPC = 151.35 + 10.40\ A + 10.15B + 21.30\ C + 0.028\ AB + 1.09AC + 1.01\ BC - 12.55A^2 + 5.41B^2 - 24.73\ C^2 \quad (5.4)$$

$$AA = 82.03 + 7.95A + 5.41B + 15.07C - 0.46AB - 6.45AC - 1.49BC - 3.74A^2 + 3.40B^2 - 14.12C^2 \quad (5.5)$$

$$VITC = 142.64 + 12.76A + 12.57B + 37.86C + 3.66AB - 5.98AC - 4.03BC - 6.58A^2 + 6.45B^2 - 45.44C^2 \quad (5.6)$$

Table 5.5: Best fit RSM model fitness parameters and ANOVA data for various cases during normal UAE of papaya pulp-water system.

Components	Total Polyphenols		Antioxidants Activity		Vitamin C	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	646.33 (Quadratic)	< 0.0001 (Quadratic)	118.96 (Quadratic)	< 0.0001 (Quadratic)	373.84 (Quadratic)	< 0.0001 (Quadratic)
A	603.79	< 0.0001	145.00	< 0.0001	226.44	< 0.0001
B	856.58	< 0.0001	107.59	< 0.0001	388.90	< 0.0001
C	3270.39	< 0.0001	569.07	< 0.0001	1206.84	< 0.0001
AB	73.29	< 0.0001	1.46	0.2547	2.25	0.1644
AC	78.84	< 0.0001	22.42	0.0008	16.90	0.0021
BC	113.93	< 0.0001	10.60	0.0086	19.78	0.0012
A^2	0.48	0.5038	0.098	0.7602	3.65	0.0853
B^2	65.20	< 0.0001	13.45	0.0043	27.42	0.0004
C^2	646.14	< 0.0001	156.07	< 0.0001	1063.49	< 0.0001
Lack of Fit	1.21	0.4186	2.20	0.2032	2.86	0.1367
R squared		0.9983		0.9907		0.9970
Adequate Precision		99.305		41.384		65.504

Components	Total Phenols		Antioxidants Activity		Vitamin C	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	411.12 (Quadratic)	< 0.0001 (Quadratic)	263.60 (Quadratic)	< 0.0001 (Quadratic)	539.28 (Quadratic)	< 0.0001 (Quadratic)
A	353.91	< 0.0001	323.87	< 0.0001	276.29	< 0.0001
B	337.17	< 0.0001	150.30	< 0.0001	268.25	< 0.0001
C	1483.80	< 0.0001	1165.39	< 0.0001	2432.57	< 0.0001
AB	1.979E-003	0.9654	0.85	0.3771	18.17	0.0017
AC	3.11	0.1083	170.78	< 0.0001	48.52	< 0.0001
BC	2.68	0.1325	9.10	0.0130	22.05	0.0008
A^2	141.65	< 0.0001	19.74	0.0012	20.23	0.0011
B^2	26.29	0.0004	16.35	0.0023	19.43	0.0013
C^2	550.28	< 0.0001	281.07	< 0.0001	964.00	< 0.0001
Lack of Fit	3.34	0.1061	0.90	0.5465	2.90	0.1338
R squared		0.9973		0.9958		0.9979
Adequate Precision		70.496		60.871		78.154

Table 5.6: Best fit RSM model fitness parameters and ANOVA data for various cases during pulsed UAE of papaya pulp-water system.

5.5 ANOVA based Identification of Best Fit Model during Normal UAE and Pulsed UAE of Papaya Peel

Table 5.7 and 5.8 summarizes the ANOVA results for the best-fit quadratic model during NUAE and PUAE of the PPE. The patterns were comparable to those being established for a typical PUAE data set of the PPU case. The model's adequacy can be confirmed by the highest R^2 (0.9990 for TPC, 0.9901 for AA, and 0.9990 for the TFC of the NUAE based PPE extraction and 0.9902 for TPC, 0.9829 for AA, and 0.9987 for the TFC of the PUAE based PPE extraction), adequate precision (119.60 for TPC, 39.078 for AA, and 107.201 for TFC for the NUAE based PPE extraction and 38.085 for TPC, 30.825

for AA, and 95.548 for TFC for the PUAE based PPE extraction), high model F value (1091.78 for TPC, 111.60 for AA, and 1074.91 for TFC for the NUAE based PPE extraction and 112.31 for TPC, 63.72 for AA, and 878.97 for TFC and for PUAE based PPE extraction), and low p value (0.0001) for all the cases. The quadratic model's fitness was confirmed by the non-significant lack of fit values of 2.20, 2.59, and 3.25 for TPC, AA, and TFC, respectively for the NUAE of PPE and 1.18, 3.27, and 1.50 for TPC, AA, and TFC, respectively for the PUAE of PPE.

For the NUAE of PPE, all linear factors (A, B, C), cross product terms (AB, BC, AC), and squared terms (B^2, C^2) were significant in altering the response variable for the TPC case. Furthermore, all linear terms (A, B, C), cross product terms (AC, BC), and squared terms (A^2, C^2) had a substantial impact on the response variable in the TFC case. The higher F value and lower p value for the identified factors confirm their strong influence. Similarly, all linear factors (A, B, C), cross product terms (AC, BC), and squared terms (B^2, C^2) were significant in influencing response variable variations in the AA case. Thus, the following have been the best quadratic models to represent TPC, AA, and TFC characteristics of the NUAE of PPE extraction system:

$$TPC = 126.55 + 9.56A + 4.60B + 16.68C + 0.61AB - 4.87AC - 2.06BC - 0.88A^2 - 1.72B^2 - 14.31C^2 \quad (5.7)$$

$$AA = 74.62 + 3.86A + 2.84B + 8.39C - 0.17AB - 2.31AC - 1.56BC - 1.23A^2 + 2.30B^2 - 10.26C^2 \quad (5.8)$$

$$TFC = 271.67 + 22.85A + 13.97B + 67.64C + 1.16AB - 11.79AC - 5.90BC + 4.85A^2 - 0.089B^2 - 86.80C^2 \quad (5.9)$$

During PUAE of PPE, all linear factors (A, B, C) and quadratic terms (A^2, C^2) except interaction terms (AB, BC, AC) were significant in influencing the response variable for the TPC case. Similarly, for the AA case, all linear factors (A, B, C), interaction terms (AC), and quadratic terms (A^2, C^2) were significant in influencing response variable variations.

Furthermore, for the TFC case, all linear terms (A, B, C), interaction terms (AC, BC) and quadratic terms (A^2, C^2) had a significant impact on the response variable. The greater F value and lower p value for the indicated factors have confirmed their major influence. As a result, the best quadratic models to

represent TPC, AA, and TFC attributes of the PUAE based PPE extraction system can be summarized as follows:

$$TPC = 134.32 + 5.47 + 3.84B + 10.49C - 0.11AB - 0.90AC - 0.90BC + 3.80A^2 - 0.48B^2 - 18.61C^2 \quad (5.10)$$

$$AA = 75.55 + 7.39A + 2.26B + 9.68C - 0.50AB - 2.19AC - 0.39BC + 2.77A^2 - 1.15B^2 - 8.59C^2 \quad (5.11)$$

$$TFC = 263.98 + 27.93A + 14.06B + 39.20C + 1.07AB + 4.59AC - 3.96BC - 3.94A^2 + 1.82B^2 - 86.21C^2 \quad (5.12)$$

These best fit models and associated parameters (section 5.4 and 5.5) affirm significance of each parameter and hence appropriate fitness of the values to fairly represent response variables. The models affirmed strong dependence of the response variables on the first order terms, first-order interaction terms, and as well as quadratic terms. Such modelling efforts will be highly beneficial for the sensitivity analysis of the available degrees of freedom and henceforth appropriate selection of the critical process variables in due course of the process design and development. Further, the modelling efforts also provide useful insights for the design, analysis and control of ultrasound assisted extraction processes and thereby assist in further understanding of the process scalability issues.

Table 5.7: Best fit RSM model fitness parameters and ANOVA data for various cases during normal UAE of papaya peel-water system.

Components	**Total Polyphenols**		**Total Flavonoids**		**Antioxidant Activity**	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	1122.30 (Quadratic)	< 0.0001 (Quadratic)	1074.91 (Quadratic)	< 0.0001 (Quadratic)	111.60 (Quadratic)	< 0.0001 (Quadratic)
A	843.28	< 0.0001	563.67	< 0.0001	101.50	< 0.0001
B	586.26	< 0.0001	210.77	< 0.0001	55.04	< 0.0001
C	6122.71	< 0.0001	4938.27	< 0.0001	479.57	< 0.0001
AB	3.72	0.0826	1.15	0.3079	0.16	0.6955
AC	474.77	< 0.0001	119.93	< 0.0001	29.11	0.0003
BC	203.58	< 0.0001	30.04	0.0003	13.36	0.0044
A^2	17.87	0.0018	6.97	0.0247	2.83	0.1233
B^2	48.08	< 0.0001	2.332E-003	0.9624	9.89	0.0104
C^2	898.36	< 0.0001	2235.95	< 0.0001	197.38	< 0.0001

Lack of Fit	3.03	0.1247	3.25	0.1109	2.59	0.1598
R squared		0.9990		0.9990		0.9901
Adequate Precision		122.262		107.201		39.078

Table 5.8: Best fit RSM model fitness parameters and ANOVA data for various cases during pulsed UAE of papaya peel-water system.

Components	Total Phenols		Total Flavonoids		Antioxidant Activity	
	***F* value**	***P* value**	***F* value**	***P* value**	***F* value**	***P* value**
Model	112.31 (Quadratic)	< 0.0001 (Quadratic)	878.97 (Quadratic)	< 0.0001 (Quadratic)	63.72 (Quadratic)	< 0.0001 (Quadratic)
A	101.26	< 0.0001	967.64	< 0.0001	165.75	< 0.0001
B	50.09	< 0.0001	245.30	< 0.0001	15.47	0.0028
C	372.52	< 0.0001	1906.79	< 0.0001	284.91	< 0.0001
AB	0.031	0.8631	1.14	0.3115	0.62	0.4505
AC	2.20	0.1692	20.91	0.0010	11.69	0.0066
BC	2.20	0.1692	15.56	0.0028	0.37	0.5555
A^2	13.42	0.0044	5.30	0.0441	6.40	0.0299
B^2	0.22	0.6500	1.13	0.3132	1.11	0.3171
C^2	322.65	< 0.0001	2535.90	< 0.0001	61.67	< 0.0001
Lack of Fit	1.18	0.4291	1.50	0.3332	3.27	0.1095
R squared		0.9902		0.9987		0.9829
Adequate Precision		38.085		95.548		30.825

5.6 Response Surface Characteristics and Relation between the Process Variables for Normal UAE and Pulsed UAE Processes

5.6.1 Influence of Normal UAE Process Parameters on Total Polyphenols

The TPC response surface plot depict similar pattern for both PPU and PPE cases. Fig. 5.1 ((a-c) and (d-f)) illustrate the impact of any two process variables on the TPC response surface characteristics of PPU and PPE extraction with the NUAE process. For PPU and PPE, the 3D surface graph clearly shows that the TPC yield enhanced for any two combinations of ST, ET, and LR. The TPC enhanced considerably from 53.35 – 140.25 mg GAE/g for PPU and 53.68 – 121.79 mg GAE/g for PPE for a variation in ST and LR from 5 – 20 min and 0.2 – 0.5 g/mL respectively at 40°C. Also, a marginal increase has been apparent in the TPC yield for a variation in the LR from 0.35 – 0.5 g/mL and marginal decline in the TPC yield is evident at the end of the 3D graph and for 0.5 g/mL value of the LR.

Likewise, for a fixed choice of LR of 0.35 mg/mL, initially there was a slow increase in the yield of TPC for both PPU and PPE cases. However, the corresponding TPC yield eventually started to increase. The TPC for PPU and PPE altered from 114.8 – 145.21 mg GAE/g and 109.79 – 119.26 mg GAE/g respectively, for the ST and ET variations from 12.5 – 20 min and 40 – 55 °C, respectively.

In addition, for a given ST of 20 min, the TPC for PPU and PPE altered from 94.76 – 145.21 mg GAE/g and 76.77 – 123.7 mg GAE/g respectively, for variations in process factors such as LR from 0.2 – 0.5 g/mL and ET from 40 – 70 °C. The 3D response surface plot shows that the TPC yield grows substantially with LR and ET and eventually reaches a maximum in the middle region and thereafter reduces marginally. Thus, for PPU and PPE, the maximum TPC yield of 145.21 ± 0.31 mg GAE/g and 126.6 ± 0.28 mg GAE/g respectively was obtained at 55 ^{o}C ET, 12.5 min ST, and 0.35 g/mL LR and 70 ^{o}C ET, 12.5 min ST, and 0.35 g/mL LR respectively. The 3D response surface plot confirmed nonlinear and quadratic variation in the TPC with respect to any two process parameters. The TPC can be analysed to the comparatively sensitive with respect to the hierarchy of LR > ST > ET in the considered degrees of freedom.

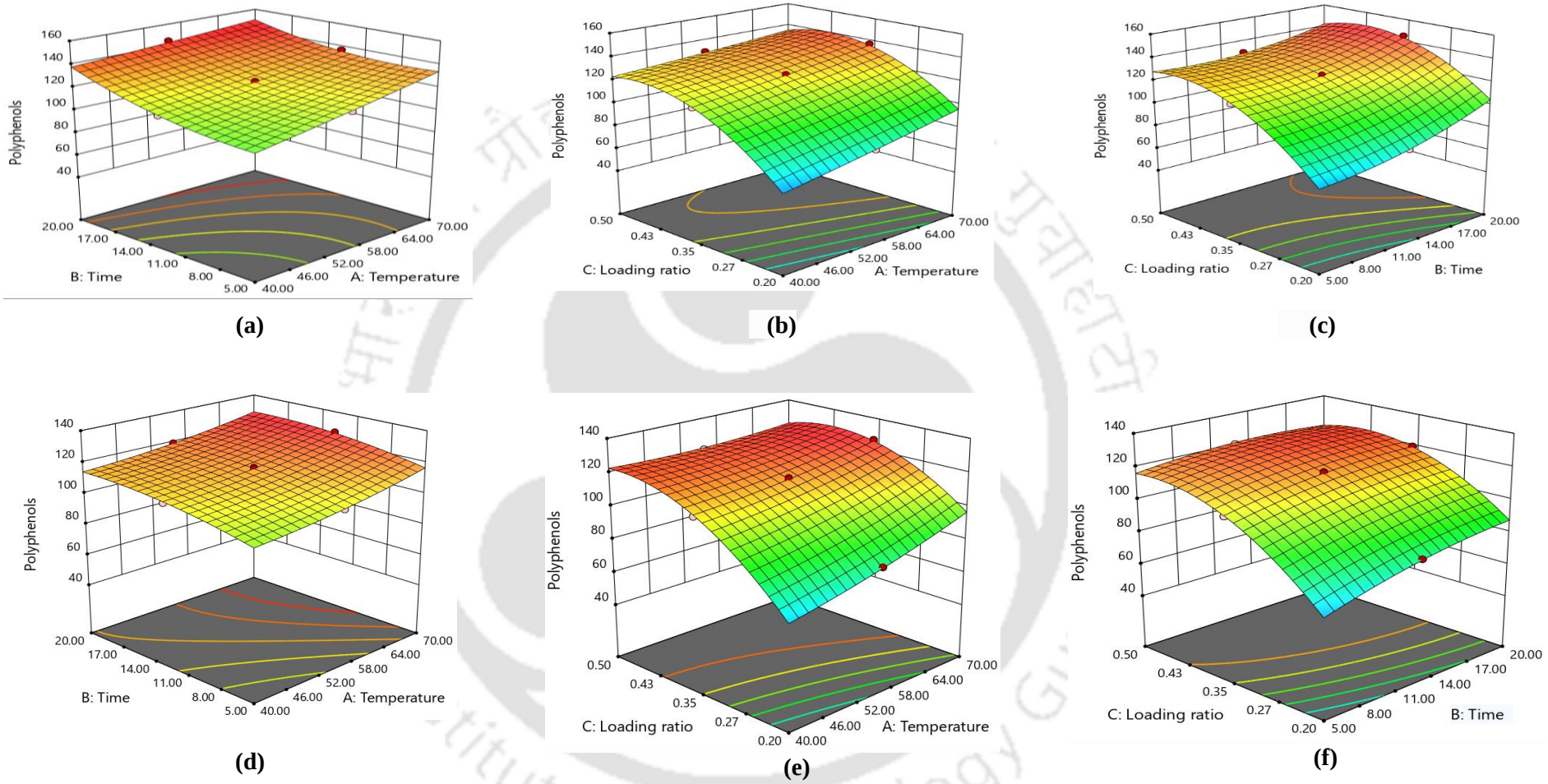

Figure 5.1: Response surface plots of total polyphenols for papaya pulp (a - c) and papaya peel (d-f) during normal UAE.

5.6.2 Influence of Normal UAE Process Parameters on Antioxidant Activity

For the AA case, the LR, followed by ET and ST, profusely influenced the response variable profiles for PPU and PPE cases (Fig. 5.2 (a-c) and (d-f) respectively). The 3D response surface graph for a given LR demonstrated that every combination of ST and ET fostered an enhanced AA value set. The AA surface plot depicts similar pattern for both PPU and PPE system. At the beginning, the AA increases with the ST and LR and eventually reaches a maximum value at 0.35 g/mL. Afterwards, a small decline in the yield of AA has been apparent in the 3D response surface plot.

Additionally, the AA considerably increased from 35.25 – 78.13 % for PPU and 47.67 – 75.3 % for the PPE case for the ST and LR variations of 5 – 20 min and 0.2 – 0.5 g/mL, respectively, and at 40 °C. Conversely, for a constant value of LR of 0.2 g/mL, the AA altered from 35.25 – 50.82 % for PPU and 47.67 – 67.85 % for PPE for the ST and ET variation from 5 – 20 min and 40 –70 °C, respectively. These variations affirmed that firstly, a linear increase prevailed for the AA with ST and ET. Thereafter, the AA yield increased and reached saturation.

Furthermore, the AA varied substantially from 35.25 – 83.61 % for PPU and 55 – 75.3 % for PPE with a variation in LR and ET from 0.2 – 0.5 g/mL and 40 – 70 ^{o}C, respectively and for a fixed choice of 20 min ST. At first, the yield increased linearly with a variation in ET and LR and eventually reached a maximum value. Thereafter, a small decline in the AA yield was evident. The highest combination of process parameters for the PPU case were 0.35 g/mL LR, 55 °C ET, and 12.50 min. For the condition, the highest AA of 85.83 ± 0.29 % was obtained. For the PPE case, the best process parameters were 0.35 g/mL LR, 55 °C ET, and 20 min ST, and thereby resulted in the maximum AA of 80.36 ± 0.17%.

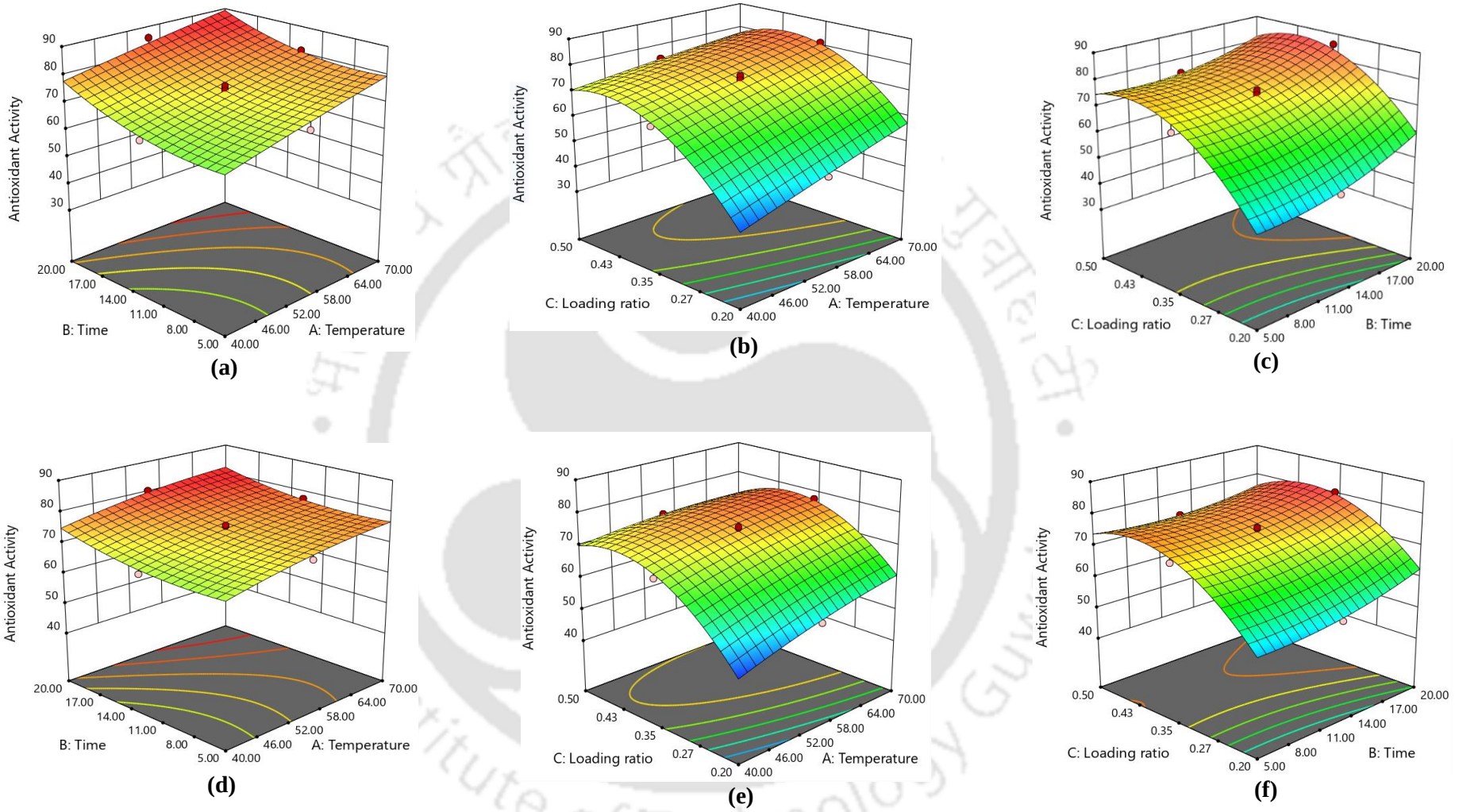

Figure 5.2: Response surface plots of antioxidant activity for papaya pulp (a - c) and papaya peel (d-f) during normal UAE.

5.6.3 Influence of Normal UAE Process Parameters on Vitamin C

At 70 °C, the VITC enhanced considerably from 16.44 – 98.87 mg/100g for ST and LR variations from 5 – 20 min and 0.2 – 0.5 g/mL respectively. The VITC yield first enhanced as linearly with respect to ST and LR variations and thereafter reached a peak and eventually declined. For a fixed ratio of LR of 0.2 g/mL, the VITC, on the other hand, varied well (16.44 – 75.23 mg/100g for a variation in ST and ET from 5 – 20 min and 40 – 70 °C, respectively). The response surface plot (Fig. 5.3 (a-c)) affirmed linear VITC yield enhancement during initial combinations of ST and ET. Eventually, the VITC yield enhanced exponentially with ST and ET.

Furthermore, for a fixed choice of ST of 20 min, the binary combination of ET and LR varied VITC significantly from 56.22 – 134.46 mg/100g. The 3D response surface plot exhibited substantial VITC yield alterations with respect to LR and ET. Thereafter, the plot affirmed a peak and subsequent sharp decline in the VITC yield upto 0.5g/mL LR. The best process parameter combination for the VITC was 0.35 g/mL LR, 55 °C ET, and 12.5 min ST. At this condition, highest VITC yield of 134.46 ± 0.25 mg/100g was obtained. The combination of LR, followed by ST and ET, has been analysed to substantially influence VITC extraction.

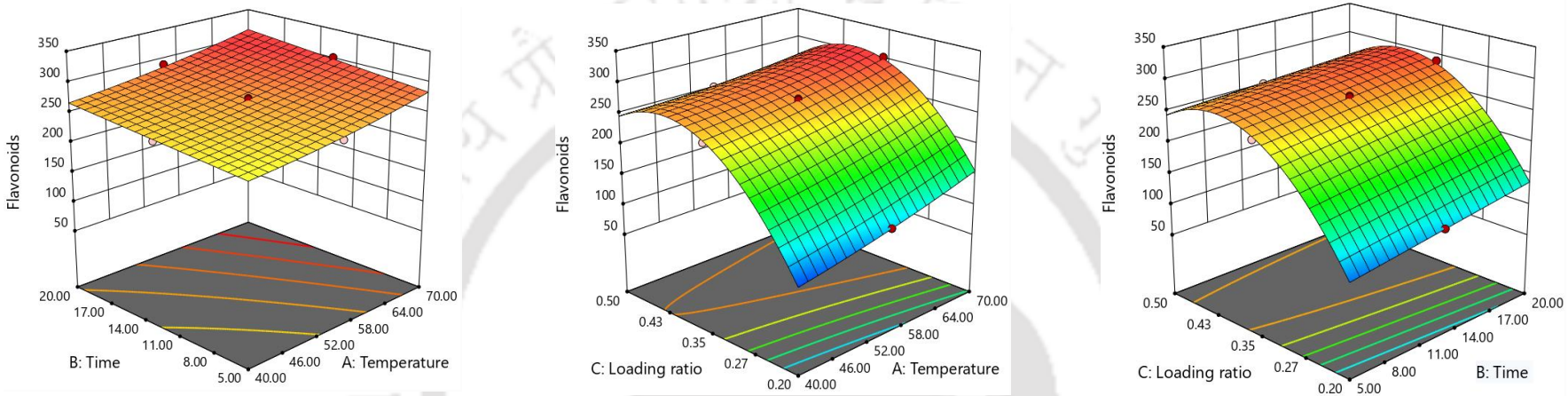

Figure 5.3: Response surface plots of total flavonoids for papaya peel (a - c) during normal UAE.

5.6.4 Influence of Pulsed UAE Process Parameters on Total Polyphenols

For both PPU and PPE, the TPC response surface plot illustrate a similar trend. The influence of any two process factors on TPC response surface characteristics for PPU and PPE extracts of PUAE has been shown in Fig. 5.4 (a – c) and Fig. 5.4 (d – f) respectively. The 3D surface graph for PPU and PPE clearly illustrate that the TPC yield increased for any two combinations of ST, ET, and LR. For an alteration in ST and LR from 5 – 20 min and 0.2 – 0.5 g/mL respectively and at 40°C, the TPC significantly enhanced from 80.87 – 140.5 mg GAE/g for PPU and 97.46 – 127.24 mg GAE/g for PPE. In addition, for the LR alteration from 0.35 – 0.5 g/mL, a marginal enhancement in the TPC yield can be observed. Also, a small decline in the TPC yield can be observed towards the end of the 3D graph (for 0.5 g/mL LR).

Similarly, at a constant LR of 0.35 mg/mL, the yield of TPC for both PPU and PPE cases increased slowly during the initial combinations of ST and ET. Then afterwards, the TPC yield began to rise. For variations in ST and ET from 12.5 – 20 min and 40 – 55 °C, respectively, the TPC of PPU and PPE varied from 126.7 – 169.16 mg GAE/g and 132.27 – 140.03 mg GAE/g respectively.

For variations in process parameters such as LR from 0.2 – 0.5 g/mL and ET from 40 – 70 °C, the TPC of the PPU and PPE varied from 97.43 – 162.5 mg GAE/g and 107.15 – 136.39 mg GAE/g, respectively, for a fixed choice of ST of 20 min. The 3D response surface plot reveals that the TPC yield increased significantly with LR and ET, and reached a peak in the centre, and thereafter reduced gradually.

Consequently, for the PPU and PPE, the maximum TPC yields of 169.16 ± 0.61 mg GAE/g and 145.44 ± 0.07 mg GAE/g were achieved at 55 ^{o}C, 12.5 min, and 0.35 g/mL and 70 ^{o}C, 12.5 mins, and 0.35 g/mL, respectively. The 3D response surface plot validated the nonlinear and quadratic variation of TPC with respect to any two process factors. The TPC was found to be the most sensitive among all degrees of freedom for both PPU and PPE and followed the hierarchy of LR > ET > ST.

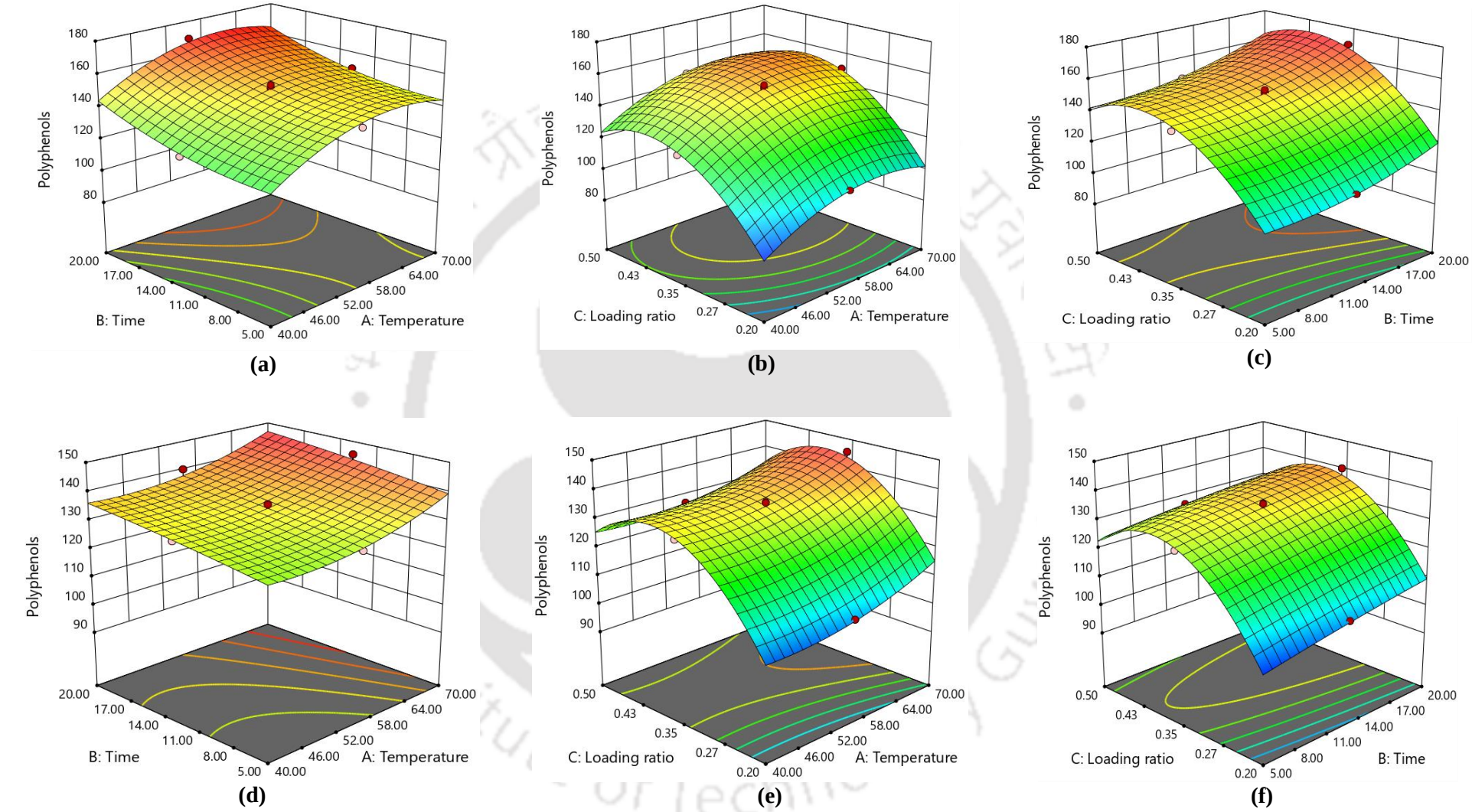

Figure 5.4: Response surface plots of total polyphenols during normal UAE of papaya pulp (a - c) and papaya peel (d - f).

5.6.5 Influence of Pulsed UAE Process Parameters on Antioxidant Activity

The LR, followed by ET and ST, significantly affected the response variable profiles for PPU and PPE in the AA case study (Fig. 5.5 (a – c) and (d – f)). For a given LR, the 3D response surface graph revealed the ST and ET combination always resulted in a higher AA. For both PPU and PPE systems, the AA surface plot shows a similar pattern. Initially, AA enhanced with the variations in ST and LR and finally reached a maximum value at an LR of 0.35 g/mL. Thereafter, a minor drop in AA yield has been confirmed by the 3D response surface plot.

Furthermore, at 40 °C, for ST and LR variations of 5 – 20 min and 0.2 – 0.5 g/mL, respectively, the AA increased significantly from 31.29 – 85.2 % for the PPU and 46.51 – 75.67 % for the PPE case. At a constant LR of 0.2 g/mL, the AA varied from 31.29 – 86.88 % for PPU and 46.51 – 69.42 % for PPE for an alteration in ST and ET from 5 – 20 min and 40 – 70° C, respectively. These variations confirmed that, the AA initially increased linearly with ST and ET and eventually attained a saturation towards the end of PUAE process.

Furthermore, with a variation in LR and ET from 0.2 – 0.5 g/mL and 40 – 70 ^{o}C, respectively, and for a fixed choice of 20 min ST, the AA varied significantly from 45.05 – 86.88 % for PPU and 52.73 – 75.67 % for PPE. Initially, the yield enhanced linearly with variations in ET and LR and thereby reached a maximum value. Thereafter, a minor reduction in the AA yield has been apparent. For the PPU case, the best combination of process parameters corresponds to 0.35 g/mL LR, 55^{o}C ET, and 12.50 min ST, for which the maximum AA of 92.9 ± 0.45% has been achieved. The best combination of process parameters for the PPE case were 0.35 g/mL LR, 70 °C ET, and 20 min ST, for which a maximum AA of 89.17 ± 0.52 % has been achieved.

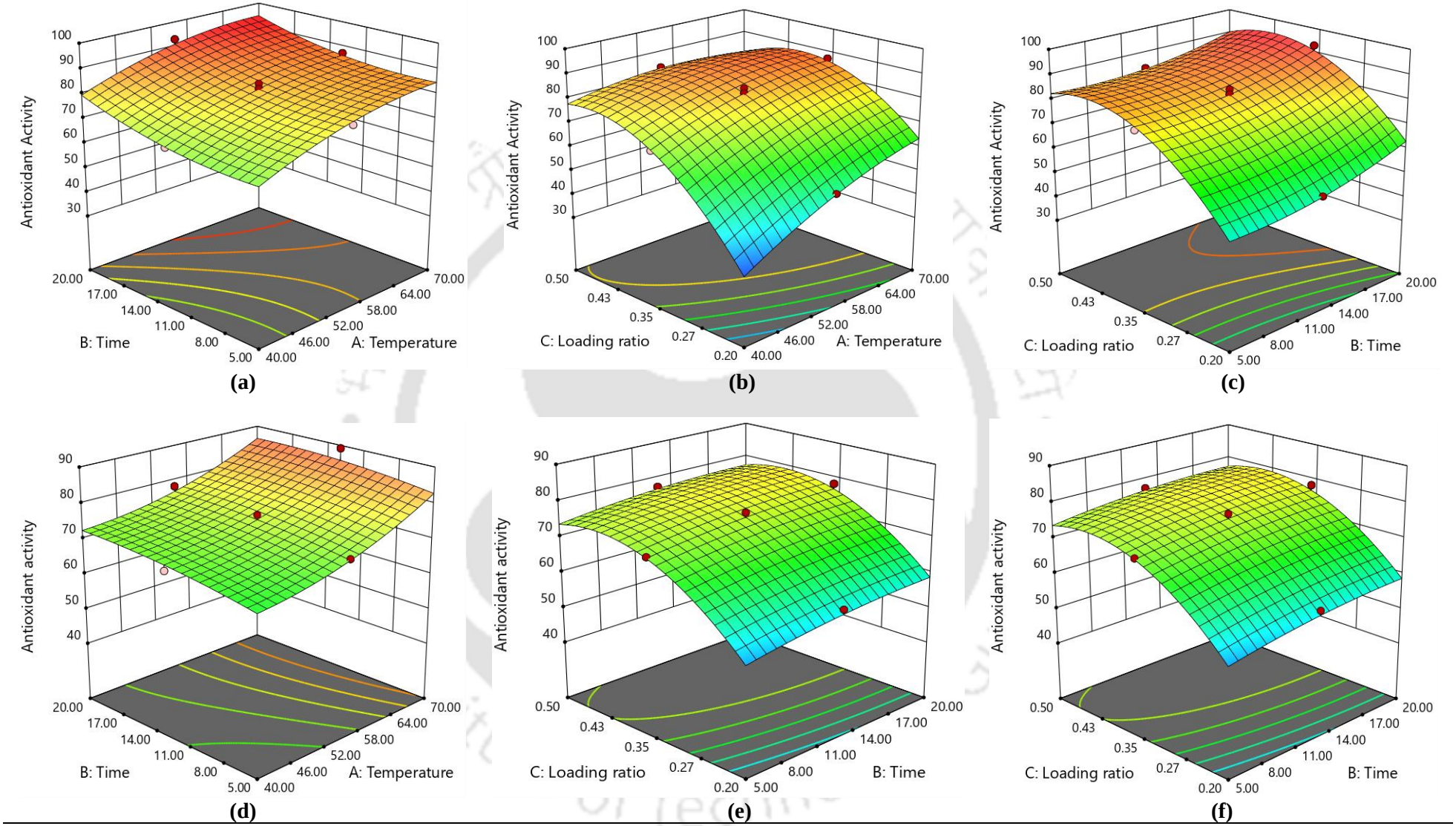

Figure 5.5 : Response surface plots of antioxidant activity during pulsed UAE of papaya pulp (a - c) and papaya peel (d-f).

5.6.6 Influence of Pulsed UAE Process Parameters on Total Flavonoids

For an increase in ST from 5 – 20 min and LR from 0.2 – 0.5 g/mL and for a fixed choice of ET (70°C), the TFC of PPE increased significantly from 139.35 – 257.17 mg QE/g. During initial alterations of ST and LR, the 3D response plot affirmed a linear increase in the TFC yield (Fig. 5.6 (a – c)). Subsequently, the TFC yield achieved its maximum value and thereafter reduced towards the end of the PUAE process.

On the other hand, the TFC yield of PPE varied considerably from 97.36 – 178.73 mg QE/g for an alteration in ST and ET from 5 – 20 min and 40 – 70 °C, respectively, and for a fixed choice of LR (0.2 g/mL). For this case also, a linear increase in the TFC yield has been apparent with respect to ST and ET alterations. Thereafter, the TFC yield increased gradually.

Furthermore, the binary combination of ET (40 –70 °C) and LR (0.2 – 0.5 g/mL) altered TFC yield of PPE from 97.36 – 234.78 mg QE/g and for a fixed choice of the ST of 5 min. The TFC yield enhanced linearly during the initial combinations of ST and LR. Thereafter, the TFC yield peaked and reduced eventually. The best process parameter combination to attain the maximum TFC yield of 292.2 ± 0.12 mg QE/g refer to 0.35 g/mL LR, 70 ºC ET, and 12.5 min ST. For the TFC, the process parameters had a hierarchical influence and in the order LR > ET > ST.

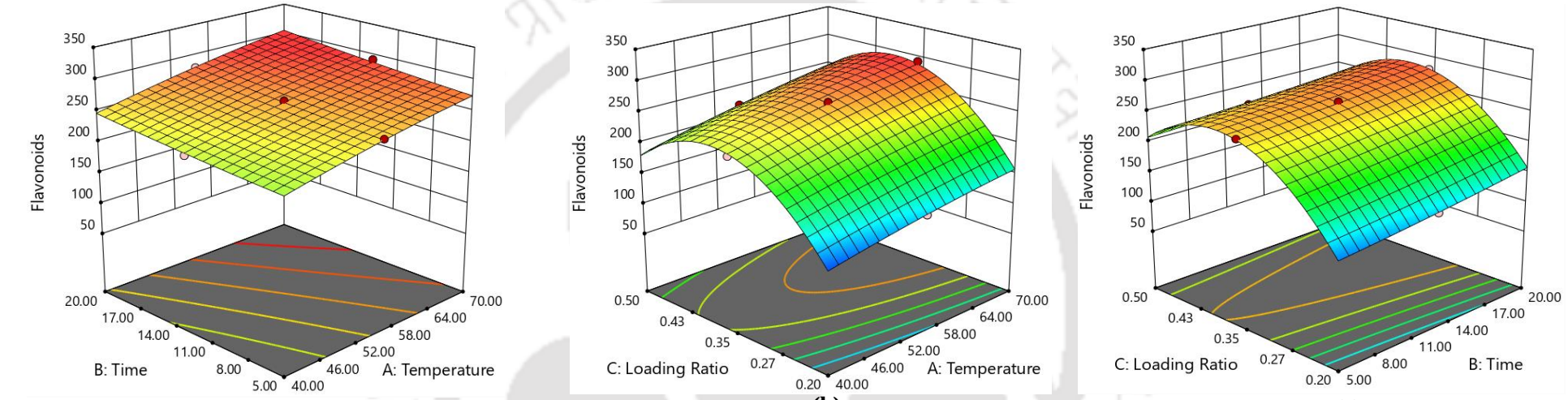

Figure 5.6: Response surface plots of total flavonoids during pulsed UAE of papaya peel (a - c).

5.6.7 Influence of Pulsed UAE Process Parameters on Vitamin C

For a variation in ST and LR from 5 – 20 min and 0.2 – 0.5g/mL respectively, the VITC yield of PPU increased significantly from 59.31 to 153 mg/100g and for a fixed choice ET of 70°C. The response surface plot of VITC depicts that the VITC yield increased initially as a linear function with respect to ST and LR. Thereafter, the yield peaked and followed by a declining trend towards the end of PUAE process.

For a constant LR of 0.2g/mL, the VITC of PPU ranged from 28.52 – 96.83 mg/100g for an alteration in ST and ET from 5 – 20 min and 40 – 70 °C, respectively. During initial combinations of ST and ET, the 3D surface plot (Fig.5.7 (a – c)) confirmed linear VITC yield enhancement. Thereafter, the VITC yield increased exponentially.

The 3D response surface plot revealed a significant VITC yield of PPU from 53.93 – 153 mg/100g at a constant ST of 20 min for the binary combination of ET (40 – 70 °C) and LR (0.2 – 0.5 g/mL). Thereafter, the response surface plot revealed a peak in VITC yield and a sharp decline thereafter for the LR altering upto 0.5 g/mL. The best process parameter combination of 0.35 g/mL LR, 55°C ET, and 12.5 min ST, yielded the maximum VITC yield of 164.94 ± 0.25 mg/100g. The conjunction of LR, ST, and ET has been analysed to have a significant influence on the VITC extraction yield.

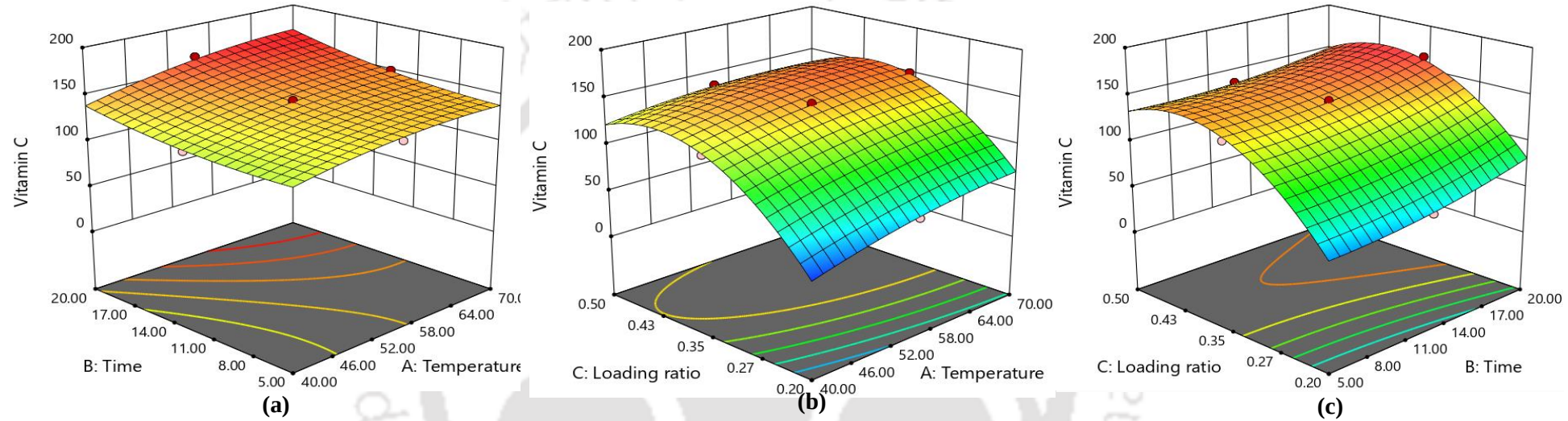

Figure 5.7: Response surface plots of Vitamin C during pulsed UAE of papaya pulp (a - c).

5.7 Optimisation

For the optimization of bioactive constitution in the aqueous extract, the optimality of all independent parameters have been determined for both PPU and PPE systems and for the NUAE and PUAE processes. For the NUAE of PPU case, the optimum values for TPC, VITC and % AA were 148.82 mg GAE/g, 148.81 mg/100 g and 87.47 % for an optimal process parametric choice of 60.33 °C ET, 19.82 min ST and 0.40 g/mL LR. For the PPE case, the optimum values for TPC, TFC and % AA were 138.58 mg GAE/g, 312.20 mg QE/g and 81.10 % for an optimum process condition of 66.60 °C ET, 18.53 min ST and 0.40 g/mL LR.

For the case of PUAE of PPU, the optimal values for TPC, VITC, and % AA were 169.11 mg GAE/g, 162.13 mg mg/100g, and 92.29 %, respectively, for an optimal process parameter set of 58.59 °C ET, 18.82 min ST, and 0.40 g/mL LR. For the PPE case, the optimal values of TPC, TFC, and % AA correspond to 146.40 mg GAE/g, 301.28 mg QE/g, and 87.88 % for the optimal process parameter set of 70 °C ET, 17.67 min ST, and 0.40 g/mL LR. Consequently, at comparable LR, both PPU and PPE demonstrated optimal extraction for both NUAE and PUAE processes. Additionally, other independent parameters such as ET and ST had different effect on the extraction of bioactives yield for both PPU and PPE systems and with NUAE and PUAE system respectively. This can be attributed to the different types of ubiquitous bioactive metabolites and their contents in the PPU and PPE samples (Vallejo-Castillo et al., 2020). As a result, it can be concluded that the PPU possessed higher proportions of bioactive compounds than the PPE.

5.8 Comparative Analysis of Normal UAE and Pulsed UAE for Papaya Pulp and Papaya Peel

Following a comprehensive analysis of the most relevant data for the NUAE and PUAE of PPU and PPE systems, it can be inferred that both PPU and PPE are excellent sources of bioactive compounds. In comparison to the NUAE, the PUAE process produced more bioactives for both PPU and PPE systems. Also PPU enabled better extraction of bioactives in comparison to PPE for both the processes. This could be ascribed due to variant cell structure and differences in chemical composition for the PPU

case in comparison with the PPE case (Vallejo-Castillo et al., 2020). As a result, in the former case, the potential of ultrasonic waves to penetrate the matrix and the potential of the solvent to permeate the sample matrix have together enhanced and thereby resulted in increased mass transfer rates of bioactives into the solvent (Uribe et al., 2015). Furthermore, experimental findings indicated that LR has been a crucial factor in improving the bioactive yield. Thereby, it has been proposed that the concentration gradient between the sample and the solvent enabled easier transport of the solute into the solvent (Anticona et al., 2021). Additionally, agitation induced by PUAE has been proven to accelerate the initiation of mechanical processes such as particle collisions and cell wall disintegration. This improves the mass transfer rates of bioactives from the interior of the cell wall to the periphery and consequent penetration of bioactives into the solvent phase (Chakraborty et al., 2020).

Both PPU and PPE yield patterns affirmed significant trends. For the PPU, at an ET of 55 °C, a LR of 0.35 g/mL, and a ST of 20 min, the NUAE indicated the highest yield of TPC, AA and VITC as 145.21± 0.31 mg GAE/g, 85.83 ± 0.29 %, 139.5 ± 0.68 mg/100g respectively. On the other hand, the PPE affirmed highest yield of TPC of 126.51 ± 0.28 mg GAE/g, and TFC of 302 ± 0.36 mg QE/g for 70 °C ET, 0.35 g/mL LR, and 12.50 min ST. However, the optimal AA of 80.36 ± 0.17 % was obtained at an ET of 55 °C, a LR of 0.35 g/mL, and a ST of 20 min.

Similarly, for the PPU case, the PUAE indicated the maximum yield of TPC, AA, and VITC as 169.16 ± 0.61 mg GAE/g, 92.9 ± 0.45 %, and 164.94 ± 0.25 mg/100g, respectively, at an ET of 55 °C, LR of 0.35 g/mL, and ST of 20 min. At 70 °C ET, 0.35 g/mL LR, and 12.50 min ST, the PUAE of PPE yielded the maximum TPC of 145.44 ± 0.07 mg GAE/g, AA of 89.17 ± 0.52 %, and TFC of 292.2 ± 0.12 mg QE/g. The lesser yield of bioactives (TPC and AA) for the PPE case could be attributed towards the sensitivity of thermo-sensitive bioactive molecules at higher ET. While a sufficiently high ET can improve solute solubility by increasing the diffusion coefficient of the solution, even greater ET can cause some thermo-sensitive compounds to degrade (Ciric et al., 2020). Upon analysing the influence of various parameters for the PPU case, it has been discovered that only LR and ET had the greatest effect on the TPC, % AA, and VITC yield. Similarly, LR, ET, in addition to ST, had a considerable influence on the response variable for the PPE case. For both NUAE and PUAE processes, the degrees

of freedom were ranked in the following order: LR > ET > ST. To summarise, the PUAE method has been superior for the extraction of bioactives from both PPU and PPE systems, and the PPU possessed more bioactives than the PPE.

5.9 Comparative Assessment with Prior Art Data

The findings of this study (NUAE and PUAE) have been compared to the prior art data (Tables 5.9 and 5.10). According to the data, the PUAE has the most effective extraction method for both PPU and PPE system. PUAE exhibited a higher extraction efficiency than NUAE and HWE for both PPU and PPE systems. Ultrasonic waves break the cell wall, allow increased interaction between sample and solvent and thereby improve the extraction efficiency (Montero-Calderon et al., 2019). In addition, increasing ET during HWE can potentially improve the permeability of the solvent into the cell, which in turn increases the solubility of the targeted ingredients within the solvent (Sillero et al., 2020). However, this can result in the deterioration of some bioactive metabolites, which in turn lead to a reduction in the effectiveness of the extraction process (Zakaria & Kamal, 2016). Therefore, this hypothesis could perhaps reflect, to a certain extent on the reduced efficacy of the HWE in conjunction with the PUAE. In addition, compared to the PPE, the RSM-based numerical optimization of the PUAE resulted in better extraction of bioactive components from the PPU. Many relevant conclusions can be drawn from the findings reported in the Table (Tables 5.9 and 5.10). Prominently, the best results were achieved with the PUAE of PPU system, which allowed for the extraction of 169.16 ± 0.61 mg GAE/mg TPC, 92.9 ± 0.45% AA, and 164.94 ± 0.25 VITC at 55 °C ET, 20 min ST, and 0.35 g/mL LR. The best PPE system UAE values were 145.44 ± 0.07 mg GAE/g TPC, 89.17 ± 0.52 % AA, and 292.2 ± 0.12 mg QE/mg TFC at 70 ^{o}C ET, 12.5 min ST, and 0.35 g/mL LR. The possible reason for this could the sensitivity of bioactive compounds at higher ET. According to Vallejo-Castillo et al. (2020), the MAE generated 93 % lesser TPC than the best data of the PUAE – PPU system being reported in this investigation. This might be due to lower LR (0.016 g/mL) used in the literature, or because of sub-optimal extraction procedures, such as MAE and pre-treatment of papaya samples at 50°C (Vallejo-Castillo et al., 2020). Cavitation during ultrasound, lower LR stated in the literature, and the use of dried powder for extraction may explain such significant variations. Among them, the last of these reasons has been

validated by our research group's investigation on HWE. For instance, the HWE conducted in our investigation under optimal conditions yielded significantly higher TPC levels than those reported in the cited literature (Vallejo-Castillo et al., 2020). Moreover, Uribe et al. (2015) affirmed that the HHPE-UE resulted in 99.2 % lower TPC and 54.35 % lower VITC concentrations than the best data of the PUAE – PPU system being reported in this investigation. This could be attributed to the substantially lower LR of 0.25 g/mL being adopted in the said literature.

Considering all of this into consideration, it is possible to conclude that PUAE with raw PPU and PPE systems is the most efficacious technique for extracting highly desired bioactive constituents rich in TPC, TFC, VITC, and AA. Consequently, the relevance and originality of this work have been validated. According to the data, the stated prior art did not emphasize upon the RSM-based PUAE extraction of bioactive components from PPU and PPE systems. Besides, the influence of process conditions on bioactive compound yield has not been addressed in any prior art.

Table 5.9: Comparative summary of optimal data for normal UAE of bioactives from papaya pulp and papaya peel system.

Extraction Process	Sample and solvent used	Temperature (ºC)	Time (min)	Loading ratio (g/mL)	Total Polyphenols (mg GAE/g)	Total Flavonoids (mg QE/g)	Antioxidant Activity (%)	Vitamin C (mg/100 g)	Literature
MAE	mixture of PPU and PPE, soy bean oil	NA	3	0.016	11.86	0.43	NA	NA	Vallejo-Castillo et al. (2013)
HHPE-UE	mixture of PPU and PPE, soy bean oil	NA	5-15	0.25	1.29	NA	NA	74	Uribe et al. (2015)
HWE of PPU	PPU, water	60.33	19.82	0.40	105	NA	76.50	89.4	This work
HWE of PPE	PPE, water	66.60	18.53	0.40	88.5	145	71.5	NA	This work
NUAE of PPU	PPU, water	60.33	19.82	0.40	148.8	NA	87.47	148.8	This work
NUAE of PPE	PPE, water	66.60	18.53	0.40	138.6	312	81.1	NA	This work

Table 5.9: Comparative summary of optimal data for pulsed UAE of bioactives from papaya pulp and papaya peel system.

Extraction Process	Sample and Solvent used	Temperature (°C)	Time (min)	Loading Ratio (g/mL)	Total Phenols (mg GAE/g)	Total Flavonoids (mg QE /g)	Antioxidant Activity (%)	Vitamin C (mg/100g)	Literature
MAE	mixture of PPU and PPE, soy bean oil	NA	3	0.016	11.86	0.43	NA	NA	Vallejo-Castillo et al. (2013)
HHPE-UE	mixture of PPU and PPE, soy bean oil	NA	5-15	0.25	1.29	NA	NA	74	Uribe et al. (2015)
HWE of PPU	PPU, water	58.59	18.82	0.45	118.4	NA	79.50	100.4	This work
HWE of PPE	PPE, water	70	17.67	0.45	100..2	167.6	73.8	NA	This work
PUAE of PPU	PPU, water	58.59	18.82	0.45	169.11	NA	92.29	162.13	This work
PUAE of PPE	PPE, water	70	17.67	0.45	146.40	301.28	87.88	NA	This work

5.10 Summary

Several useful inferences can be deduced from this work. These have been presented as follows.

- The study revealed that extraction conditions had a significant influence on the recovery of bioactive compounds.
- The study also indicated that the LR had a substantial influence on the bioactive constituent's recovery followed by ET and ST.
- Also, second-order models have been successful used to elucidate the correlation between the independent process parameters and response variables.
- For the NUAE of PPU, the optimum values for TPC, VITC and % AA were 148.82 mg GAE/g, 148.81 mg/100g and 87.47 % at optimum process condition of 60.33 °C ET, 19.82 min ST and 0.40 g/mL LR.
- For the NUAE of PPE case, the optimum values for TPC, TFC and % AA were 138.58 mg GAE/g, 312.20 mg QE/g and 81.10 % for optimum process condition of 66.60 °C ET, 18.53 min ST and 0.40 g/mL LR
- During PUAE, the PPU case resulted in higher TPC and AA yield than PPE. The optimum PUAE process conditions for PPU were obtained as 58.59 °C ET, 18.82 min ST, and 0.45 g/mL LR. Under these conditions, the optimum values for TPC, VITC, and % AA of PPU extract were obtained as 169.11 mg GAE/g, 162.13 mg mg/100g, and 92.29 %. For the PPE case, the optimal values for TPC, TFC, and % AA were 146.40 mg GAE/g, 301.28 mg QE/g, and 87.88 % for the optimal process condition of 70 °C ET, 17.67 min ST, and 0.45 g/mL LR.
- Furthermore, the study affirmed that PUAE has been an efficient green extraction technique in the extraction of desired bioactive components in comparison to a HWE process (105 mg GAE/g, 89.4 mg/100g, and 76.50 %, for PPU case and 88.5 mg GAE/g, 145 mg QE/g and 71.5 % for PPE case).

In summary it can be concluded that both PPU and PPE systems are potential sources of bioactive compounds and ultrasound-assisted extraction can be both feasible and environmentally friendly

technique for the efficient utility and application in food processing and pharmaceutical industry involving functional and herbal resources.

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Chapter 6:

Continuous and Pulsed Ultrasound-Assisted Extraction of Pectin from Pomelo Fruit Peel using Citric Acid

Chapter 6

Continuous and Pulsed Ultrasound-Assisted Extraction of Pectin from Pomelo Fruit Peel using Citric Acid

In this chapter, the findings associated to the optimization of UAE (NUAE and PUAE) process parameters during extraction of pectin from pomelo peel have been elucidated. After a brief introduction in section 6.1, the section 6.2 elaborates on the experimental data achieved through RSM modelling based design of experiments approach. Sections 6.3.1 and 6.3.2, respectively present findings associated to the parametric analysis and Pareto chart analysis for the UAE of pectin from pomelo peel. Thereafter, sections 6.4 and 6.5 respectively summarize the outcomes of the model fitness studies and ANOVA data summary for NUAE and PUAE processes respectively. Section 6.6 addresses the response surface characteristics of NUAE and PUAE of pectin from pomelo peel. Thereafter, section 6.7 details upon the optimal findings of the NUAE and PUAE process parameters during pectin extraction. A comparative analysis of electrical consumption and CO_2 emission for PUAE and AHWE process has been addressed in section 6.8. Section 6.9 elaborates on the physiochemical properties of extracted pectin. Following this, section 6.10 devotes to the nutritional characteristics of extracted pectin. Characterisation of pectin from PUAE and AHWE process have been outlined in section 6.11. Thereafter, the influence of alternate acids on the pectin yield (PY) and degree of esterification (DE) have been discussed in section 6.12. Later, sections 6.13 and 6.14 details upon the comparative findings of the AHWE and HWE and NUAE and PUAE processes in terms of PY and DE. A comparative assessment with best literature data has been conveniently presented in section 7.15. Finally, section 7.16 presents key findings of the conducted investigations.

Overview

The conducted research work targeted the citric acid (CA) aided extraction of pectin from pomelo peel using bath type sonication system. The investigation was carried out using the RSM based FCD of the NUAE and PUAE processes. The influence of process factors such as sonication time (ST) and pH on the recovery from pomelo peel was assessed. Additionally, the influence of continuous UAE (NUAE) and pulsed UAE (PUAE) on the pectin recovery were studied in a comparative frame work. The optimal PUAE process findings was eventually compared with the AHWE process.

6.1 Introduction

This chapter elaborates upon the acidic extraction of pectin from pomelo peel using UAE and conventional acidic hot water extraction (AHWE) processes. The influence of ST and pH on the PY and DE were evaluated using FCD based RSM design approach with two process variables (ST and pH) and three levels. The extraction was performed with fixed choice of dried pomelo peel powder to water ratio (LR) (1:15 g/mL) and ET (65°C). A comparative analysis of the influence of PUAE and AHWE on various properties of extracted pectin have also been addressed. The study also targeted criticality of citric acid (CA) on the pectin yield (PY) and degree of esterification (DE) in comparison with other acids used in this investigation and efficacy of the PUAE process in comparison with both NUAE and AHWE processes.

6.2 Experimental Data Analysis

Among all process variables, the pH was critical in the UAE (NUAE and PUAE) based production of pectin from pomelo peel, followed by ST. The pH (1.5 – 3.5) had a direct influence on the PY and DE (Tables 6.1 and 6.2). For the NUAE (Table 6.1), for a variation in ST and pH from 5 – 30 min and 1.5 – 3.5 pH, respectively, the PY and DE altered from 3 – 35.9 % and 40.73 – 84.9 % %, respectively. For the PUAE, these varied as 5.5 – 40.41% and 47.6 – 88.6 %, respectively (Table 6.2). The PY enhanced due to acidic condition in the solution that favoured hydrolysis of cellulose to produce additional pectin. While pectin does not generate from cellulose, the prevalent acidic conditions in the solution can contribute to the breakdown of plant cell walls that contain both cellulose and pectin. Under these acidic

conditions, the cellulose in the plant cell wall undergoes partial hydrolysis. Henceforth, it prompts the greater release of pectin in the solution. Such pectin release is from the cellular wall matrix. Thus, its release is partially related to the breakage of the cellulose fibers. Further, pH reduction released more H^+ ions and thereby fostered hydrolysis and enhanced PY (Hosseini et al., 2019). The DE of the pectin obtained at a relatively higher pH (3) possessed higher DE than that being obtained at a lower pH (2). In this regard, it is well known that the acidic extraction process typically yields a high constitution of methoxyl pectin (DE > 50%) in comparison with hot water or basic media system that yields a low methyl pectin content (Methacanon et al., 2014). The degree of esterification of the pectin has been evaluated to be about 60%. Also molecular weight of pectin, an important property of pectin and its gelling index have not been evaluated in the thesis and shall be considered in the near future to further enhance subjective and commercial aspects of the UAE based pectin extraction. Shivamathi et al. (2019) confirmed that the PY increased with reducing pH conditions and the maximum yield of pectin from lemon albedo and HCl system was achieved at a lower pH of 1.45. In tables 6.1 and 6.2, molecular weight, another important property of the pectin can also be considered as a dependent variable. However, its evaluation is tedious and requires regulated access to advanced instrumentation facility. Hence, such studies have not been considered in the targeted perspectives of the PhD thesis.

Table 6.1: FCD-RSM based parametric and response variable data summary of pectin extraction through normal UAE of pomelo peel.

S.No	pH (A)	Time (min) (B)	Pectin yield (%)	Degree of esterification (%)
1.	2.50	17.50	22.7 ± 0.25	70.25 ± 0.21
2.	3.50	5.00	3.0 ± 0.21	76.92 ± 0.33
3.	2.50	17.50	23.0 ± 0.47	72.25 ± 0.12
4.	2.50	5.00	12.51 ± 0.19	63.26 ± 0.16
5.	2.50	30.00	20.72 ± 0.24	67.6 ± 0.28
6.	3.50	17.50	10.7 ± 0.31	84.9 ± 0.42
7.	1.50	5.00	22.55 ± 0.11	40.73 ± 0.20
8.	2.50	17.50	24.1 ± 0.37	72.2 ± 0.09
9.	1.50	30.00	35.35 ± 0.08	47.06 ± 0.25
10.	3.50	30.00	6.35 ± 0.14	80.3 ± 0.14

11.	2.50	17.50	23.7 ± 0.23	71.7 ± 0.33
12.	2.50	17.50	23.5 ± 0.24	72.25 ± 0.65
13.	1.50	17.50	33.9 ± 0.42	52.09 ± 0.40

Table 6.2: FCD-RSM based parametric and response variable data summary of pectin extraction through pulsed UAE of pomelo peel.

S.No	pH (A)	Time (min) (B)	Pectin yield (%)	Degree of esterification (%)
1.	2.50	30.00	23.0 ± 0.82	72.7 ± 0.36
2.	3.50	17.50	13.7 ± 0.22	88.6 ± 0.31
3.	2.50	17.50	26.4 ± 0.37	75.2 ± 0.75
4.	1.50	30.00	42.55 ± 0.14	52.9 ± 0.37
5.	2.50	17.50	25.5 ± 0.19	74.7 ± 0.33
6.	2.50	5.00	16.79 ± 0.04	67.8 ± 0.43
7.	3.50	30.00	8.77 ± 0.11	85.4 ± 0.71
8.	1.50	17.50	46.4 ± 0.19	55.0 ± 0.47
9.	1.50	5.00	33.46 ± 0.25	47.6 ± 0.25
10.	2.50	17.50	25.0 ± 0.38	74.0 ± 0.66
11.	2.50	17.50	27.0 ± 0.74	74.5 ± 0.22
12.	3.50	5.00	5.5 ± 0.24	82.43 ± 0.24
13.	2.50	17.50	26 ± 0.47	75.2 ± 0.62

The pectin content in the peels does vary and depends on the type of fruit or vegetable, its ripened state of maturity and other factors. The prior art conveyed 10-15% pectin constitution on a dry matter basis in the apple pomace peel. The thesis work affirmed 35-43% pectin extraction from the pomelo peel. Since continuous pectin extraction was facilitated in the conducted investigations, no precipitation occurred with the supernatant.

6.3 Model Adequacy

6.3.1 Parametric Analysis

Diagnostic plots that depicts the predicted and actual data were prepared to judge upon the model adequacy in terms of predicted vs actual plots (Fig. 6.1 (a – d). As confirmed in Fig. 6 (a – d), several data points do exist near to the straight line and affirm good agreement between the experimental data

and best fit model data. Therefore, UAE of pectin from pomelo peel system can be very precisely predicted using best fit models of the data obtained with the FCD-RSM design approach.

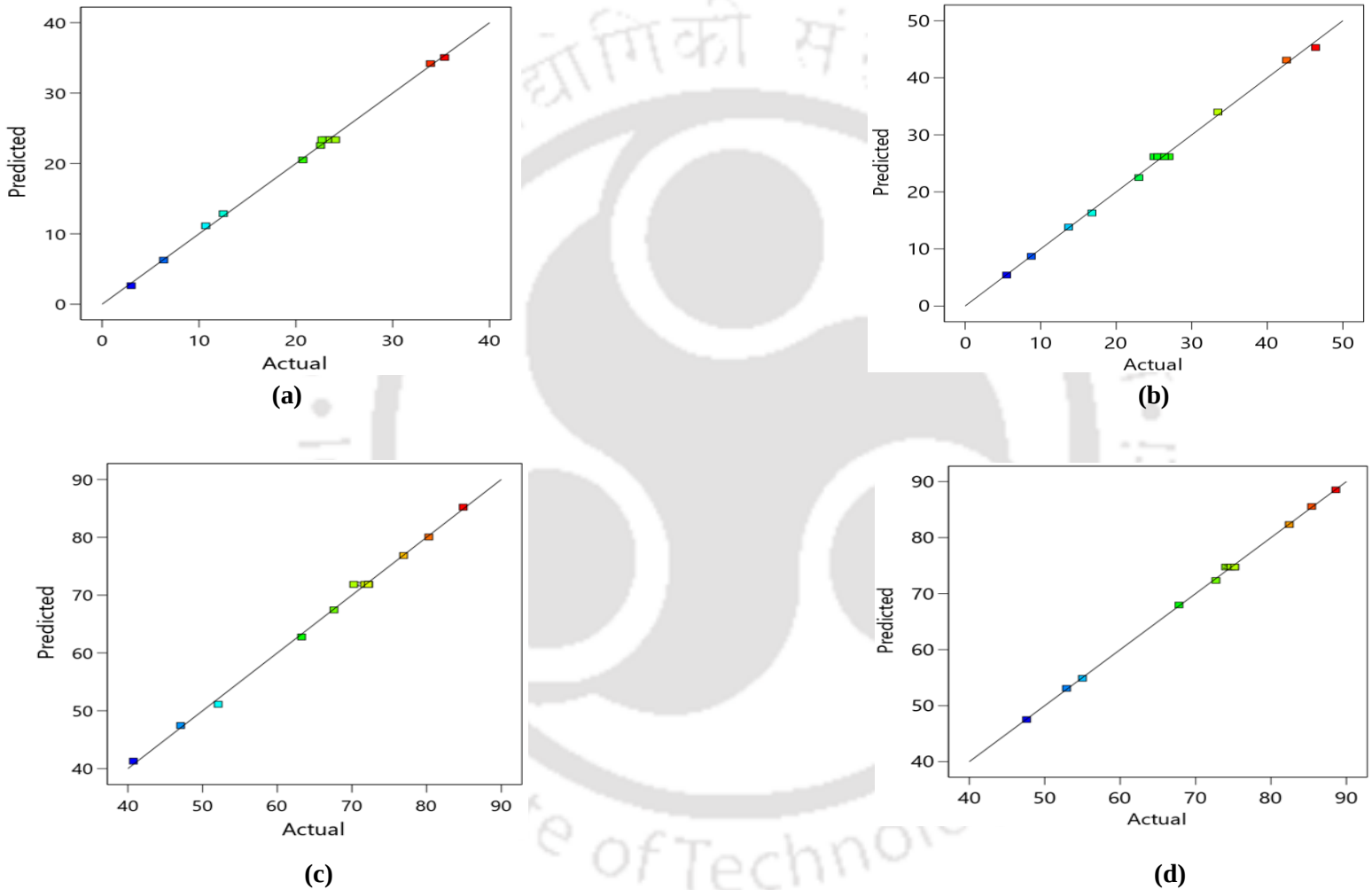


Figure 6.1: Predicted vs normal plots for normal UAE and pulsed UAE of pectin yield (a and b) and degree of esterification (c and d), respectively.

6.3.2 Pareto Chart Analysis

A detailed discussion with respect to the Pareto chart has been presented in our earlier work (Wani and Uppaluri, 2022). Fig. 6.2 (a – d) portraits a Pareto chart for the determination of positive and negative significant and non - significant factors associated to the PY extracted from pomelo peel. For the NUAE case, Fig. 6.2 (a and b) depict the Pareto chart for PY and DE respectively. The linear term (A), and quadratic term B^2 and cross product term AB confirmed negative but significant influence on the PY. However, for the case, the quadratic term A^2 had a negative but insignificant influence on the response variable. Further, the linear B term affirmed a significant and positive influence on the PY.

Similarly, for the DE case (Fig. 6.2(b)), pH (A), followed by time (B), indicated positive and significant influence on pectin DE, whereas quadratic terms (A^2 and B^2) affirmed significant negative influence on the DE of pectin. On the other hand, the cross product term AB indicated negative insignificant influence on the DE of pectin. For the PUAE case, similar response trends have been observed (Fig. 6.2 (c and d)) and were hence not further delineated in the Ph.D. thesis.

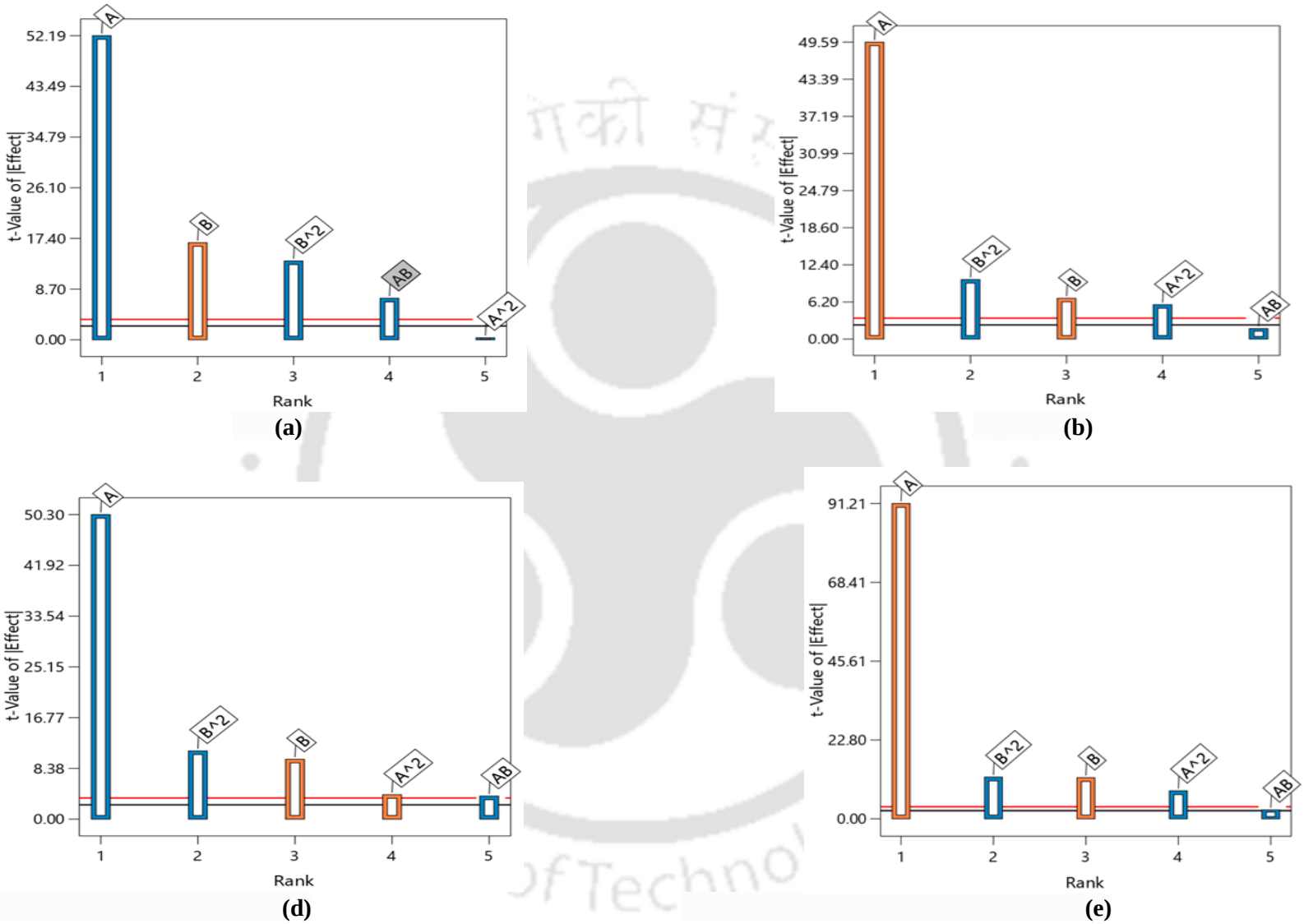


Figure 6.2: Pareto chart for normal UAE and pulsed UAE of pectin yield and degree of esterification (a and b) and (c and d), respectively.

6.4. Model Fitting and Statistical Analysis for Normal UAE

Table 6.3 presents the results of the analysis of variance (ANOVA) approach being used to determine the statistical significance of the model terms. Low p-values for PY ($p < 0.0001$) and DE ($p < 0.0001$) suggested that the established model has been significant and has good fitness. Corresponding fitness of the best model has been affirmed with very good values of regression coefficients (R^2 values of 0.9958 and 0.9976 for PY and DE, respectively), higher F values (333.44 for PY and 584.33 for DE), higher adequate precision values (59.552 for PY and 77.547 for DE). Further, for all cases, the overall model p-value was low (< 0.0001).

The lack of fit for the best fit quadratic model corresponds to the values of 3.79 and 0.86 for PY and DE, respectively. Thereby, the lower F values for the lack of fit affirmed that the lack of fit was not significant. These observations affirmed very good model significance for each case. The coefficient of the variance was 4.10 % and 1.24 for PY and DE respectively, and thereby convey good accuracy of the experimental data. Among all best fit quadratic models, the F-values of various terms representing PY indicated all linear (A, B), bi-linear B^2 (except A^2), and interaction AB term to be significant. Similarly, for the DE case, the significance corresponds to all linear (A, B), all bilinear, A^2 and B^2 terms to influence critically the measured response variable. Further, associated p-values have been lower than 0.0001 and thereby confirmed upon the best fitness of the quadratic model.

A second order polynomial equation was confirmed to be the best for the evaluation of optimal conditions for the maximum pectin extraction yield and DE, and pertinent process factors. Eqs. (13) and (14) refer to the best fit model expressions in terms of the coded factors:

$$PY = 23.32 - 12.05\,A + 3.85\,B - 0.1.73\,AB + 0.17A^2 - 7.15\,B^2 \quad (6.1)$$

$$DE = 71.86 + 17.04\,A + 2.34\,B - 0.74\,AB - 3.69\,A^2 - 6.76\,B^2 \quad (6.2)$$

Table 6.3: ANOVA data of best fit models and their parameters representing normal UAE of pectin.

Components	Pectin yield		Degree of esterification	
	***F* value**	***P* value**	***F* value**	***P* value**
Model	809.66 (Quadratic)	< 0.0001 (Quadratic)	584.33 (Quadratic)	< 0.0001 (Quadratic)
A	3127.71	< 0.0001	2507.38	< 0.0001
B	318.88	< 0.0001	47.35	< 0.0001
AB	58.32	0.6389	3.13	< 0.0001
A^2	0.75	0.0489	54.12	0.0017
B^2	449.74	< 0.0001	181.38	0.0342
Lack of Fit	0.73	0.5849	0.86	0.5295
R squared		0.9983		0.9976
Adequate Precision		91.108		77.547

6.5 Model Fitting and Statistical Analysis for Pulsed UAE

The ANOVA data of the best fit quadratic model have been presented in Table 6.4. The trends have been similar to those being elaborated for the NUAE case. The evaluated R^2 (0.9975 for PY, and 0.9993 for DE), adequate precision (77.284 for PY and 141.905 for DE), high F value (562.21 for PY and 2056.03 for DE) and low p value (< 0.0001) for all cases affirmed upon the model adequacy. The non-significant lack of fit of 0.40 and 0.31 for PY and DE respectively affirmed a good fitness of the quadratic model. The coefficient of the variance (2.83 % and 0.60% for PY and DE, respectively) indicated a very good accuracy of the experimental values. For the PY case, all linear terms, interaction terms and A^2 and B^2 term have been significant to influence upon the response variable. Similarly, for the DE case, all linear terms, interaction terms and A^2 and B^2 term have been highly prominent to influence upon the variations of the response variable. These dominant influences have been assured by the higher F value and lower p value for the mentioned variables. Thereby, the best fit quadratic models to represent PY and DE characteristics of the PUAE process can be represented with the following expressions:

$$PY = 26 - 13.55\,A + 2.66\,B - 0.81\,AB + 0.99\,A^2 - 6.17\,B^2 \quad (6.3)$$

$$DE = 74.75 + 16.82\,A + 2.19\,B - 0.58\,AB - 3.04\,A^2 - 4.59\,B^2 \quad (6.4)$$

Table 6.4: ANOVA data of best fit models and their parameters representing pulsed UAE of pectin.

Components	Pectin yield		Degree of esterification	
	***F* value**	***P* value**	***F* value**	***P* value**
Model	483.51 (Quadratic)	< 0.0001 (Quadratic)	2056.03 (Quadratic)	< 0.0001 (Quadratic)
A	2138.09	< 0.0001	9382.18	< 0.0001
B	82.67	< 0.0001	159.75	< 0.0001
AB	12.18	0.6389	7.50	< 0.0001
A^2	45.83	0.0489	141.00	0.0017
B^2	181.45	< 0.0001	321.48	0.0342
Lack of Fit	1.36	0.3744	0.31	0.8186
R squared		0.9971		0.9993
Adequate Precision		70.399		141.905

6.6 Response Surface Characteristics

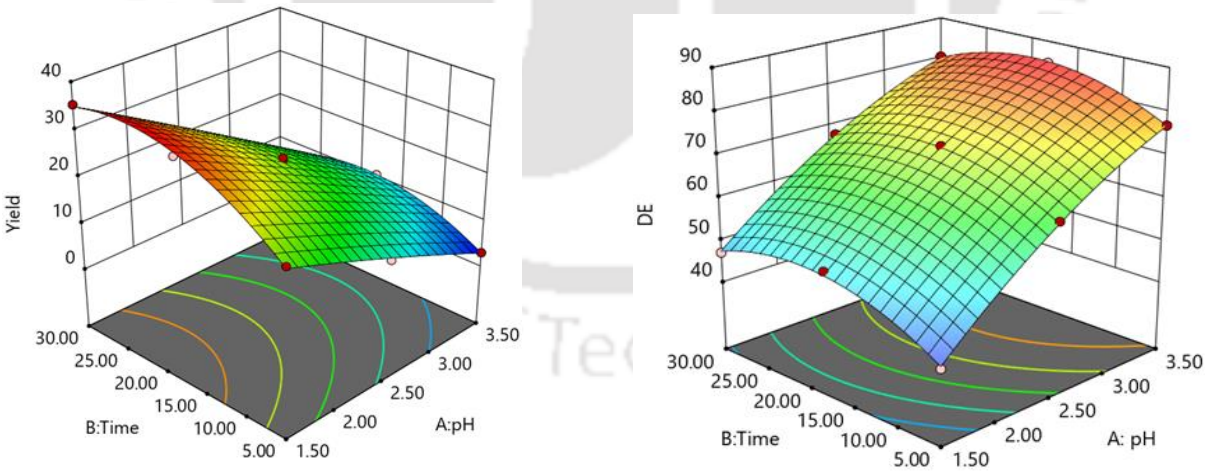
The response surface plots for NUAE and PUAE of pectin extracted from pomelo peel have been illustrated in Fig. 6.3 and Fig. 6.4, respectively. The response surface plot depicts the influence of alternate process factors and their interactions on the yield and DE of pectin.

6.6.1 Effect of Normal UAE Process Parameters on Pectin Yield (PY)

Fig. 6.3(a) depicts the response surface plot for extraction yield as a function of ST and pH. As illustrated in Fig. 6.3(a), the yield has been strongly dependent on the pH followed by the ST. For a variation in ST and pH from 5 – 30 min and 3.5 – 1.5 respectively, the PY increased from 3 – 33.35 % with maximum yield value being achieved as 35.35 ± 0.08 mg GAE/g (extraction pH of 1.5 and 17.5 min ST). These findings confirmed that the pH is an important parameter for pectin extraction and could be explained with the enhanced pectin solubility with reducing pH. These findings are also consistent

with prior research that confirmed the pH had a profound influence on the extraction yield of pectin from fruit by-products (Shivamathi et al., 2019).

Further, with respect to variation in ST from 5–30 min, and for a fixed choice of pH of 1.5, the PY increased from 22.55 – 33.35. A reduction in the PY has been indicated in the response surface plot for the case of ST approaching 30 min. Such a reduction in the PY at higher ST is consistent with the findings of relevant prior art (Xu et al., 2014). The authors demonstrated that the pectin degrades in due course of prolonged ST. According to their findings, the yield improved within the first 20 min of the extraction and thereafter started to decline for extended periods of time. This could be also due to the excessive heating effect and prolonged exposure to ultrasound treatment that together caused structural changes and disintegration of the pectin (Methacanon et al., 2014). Thus longer duration of sonication has been detrimental to extraction yield and renders infeasible process economy. Therefore, for the conducted investigations, a ST of 17.5 min was sufficient to achieve maximum yield of pectin during ultrasound extraction. Additionally, response surface plot indicated significant interaction effect between ST and pH on the PY. The maximum extraction yield (35.3 %) was obtained for the most acidic condition (1.5 pH and 17.5 ST).



(a) (b)

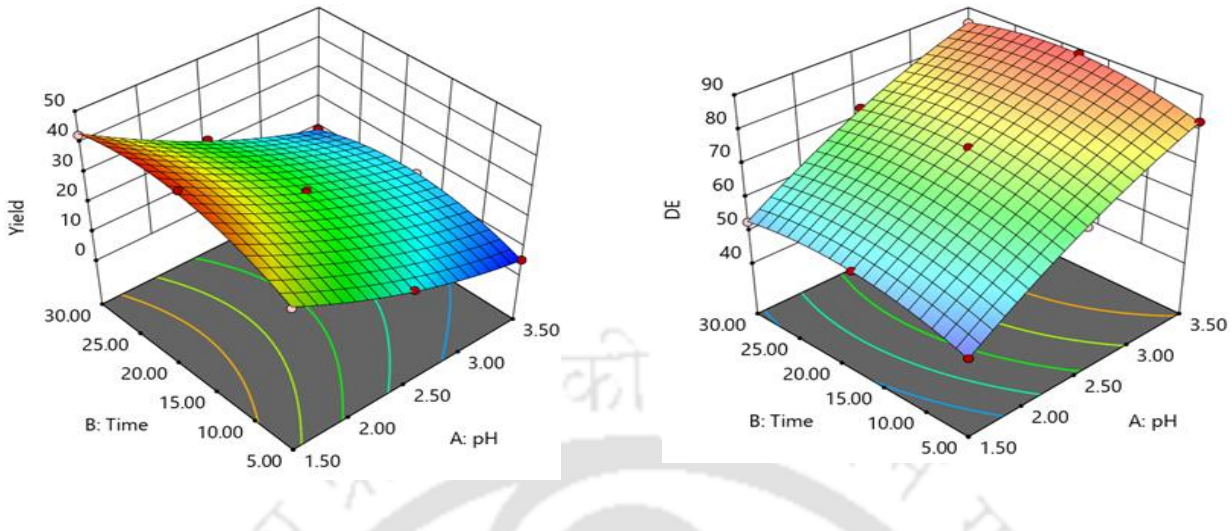
Figure 6.3: Response surface plots of pectin yield and degree of esterification during normal UAE process.

6.6.2 Effect of Normal UAE Process Parameters on Degree of Esterification (DE) of Pectin

Due to its strong influence upon several functional properties such as gelling, solubility etc., the DE pf the pectin has been a critical factor for analysis and introspection. The degree of esterification has a considerable influence on the quality, application, and extraction method of pectin. The DE represents carboxyl groups of the main chain of galacturonic acid that underwent esterification with methyl or acetyl groups. The pH has been the most critical factor to influence the DE (Methacanon et al., 2014). The 3D response surface plot (Fig. 6.3 (b)) demonstrates the effect of pH and ST on DE for the NUAE case. The 3D surface plot confirmed that the DE of extracted pectin enhanced from 47.06 – 80.3 % for a pH variation from 1.5 – 3.5 and ST variation from 5 – 30 min. For a fixed choice of ST of 5 min, the DE of pectin enhanced from 40.73 –76.92 % for a variation in pH from 1.5 – 3.5. Such a trend has been supported by the prior studies that also confirmed pectin production with higher DE at higher pH conditions.

Also, for a fixed choice of 3.5 pH, the DE of pectin increased from 76.92 – 84.9% for a variation in extraction from 5– 17.5 min. Thereafter, the surface response plots indicated a marginal reduction in the DE for an increase in the ST upto to 30 min. Additionally, no significant interaction effect was observed between ST and pH on the DE of pectin. Also, it is worth noting that the extraction yield had no correlation with the DE. Moorthy et al. (2015) reported low DE values for a lower pH combined with a longer ST (i.e. pH 2.0 and 60 min ST). Such conditions may have possibly caused pectin de-esterification. In the thesis work, the highest DE (84.9 ± 0.42 %) was obtained for a combination of pH and ST of 3.5 and 17.5 min, respectively.

Similar response patterns have been analysed for the PUAE of Pectin (Fig. 6.4 (a and b)), and have not been further explained. For the PUAE, the lowest and highest PY and DE (5.5 ± 0.24 % and 42.55 ± 0.14 %; and 47.6 ± 0.25 % and 88.6 ± 0.31 %, respectively) have been obtained at a pH of 1.5 and 3.5 and 5 and 30 min ST, respectively. Also, it shall be noted that, depending on the source, and process conditions being applied during pectin extraction process, the product would have specific characteristics (Moorthy et al., 2015).



(a) **(b)**

Figure 6.4: Response surface plots of pectin yield and degree of esterification during pulsed UAE process.

6.7. Determination and Validation of Optimal Extraction Conditions

For both NUAE and PUAE cases, the response surface quadratic polynomial models have been effective for the prediction of extraction yield and pectin DE. Therefore, the extraction conditions for PY and the DE of pectin were optimised based on the prediction profiles of the variables being confirmed by the Design Expert software. The best extraction conditions for the NUAE method were found at 22.33 min ST, 1.72 pH, and a fixed choice of 65°C ET, and 15 mL/g LR. Under these optimal conditions, the predicted yield and DE were 33.76 % and 56.8 %, respectively. For the PUAE technique, the optimal extraction conditions were 16.11 min ST and 1.70 pH, for a fixed choice of the LR and ET as 15g/ mL and 65°C respectively. Under these optimal conditions, the predicted yield was the highest (40.41 %) along with an appropriate DE of 59.95%.

Thus, comparatively, the yield of pectin obtained from PUAE (41.41 %) was higher and the best than that obtained from NUAE (33.76 %). Corresponding DE for PUAE (59.95 %) was higher than that of the DE for NUAE (56.44 %). Despite conducting both extraction methods at similar conditions of LR (1:15 g/mL) and ET (65 ºC), the PUAE resulted in a remarkable increase in extraction yield with lower

ST and marginally higher DE in comparison to NUAE. Thereby, this confirmed upon the promising performance of the PUAE in conjunction with the NUAE technique and is in agreement with the inferences provided for various fruit by-products (Wani & Uppaluri, 2022a).

6.8. Comparative Analysis of Electric Consumption and CO_2 Emission between pulsed UAE and Acidic Hot Water Extraction (AHWE) for Pectin Extraction

The electrical consumption for the pectin extraction from pomelo peel has been evaluated for the optimal values obtained for the PUAE. It is presumed that GHG were generated fully from the fossil fuel feed stock based electrical power generation system. The amount of electricity needed to extract pectin from pomelo peel utilising the PUAE and AHWE processes for a 16.11 min ST were 0.073 and 0.26 kWh, respectively. Thus, in comparison to the PUAE, AHWE consumed 3.71 times more energy to extract pectin from pomelo peel. Therefore, it is conceivable that the PUAE approach in comparison with the AHWE required lower operating cost to extract pectin. In addition, the PUAE of pectin contributed lower CO_2 production (0.056 kg) than the AHWE (0.21 kg). As a result, the PUAE approach can be considered to be a more environmentally friendly and sustainable process than the AHWE method for the pectin extraction from pomelo peel.

6.9. Physicochemical Properties of Extracted Pectin

Table 6.5 illustrates the physicochemical properties of pectin extracted under optimal PUAE and AHWE conditions. The physicochemical properties do get significantly affected with the type of extraction method being followed. The % moisture, % ash and % fat content for the PUAE and AHWE based pectin product were 12.14 ± 0.43 % and 16.42 ± 0.36 %; 1.11 ± 0.3 % and 1.41± 0.12 %; and 0.04 and 0.07 %, respectively. In terms of solubility, pectin extracted from PUAE (79 ± 0.5 %) had better solubility in water in comparison to that produced with the AHWE (72.3 ± 0.15%) method. Such excellent pectin solubility could further extend its utility in various applications in the food industry. The total soluble protein content of PUAE pectin (6.28 ± 0.23 mg/g) was marginally higher than that obtained with the AHWE (4.13 ± 0.7 mg/g). In a prior investigation, similar findings have been reported for these two methods (Hosseini et al., 2019). Protein is one of the most favourable hydrophobic

components to influence pectin's emulsification ability and this is achieved through the lowering of the interfacial tension at the oil-water interface (Chen et al., 2016). Thus, a larger protein content is very likely to improve emulsification characteristics of the pectin being extracted with the UAE method (Maran & Priya, 2015).

WHC, a fundamental property in many food systems, is defined as the quantity of bound water/gram of sample. Compared to the AHWE, the PUAE affirmed significantly higher WHC and OHC values in the pectin product. The WHC for the pectin extracted with the PUAE method was 8.5 ± 0.1 g/g and for the AHWE, it was 6.1± 0.05 g/g. For both cases, the values were higher than the extracted pectin from pistachio green hull (4.11 ± 0.34 g water/g pectin). In a study, Ke et al. (2020) explained that many parameters do influence the WHC. These include the amount of free hydroxyl groups in pectin structure, porosity of pectin powder etc. Compared to the AHWE method (3.20 ± 0.21 g/g), the OHC of pectin extracted with the PUAE was 5.45 ± 0.77 g/g. This has been higher than the OHC reported for the *Opuntia ficus indica cladodes* based pectin (1.23 ± 0.42 g oil/g pectin) and pistachio green hull pectin (2.02 ± 0.19 g oil/g pectin) (Methacanon et al., 2014). In this regard, it shall be noted that many factors such as total charge density do influence the OHC.

Emulsification properties of food products are essential in due course of product design and development. In the thesis work, two parameters namely, EC and ES have been studied. EC rate has been defined as the ratio of the emulsified layer volume to the total volume. After emulsion formation, three different phases could be observed, namely a pectin solution, an emulsified layer phase and an oil phase (in the order from bottom to top). The properties of these phases do influence the characteristics of pectin product (Hosseini et al., 2019). Pectin extracted with the PUAE method demonstrated higher EC (30.3 ± 0.04 %) than the pectin extracted with AHWE (23. 7 ± 0.11 %). In addition, both pectin's obtained from PUAE and AHWE demonstrated similar ES values (80.8 ± 0.24 % and 80. 27 ± 0.12 %, respectively). These findings have been also in accordance with studies published previously in the literature for the extraction of pectin from *citrus medica* peel (Pasandide et al., 2017). These observations provide further evidence for the notion that pomelo pectin may have a greater potential for application as an emulsifier and stabilizer in the food industry.

Table 6.5: Physicochemical and functional properties of extracted pectin.

S.No	Parameters	PUAE	AHWE
1	Moisture (%)	12.14 ± 0.43	16.42 ± 0.36
2	Ash (%)	1.11 ± 0.3	1.41 ± 0.12
3	Fat (%)	0.04	0.07
4	TPC (mg GAE/g)	50.90 ± 0.66	28.18 ± 0.33
5	TFC (mg QE/g)	44 ± 0.14	30 ± 0.23
6	AA (%)	69.6 ± 0.2	60.1% ± 0.35
7	OHC (g/g)	5.45 ± 0.77	3.20 ± 0.21
8	WHC (g/g)	8.5 ± 0.1	6.1 ± 0.05
9	EC (%)	30.3 ± 0.04	23. 7% ± 0.11
10	ES (%)	80.8 ± 0.24	80. 27 ± 0.12
11	Protein content (mg/g)	6.28 ± 0.05	4.13 ± 0.07
12	Solubility (%)	79 ± 0.5	72.3 ± 0.15

6.10 Nutritional Characteristics of Extracted Pectin

TPC and TFC are the key characteristics of the pectin that critically influence functional properties such as antioxidant activity (Nishad et al., 2019). Thus, the TPC and TFC of extracted pectin were determined under optimal extraction conditions of the PUAE. The findings have been summarised in Table 6.5. For the PUAE case, the TPC and TFC were 50.90 ± 0.66 mg GAE/g pectin and 44 ± 0.14 mg QE/g, respectively. In this regard, it shall be noted that very few researchers addressed these characteristics of the pectin. On the other hand, for the AHWE case, these were significantly lower (28.18 ± 0.33 GAE mg/g and 30 ± 0.23 QE mg/g, respectively). For this case, it is well known that while conventional extraction method degrades phenolic compounds due to higher processing temperature, the UAE effectively retains higher TPC and TFC in the pectin due to breakage of cell wall that enhanced the rate of diffusion across the cell wall and thereby promoted better release of phenolic and flavonoid compounds into the extract (Wani & Uppaluri, 2022c).

DPPH scavenging is an excellent measure of antioxidant capacity and it is the most feasible and simplest method for the determination of scavenging activity. Pectin possesses antioxidant properties due to the presence of hydroxyl groups (Wang et al., 2016). The findings indicated that there has been significant DPPH radical scavenging capacity of the pectin extracted with both PUAE (69.6 ± 0.2 %) and AHWE (60.1% ± 0.35%) techniques.

The conducted studies affirmed that the pectin with higher TPC and TFC content exhibited greater antioxidant activity. This is due to the reason that the phenolic and flavonoid compounds in the pectin acts as antioxidants through the neutralization of free radicals and prevention of the oxidative damage to the cells. Several studies reported a comparative analysis of the antioxidant activity of pectin extracted by normal and pulsed UAE and for the commercial pectin (Methacanon et al., 2014). It was found that pectin extracted with the UAE exhibited higher antioxidant activity than commercial pectin. This could be due to the UAE process ability to increase the release of phenolic and flavonoid compounds from pectin. This occurs due to the cellular wall breakage and promoted effective diffusion of the compounds into the solvent.

6.11 Characterisation of Pectin Extracted by Pulsed UAE and AHWE Process

In this section, the pectin extracted with PUAE at optimal condition and with AHWE process at optimal condition of the PUAE process have been analysed and compared in terms of alternate characterization such as XRD, FTIR, thermal (DSC and TGA) and morphological analysis (FESEM). A detailed account of the associated findings for both PUAE and AHWE based pectin samples has been delineated in the following sub-sections.

6.11.1 X-ray Diffraction (XRD) Analysis

The pectin samples obtained with the PUAE and AHWE processes were subjected to XRD analysis and eventually infer upon their amorphous or crystalline structure. It is well known that for crystalline matrices, a series of distinct peaks exist in the XRD pattern. On the other hand, for the amorphous materials, the XRD pattern depict a broad background like pattern with much noise and without distinct peaks. Fig.6.5 depicts the XRD pattern of both pectin samples confirm upon the amorphous structure for both pectin samples. A little crystallinity exists for the PUAE based pectin sample and with major

peaks at 13.2° and 20.3°. These findings do corroborate with the reported findings of Hosseini et al. (2019) and Wang et al. (2016). Also, both pectin samples affirmed minor crystalline peaks at 8.02°, 23.6°, 28.42°, 36.2°, 7.6°, and 22.2°. Further, the spectra of PUAE pectin exhibited sharper and stronger peaks than those of the AHWE based pectin and especially at 18.33° and 29.04°. This demonstrated minor crystallinity in the structure of the pectin obtained with the PUAE process. Such an alteration can be attributed to the variation in the molecular weight of pectin under different extraction conditions. This is in agreement with the reported findings of pectin extraction from black carrot pomace (Misra & Yadav, 2020). Thereby, the higher peak intensity of the pectin extracted with the UAE could be correlated with the retention of native pectin structure during the PUAE process.

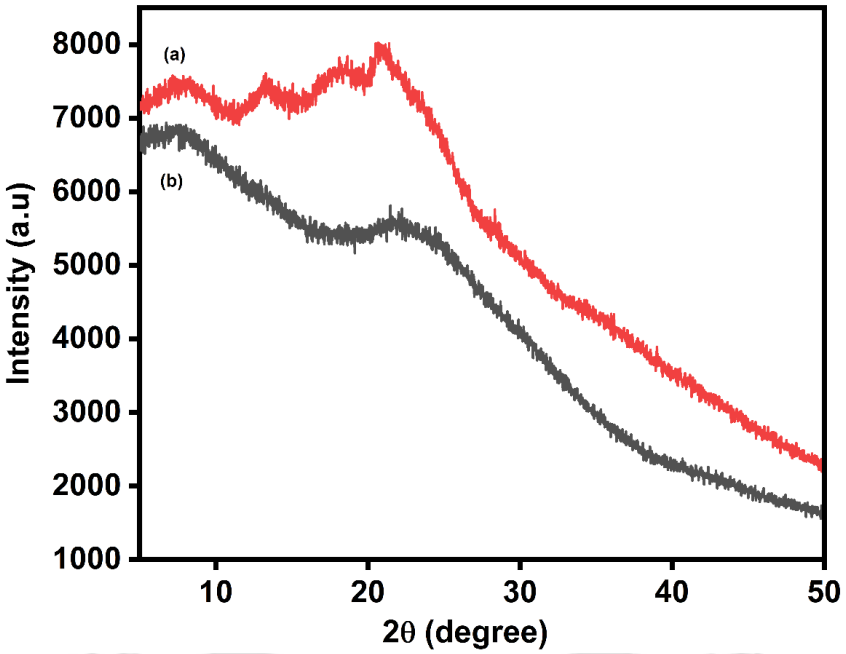


Figure 6.5: XRD diffraction pattern of (a) pulsed UAE and (b) acidic hot water extraction of pectin's from dried pomelo peel powder.

6.11.2 FTIR Analysis of Extracted Pectin

Fig. 6.6 depicts the FTIR spectra of pectin samples obtained with AHWE and PUAE processes. In the FTIR spectra, the peak at 3200 – 3500 cm^{-1} confirmed upon the presence of OH groups in the pectin molecule. The peak at 2800 – 3000 cm^{-1} attributed to CH vibrations and especially for CH, CH_2, and CH_3 stretching and bending patterns (Van Hung et al., 2021). The peaks near 1734 cm^{-1} can be linked

to CO from the carboxylic acid methyl ester group. Two additional peaks that corroborate with the stretching vibrations of the CO group (free carboxyl group) and these refer to the peaks between 1400 and 1650 cm^{-1}. All these observations affirmed pectin extraction from pomelo peel with the fact that the mentioned peaks for the key functional groups correspond to that of pectin (Santos et al., 2020). Additionally, similar spectral characteristics have been discovered for AHWE and PUAE based pectin samples. For this, distinctive peaks exist at intervals of 3200-3500 cm^{-1}, 2929 cm^{-1}, 2929 cm^{-1}, 1736 cm^{-1}, 1734 cm^{-1}, 1400-1650 cm^{-1}, and 800 – 1250 cm^{-1}. Further, PUAE pectin samples had stronger absorption areas than those pertinent in the AHWE based pectin sample. This affirmed that the pectin extracted with PUAE had higher degree of esterification than that obtained with the AHWE process (Shivamathi et al., 2019). All these observations are in good agreement with the insights provided for the pomelo peel based pectin extraction with the intermittent sonication method (Methacanon et al., 2014). Thus, from the spectrum analysis, it can be concluded that both AHWE and PUAE pectin represent similar types of pattern, peaks, functional groups and bands.

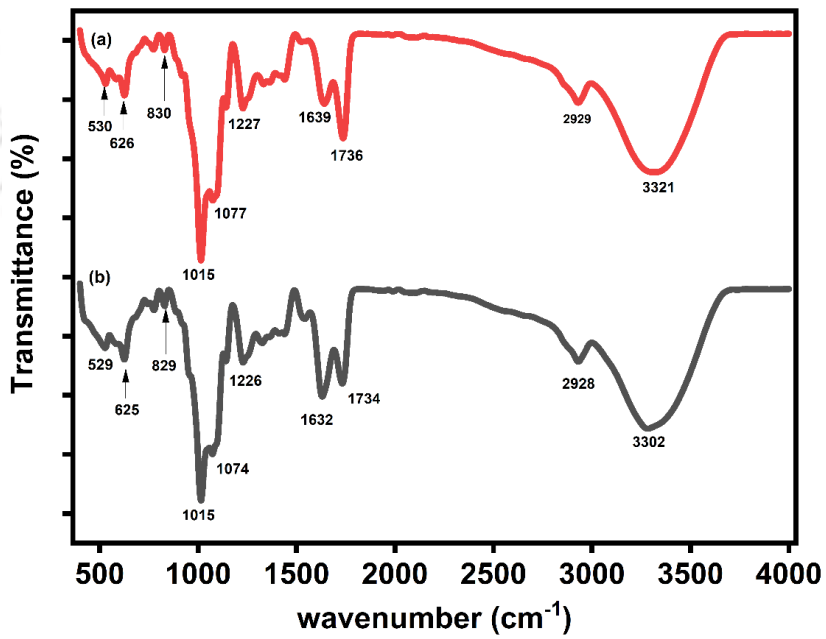


Figure 6.6: FTIR spectrum of pectin extracted by (a) pulsed UAE and (b) acidic hot water extraction from dried pomelo peel powder.

6.11.3 Thermal Properties of Extracted Pectin

DSC and TGA analyses were targeted to examine the thermal properties of the pectin obtained from PUAE and AHWE processes. Thereby, associated alterations during thermal degradation can be understood. Fig. 6.7 and 6.8 respectively depict the DSC and TGA thermograms.

6.11.3.1 Differential Scanning Calorimetry (DSC) Analysis Pectin

Differential scanning calorimetry (DSC) analysis was conducted out to examine the thermal behaviour of pectin extracted with PUAE and thereby compare it with that of the pectin extracted with the AHWE process (Fig. 6.7). The DSC curves exhibited major endothermic peaks for the AHWE and PUAE based pectin samples and at 94.71 and 99.2 °C respectively. The minor shift of this peak for the AHWE and towards lower temperature is due to the greater water content and altered conformation. Additionally, these endothermic peaks can be attributed to the presence of water, hydrogen bonds between galacturonic acid units, and structural changes that involved the transformation of galacturonan ring from the more stable $^{4}C1$ chair conformation to the inverted $^{1}C4$ chair conformation (Wang et al., 2016). Additionally, the amount of heat flow for AHWE was significantly greater in comparison to the pectin extracted with the PUAE. This affirmed more severe modifications in the pectin achieved with AHWE and hence a reduced degree of thermal stability for the sample (Methacanon et al., 2014). The exothermic transition for the PUAE based pectin occurred pectin at 245.7 °C and refers to the pectin degradation at this temperature. On the other hand, the associated transition was marginally lower for the AHWE based pectin sample (239 °C). Wang et al. (2016) reported that the pectin extracted with the ultrasound indicated marginally higher thermal stability than the pectin obtained with the conventional heating process. In this regard, it can be noted that the pectin can be used as an additive in food products being prepared at higher temperatures such as cakes, bread and pastries (Liew et al., 2016). Therefore, the high temperature resistant pectin can have better preference and choice in the food industry sector. In the current study, the thermal analysis revealed that the PUAE based pectin had greater thermal stability than the AHWE based pectin. Hence, the PUAE could be better preferred than the AHWE for such mentioned applications.

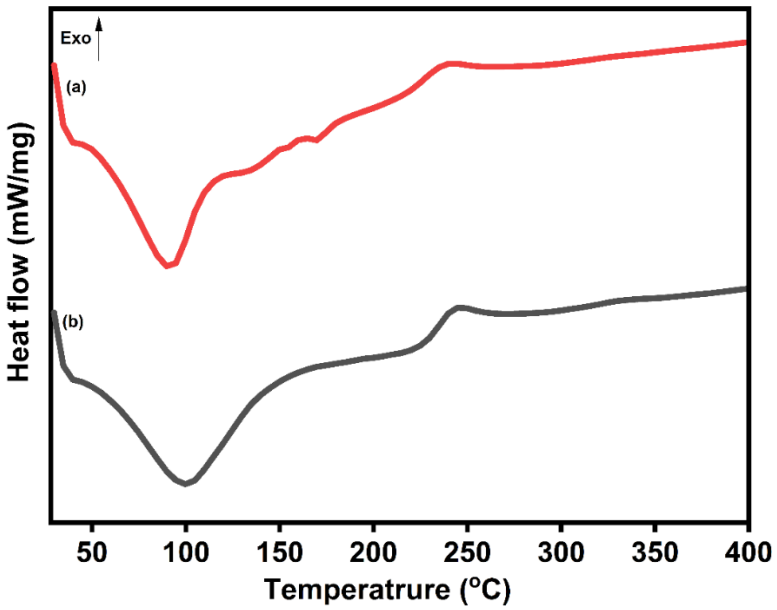


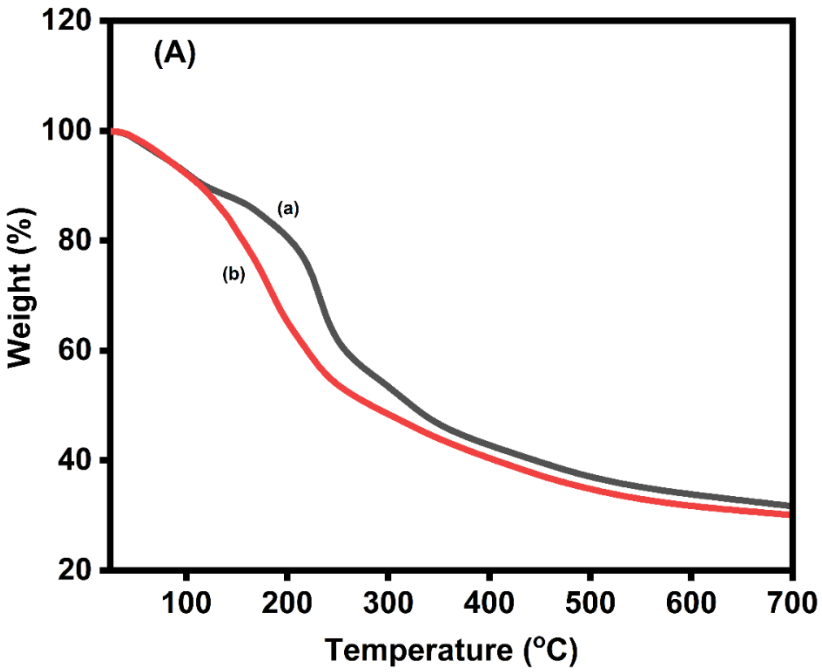
Figure 6.7: DSC thermograms of pectin extracted from dried pomelo peel powder by (a) acidic hot water extraction and (b) pulsed UAE.

6.11.3.2 Thermogravitic Analysis (TGA) of Pectin

Fig. 6.8 ((A) and (B)) respectively illustrate the TGA and first derivative TGA (DTGA) curves for both PUAE and AHWE based pectin samples. Through such analysis, the thermal behaviour of the pectin obtained with the PUAE and AHWE processes can be compared. The TGA curves represent three distinct regions at 25-140, 140-290, and 290-700 °C during thermal degradation. The first region (25-140 °C) is due to evaporation based water loss. Similar region has also been reported in the literature (Wang et al., 2016). The second stage that exists between 190 and 290 °C corroborates with the significant mass loss (50%) due to the pyrolytic decomposition of polysaccharides (Wang et al., 2016). During this phase, the galacturonic acid chains begin to undergo rigorous thermal degradation and thereby generate various gaseous products and a solid char. The third region (290 – 700 °C) accounts to a slower mass loss due to the volatilization of other compounds (char thermal decomposition) and water removal (Zhou et al., 2011). Under these circumstances, the solid char containing poly-aromatic structures grafted by aliphatic and ketonic groups would undergo partial degradation and would get compacted with the increasing pyrolytic temperature (Talekar et al., 2018).

In addition to the peaks at about 100 °C due to the release of free water, the PUAE based pectin samples confirm upon two unique mass losses in the DTGA curve. The double-stage decomposition for the PUAE pectin sample has been demonstrated with two DTGA peak temperatures (first at 230 °C and the second at 317 °C). For the AHWE based pectin sample, a single peak only appears at 182 °C. Thus, it can be concluded that the thermal degradation of AHWE based pectin sample started earlier than the PUAE based sample and can be henceforth inferred to be comparatively with lesser thermal stability. Also, it has been found that the PUAE based pectin sample conveyed larger peak in the thermogram than the thermogram obtained with the AHWE based pectin sample.

In summary, with the DSC and DTGA analyses, it can be concluded that the AHWE based pectin samples affirmed lower thermal stability in comparison to the pectin extracted with the PUAE process.



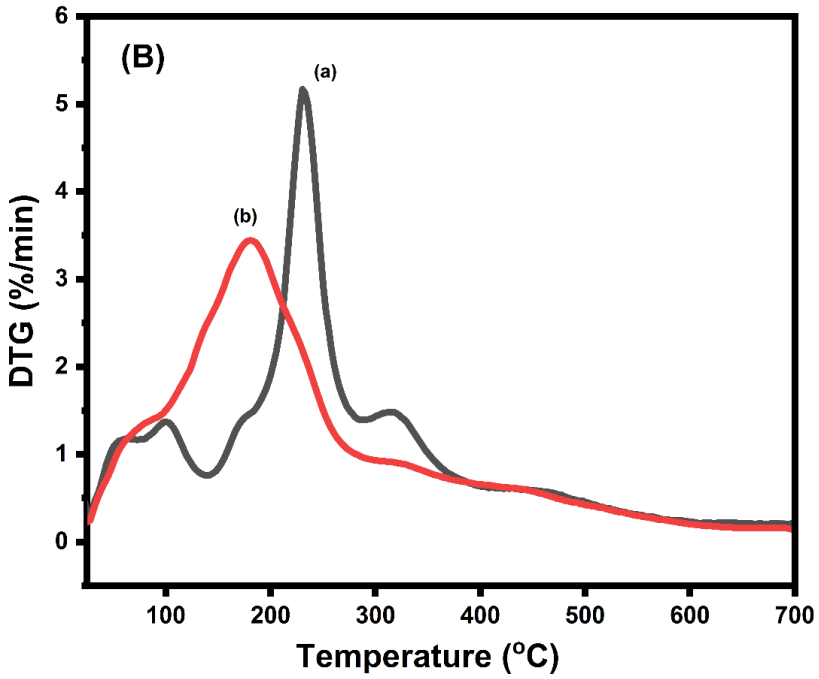
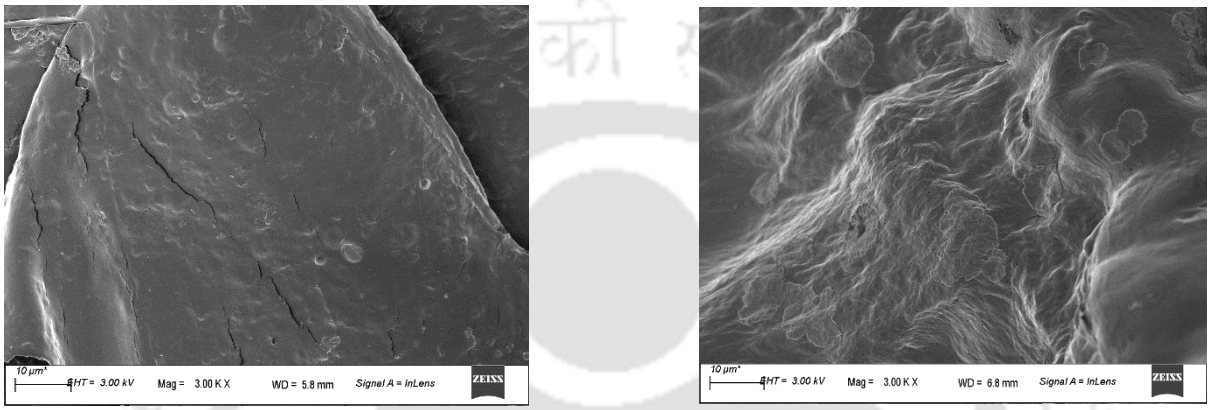


Figure 6.8: TGA (A) and DTGA (B) curves of pectin extracted from dried pomelo peel powder by (a) pulsed UAE and (b) acidic hot water extraction process at a heating rate of 5 °C/min under nitrogen atmosphere.

6.11.4 FESEM Analysis of Pectin

FESEM analysis was carried out to examine the morphological alterations in the pectin samples obtained with the PUAE and AHWE processes. Fig. 6.9 depicts the morphological variations of pectin obtained with the PUAE and AHWE processes. From the study, it can be seen that the pectin obtained with PUAE process affirmed smoother, compact, and flatter surface. Wang et al. (2015) reported similar findings and conveyed that sonication caused disruption between pectin molecules and as well facilitated reorganisation of the pectin matrix during UAE process. A similar result has been reported through the SEM analysis of pectin extracted from passion Gac pulp powder (Tran et al., 2021). On the other hand, the pectin extracted with AHWE process possessed a comparatively rough, uneven and irregular surface. This has been attributed to the sudden enhancement in the temperature during AHWE process and its influence on the pectin's morphology (Kazemi et al., 2019). In general, the structure of PUAE pectin was smoother with more integrity than that obtained for the AHWE pectin sample.

Thus, in summary, it can be concluded that the structural differences do exist in the extracted pectin's through AHWE and PUAE processes. It is hypothesized that the extracted pectin with smoother and flatter surface have smaller and narrow particle size distribution (Kulkarni & Vijayanand, 2010). Thereby, this promising feature of the PUAE based pectin is responsible for its enhanced solubilisation and can be particularly beneficial for applications in food, cosmetic and pharmaceutical industries.



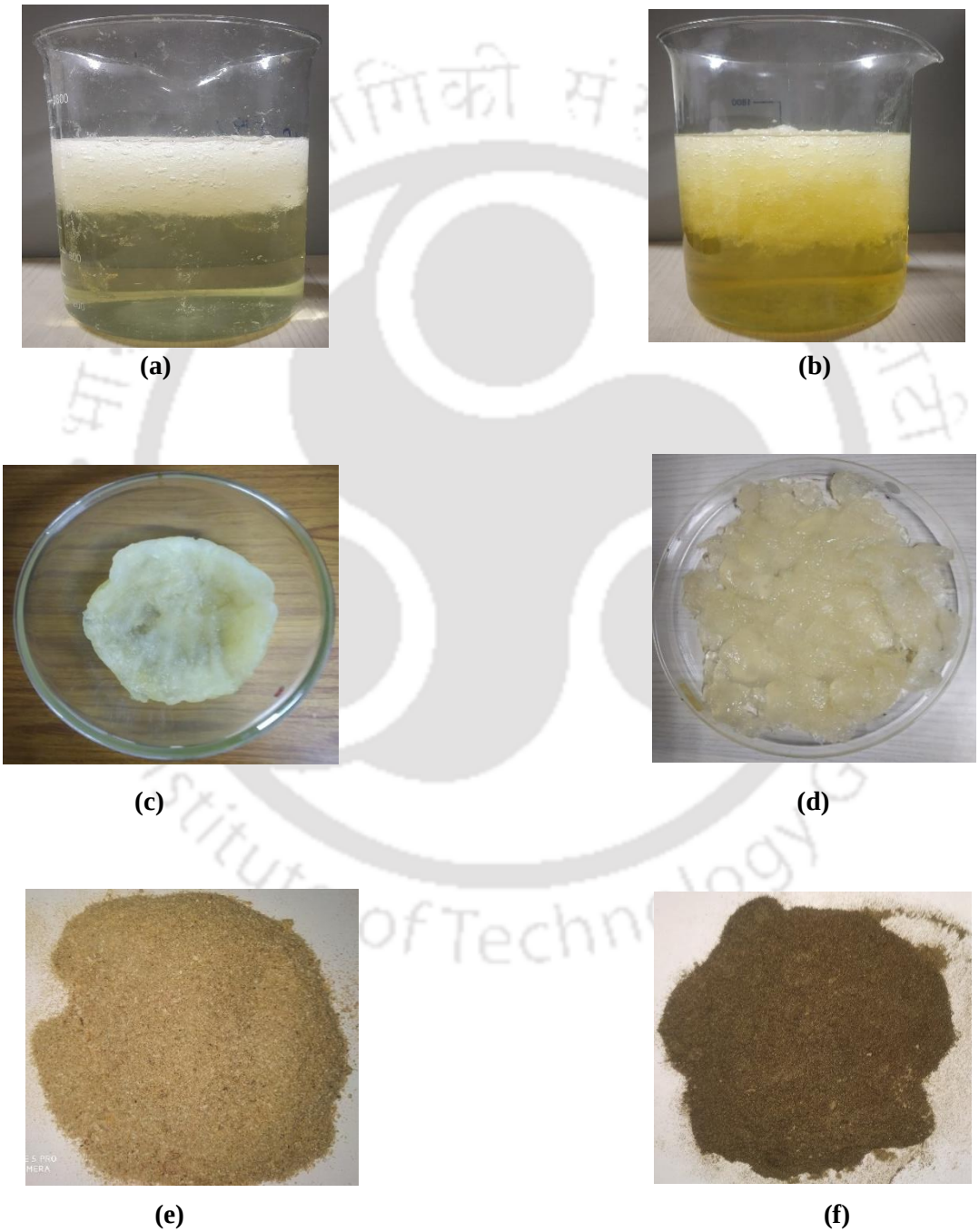
(a) **(b)**

Figure 6.9: FESEM micrograph of pectin extracted from dried pomelo peel powder by (a) pulsed UAE and (b) acidic hot water extraction process.

6.11.5 Macrographic Images of Extracted Pectin

Fig. 10 (a – f) depicts the macrographs of precipitated pectin, wet pectin and dried pectin powder samples obtained respectively with the PUAE and AHWE processes. It can be seen that the wet pectin (Fig. 10 (c)) obtained from the PUAE process possessed a lighter white colour in comparison to the pectin obtained with AHWE process (Fig. 10 (d)) The yellowish darker colour observed in AHWE pectin could be due to extended exposure at moderately high temperature of the AHWE process. The respective wet pectin samples obtained with PUAE and AHWE processes were eventually dried at 50 °C in an oven and the grinded powder macrograph have been depicted in Fig. 10 ((e) and (f)) respectively. Thereby, the PUAE based wet pectin yielded as a fine brownish powder and the AHWE based wet pectin yielded a dark brown coloured powdered sample. Hot water extraction involves heat based breakage of the cell wall for the release of the pectin from the plant material. Higher temperature and prolonged heating are bound to encourage the degradation of the pectin molecules. For this reason,

the extracted pectin could result in a dark brown coloured appearance. However, further characterizations are required for the commercial acceptance of the thus produced pectin, as the commercial pectin is in light brown colour. Similar findings have been reported by Liew et al. (2019) for the extraction of pectin from pomelo peel powder.



(a) **(b)** **(c)** **(d)** **(e)** **(f)**

Fig. 6.10: Macrographs of precipitated pectin (a and b), wet pectin (c and d) and dried blended pectin (e and f) obtained by pulsed UAE and acidic hot water extraction process respectively.

6.12 Effect of Different Acids on PY and DE

Table 6.6 presents the PYs and DEs of pectin extracted from pomelo peel with the PUAE method under optimal conditions (pulsed mode) and with three different organic acids viz., HNO_3, HCl and CH_3COOH. The results affirmed that both PY and DE of pectin got significantly influenced with the HNO_3 followed by CH_3COOH and HCl. The PY was significantly higher for HNO_3 (27.87 ± 0.2 %) in comparison to HCl (23.45 ± 0.4 %) and CH_3COOH (13.12 ± 0.1 %). This might be due to the higher ability of HNO_3 acid to break down cell wall components and separate cellular contents for simpler extraction. According to Methacanon et al. (2014), the PY obtained using nitric acid extraction at 2.0 pH was substantially higher than that obtained with the HCl. Thus, the obtained results were comparable with the extraction yields reported in the literature. According to the results presented in this work, it can be concluded that HNO_3 at optimal pH proved to be better extracting solvent for pectin solubilisation than HCl and CH_3COOH.

In addition, under optimal condition, the DE (69.4 ± 0.13%) of the pectin extracted with CH_3COOH was greater than those extracted with HCl (58.9 ± 0.37 %) and HNO_3 (53.2 ± 0.08%). This is due to the fact that pectin extracted with weak acids have greater DE than the strong acids acid (Liew et al., 2018). Despite conducting extraction at optimal pH for all organic acids, due to CH_3COOH being the weakest acid, it demonstrated lesser degradation of the extracted pectin in comparison to the HNO_3 and HCl. Similar findings have been demonstrated by Methacanon et al. (2014) for pectin extraction from pomelo using acidic solution. In summary, it can be concluded that both PY and DE of the extracted pectin do get influenced with solvent, pH, and type of acid. A higher pH of 3.5 (lesser acid strength) favoured higher DE in the pectin in comparison to that obtained at a lower pH of 2.0 (higher acid strength) (Tables 1 and 2).

Table 6.6: Yield and degree of esterification of pectin extracted under optimal conditions using different acids.

S.NO	Type of Acid	PY (%)	DE (%)
1	HCl	23.45 ± 0.4	53.2 ± 0.08
2	HNO_3	27.87 ± 0.2	58.9 ± 0.37
3	CH_3COOH	13.12 ± 0.1	69.4 ± 0.13

6.13 Comparison between AHWE and HWE for DE and PY

The findings of pectin extraction from pomelo peel with acid (AHWE) or without acid (HWE) have been summarized in Table 6.7. It can be observed that the acidic extraction yielded higher DE and pectin content than the HWE. Significantly lower PY has been obtained for the HWE case (2.6 ± 0.04%) in comparison with the AHWE case (14.8 ± 0.11 %). For this, the acid has been analysed to prompt higher pectin production through the hydrolysis of protopectin. For this, the reason has been discussed in section 6.3.2 of the thesis. These findings are in good agreement with the findings reported in earlier investigations (Hosseini et al., 2019). The authors affirmed that the pH significantly influenced PY extraction from fruit by-products. The maximum extraction yield was achieved under the most acidic condition. This can be attributed to the breakage of hydrogen bonds and ester linkages between pectin and cell wall caused by the low pH. Such an effect enhanced cell wall disruption and hence greater pectin extraction (Methacanon et al., 2014). Besides, the pH also had a prominent influence on the DE of pectin and this has been already discussed for both NUAE and PUAE case. The pectin extracted with the HWE without acid (CA) indicated lower DE (38.51 ± 0.45 %) in comparison to the AHWE (51.5 ± 0.16 %). This can be explained with the fact that under pH induced environment, enhanced de-esterification occurs in the poly-galacturonic chain (Patience et al., 2021). For both cases, the obtained values were lower than the optimal findings reported in this work for the PUAE case.

Table 6.7: Yield and degree of esterification of pectin extracted with hot water extraction process under optimal conditions with and without acid.

S.NO	Type of extraction	PY (%)	DE (%)
1	HWE	2.6 ± 0.04	38.51 ± 0.45
2	AHWE	14.8 ± 0.11	51.5 ± 0.16

6.14 Comparison between Normal UAE and Pulsed UAE of Pectin Extracted from Pomelo Peel

A detailed analysis of the best data for both NUAE and PUAE cases affirmed that there has not been significant variation in the optimal data sets. Both NUAE and PUAE methods confirmed upon substantial influence on the greatest influence on PY and DE properties of extracted pectin. However, while operating under optimal conditions of ST and pH, the PUAE technique gave better results than the NUAE. For the NUAE technique, the optimal extraction conditions were 22.33 min ST, 1.72 pH and for a fixed choice of ET (65°C), and LR (15 g/mL). Under these optimal conditions, the predicted yield was 33.76 % and the DE was highest (56.80 %). For the PUAE technique, the optimal extraction conditions correspond to 16.11 min ST, 1.70 pH and for a fixed choice of LR and ET as 15g/mL and 65°C respectively. Under these optimal conditions, the predicted yield was highest (40.41 %) and the DE was 59.95%. It should be emphasized that during PUAE, the sonication was periodically turned on and off and this resulted in lesser heat generation than the NUAE. Under these circumstances, the PUAE may be preferred than the NUAE for the extraction of pectin with maximum yield and DE (Christou et al., 2021). Furthermore, PUAE-induced agitation has been demonstrated to expedite the optimal onset of mechanical processes such as particle collisions and cell wall collapse. Such phenomena increased mass transfer rates of pectin from the cell wall's interior to the periphery and, as a consequence, their penetration into the solvent phase. These findings corroborate to previous research findings with respect to successful recovery of polysaccharides, including hemicelluloses, pectin and other water-soluble polysaccharides by deploying a short duration based ultrasonic extraction process (Moorthy et al., 2015).

For both NUAE and PUAE, the recovery of PY and DE enhanced as the extraction period advanced from 5 to 17.5 min, and subsequently reduced. This might be due to the cavitational effect of ultrasound waves in the solvent medium, and subsequently higher solvent penetration into the fruit peels and for greater pectin release into the solvent (Marić et al., 2018). After 17.50 min ST, the pectin polysaccharide structure underwent modification and fragmentation and thereby limited pectin recovery. Thus the investigations confirmed that the modified pH has been a key parameter in enhancing yield and DE of extracted pectin. The reason for this has been cited in section 6.6.2 of the thesis. The degrees of freedom of the PUAE process can be stated as per the following order: pH > ST. To summarise, the PUAE method of sonication has been marginally better than the NUAE for the extraction of pectin from pomelo peel.

6.15 Comparative Analysis with Prior Art

Table 6.8 presents the literature comparison of the available prior art with respect to the optimal findings of this work. The findings suggested that the PUAE was the most efficient extraction technique for the extraction of pectin from pomelo peel. PUAE had a greater extraction efficiency than both AHWE and HWE. Under similar operating conditions, both AHWE and HWE techniques performed with 63.37% and 14.09 % and 86.11 % and 27. 42 % lesser PY and DE respectively, in comparison to the optimal findings reported for the PUAE in this work. This is due to the influence of ultrasonic waves in the sonication step of extraction, which causes cell wall breakage and subsequent release of the desired compounds in greater proportion. Thereby, surface area between the targeted compound and the extraction solvent got enhanced (Anticona et al., 2021). In addition, PUAE has been reported to cause lesser degradation of heat sensitive bioactives (Wani & Uppaluri, 2022b). Further, in comparison with the study conducted by Liew et al. (2016), the PUAE indicated 6.02 % higher PY and 5.12% higher DE. Also, with respect to the study conducted by Methacanon et al. (2014), this study affirmed 42.61 % higher PY and 3.47 % higher DE. This might be due to several factors such as higher temperature, lower LR, severe pH, higher ST etc., in the literature. All these factors may have influenced the PY and DE of pectin.

These findings suggest that both PUAE and NUAE are promising ecologically friendly green extraction techniques to extract pectin from pomelo peel sources. Furthermore, the findings strongly indicated that pomelo peels could be a viable industrial resource for the extraction of pectin. Thereby, the suggested process can serve as an effective waste management solution for the discarded pomelo peels.

Table 6.8: Literature and reported optimal data of pectin extraction from pomelo peels through acidic hot water extraction and UAE processes.

Extraction Process	Acid used	Temperature (°C)	Time (min)	Loading ratio (g/mL)	pH	Yield (%)	DE(%)	Literature
UMAE	Citric acid	NA	27.52 (sonication) 6.40 (microwave)	1:29 (Fixed)	1.80	38.0	56.9	Liew et al. (2016)
AHWE	Nitric acid	90	90	1: 30 (Fixed)	2 (Fixed)	23.2	57.9	Methacanon et al. (2014)
HWE	NA	65	16.11	1: 15 (Fixed)	1.70	2.6	38.51	This work
AHWE	Citric acid	65	16.11	1: 15 (Fixed)	1.70	14.8	51.5	This work
NUAE	Citric acid	65	22.33	1:15 (Fixed)	1.72	33.76	56.44	This work
PUAE	Citric acid	65	16.11	1: 15 (Fixed)	1.70	40.41	59.95	This work

6.16 Summary

This chapter addressed the efficacy of CA – UAE process to achieve better yield of pectin characteristics from pomelo peel extracts in comparison with the AHWE process.

Important findings of the carried out experimental investigation are as follows:

- Among the process parameters, pH was the most significant process parameter to influence the PY and DE of pectin.
- Using response surface methodology, the optimal conditions for the PUAE of pectin correspond to 1.70 pH and 16.11min ST. Under these conditions, the PY was around 40.41%, and the extracted pectin had a DE of 58.95 %.
- For the NUAE case, the optimal values for PY and DE correspond to 33.76 % and 56.44 %, respectively, for optimal process condition of 1.72 pH and 22.33 ST.
- The PUAE method affirmed to be more efficient than NUAE and AHWE processes for PY and DE, along with the added benefit of a shorter process time.
- Further, the study indicated that the CA had a greater influence on the extraction yield and DE of pectin in comparison to other acids used in this investigation.
- FTIR spectral analyses indicated similar band pattern for both PUAE and AHWE based pectin samples. However, the PUAE based pectin was found to have more intense peaks in comparison to the AHWE based pectin sample.
- Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) affirmed comparatively higher thermal stability for the pectin obtained with the PUAE process.
- X-ray diffraction (XRD) analysis further confirmed that the pectin from both PUAE and AHWE processes possessed amorphous structure. The XRD analysis of pectin extracted with PUAE process also affirmed retention of native pectin structure during UAE based pectin extraction.

- The FESEM images revealed that the pectin extracted with the PUAE possessed a smoother and flatter surface in comparison to the AHWE based pectin. Further, pectin macrographs affirmed light brown coloured pectin sample with the PUAE process.

In summary, CA can be considered as a prospective extraction agent for pectin product with better acceptable scenarios in the food industry. Also it can be concluded that in comparison to AHWE process, the UAE is promising with short extraction time, moderate operating temperature and more efficient recovery of pectin and with better properties such as improved thermal stability and smoother structure with finer particle size distribution.

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Chapter 7:

Efficacy of Ionic Gelation based Encapsulation of Bioactives from Papaya Leaf Extract

Chapter 7

Efficacy of Ionic Gelation based Encapsulation of Bioactives from Papaya Leaf Extract

In this chapter, the encapsulation of bioactives from papaya leaf (PL) extract has been targeted using ion gelation method. FCD based RSM was adopted to design and optimize the associated process parameters. After a brief introduction in section 7.1, the following sections elaborate upon the findings of the carried research work. Section 7.2 addresses the experimental data obtained through RSM modelling based design of experiments approach. Following this, section 7.3 summarizes the ANOVA findings of experimental data. Thereafter, section 7.4 details upon the response surface characteristics of the encapsulation of bioactives from PL extract. Subsequently, optimization study and encapsulation efficiency, antioxidant activity of dried and wet Ca-Al pectin beads have been addressed in sections 7.5 and 7.6 respectively. Section 7.7and 7.8 presents a brief account of the size of the beads and moisture content of dried beads. Findings of the storage stability studies of the beads at refrigerated and room temperature conditions have been presented in section 7.9. Release kinetics of total polyphenols (TP) from encapsulated wet and dry beads in water have been elaborated in section 7.10. In the following section 7.11, the characterisation results of beads with and without pectin have been delineated. A comparative assessment with best literature data has been conveniently presented in section 7.12. Finally, a summary of the key findings of the conducted research has been outlined in section 7.13.

Overview

The objective of the research investigation was to evaluate the effect of process parameters on the encapsulation efficiency of bioactives from PL extract in sodium alginate-pectin matrix using ion gelation method. For optimization purpose, face centred design (FCD) based response surface methodology (RSM) was deployed. It was observed that the encapsulation efficiency was significantly affected by flow rate of syringe pump followed by calcium chloride and sodium alginate concentration. The obtained beads were characterised for morphology, particle size distribution, FTIR and DSC studies. Also, the prepared beads were evaluated for storage stability under different storage conditions. Thereafter, in-vitro release profiles of the TP content from the encapsulated Ca-Al pectin beads have also been delineated.

7.1 Introduction

With a diverse set of constituent bioactives, the raw papaya fruit (*Carica papaya L.*) can be used as an important component in the product developed by food and nutraceutical industries. This chapter addresses the encapsulation of bioactives molecules from PL extraction using ion gelation method. For this purpose, polymers such as sodium alginate and pectin have been used as wall material and filler respectively. FCD based RSM has been utilised for the optimization of the process parameters to achieve best encapsulation efficiency as the response variable. The obtained Ca-Al beads loaded with pectin (ALEXPB) have been subjected for various characterisation studies such as storage stability, release profile, thermal analysis (DSC), morphological analysis (FESEM) and Fourier transform infrared (FTIR) spectroscopy. This study also elaborates on the comparative encapsulation performance of beads loaded with and without pectin.

7.2 Experimental Details

In this study, the combined effects of concentration of sodium alginate (% w/v), $CaCl_2$ (% w/v) and flow rate of syringe pump (mL/min) using 22G stainless needle size on the encapsulation efficiency of total TPs from PL extract using ion gelation method was targeted using FCD based RSM approach (Table 7.1). For a fixed concentration of pectin (0.5%, which was chosen based on the preliminary

experiments), the encapsulation efficiency of the Ca-Al pectin beads (ALEXPB) varied from 32.52 – 85.6 % for a variation in sodium alginate concentration (% w/v), $CaCl_2$ concentration (% w/v) and flow rate (mL/min) from 1 – 3, 3 – 9 and 0.5 – 3.5, respectively. The upper and lower limits of the chosen process parameters have been set based on the few preliminary experiments. These investigations affirmed that the encapsulation efficiency was significantly affected by the flow rate of syringe pump followed by $CaCl_2$ and sodium alginate concentration. The highest encapsulation efficiency of 85.6 % was achieved at 6 % (w/v) $CaCl_2$ concentration, 2% (w/v) sodium alginate concentration and 3.50 mL/min flow rate. It was found that the enhanced concentration of sodium alginate increased the encapsulation efficiency of the beads up to a certain concentration of sodium alginate. However, beyond this, higher concentration was not feasible as the alginate solution was difficult to pump through the stainless stain needle. The results also revealed that the $CaCl_2$ concentration had a significant effect on the encapsulation efficiency. Furthermore, the findings demonstrate that beads could not be produced at lower concentration of sodium alginate and $CaCl_2$ concentrations (below 1.0 % (w/v) and 2% (w/v), respectively). Thereby, to produce beads, 1% w/v sodium alginate concentration was adopted. This is due to the reason that alginate gels have been produced through the ionotropic gelation based cross linking of calcium ions. The low concentration of alginate could refer to the lack of enough carboxyl groups to form the spherical beads with the calcium ions (Zhang et al., 2021). Additionally, with increasing $CaCl_2$ concentration in the beads, the smoothness of the bead surface also improved. This could be due to the increased interaction between Ca^{2+} ions and alginate at the surface that prevented more material to enter the capsule core. Thereby, the bead structure got stabilized and the bead strength got enhanced (Pasukamonset et al., 2016).

Based on the optimum process parametric conditions obtained for optimum encapsulation efficiency of ALEXPB, four types of beads were produced viz., empty alginate beads (EALB), empty alginate pectin beads (EALPB), alginate beads with PL extract without pectin (ALEXB) and alginate beads loaded with PL extract and pectin (ALEXPB). The obtained beads were thereafter studied for different analysis and characterizations.

7.2.1 Preliminary Experiments

Initially, the preliminary experiments were conducted to infer upon the size of the stainless steel needle for the extrusion of the Ca-Al pectin solution. To do so, needles of various sizes (18 – 24 G) were employed and the needle selection was based on the encapsulation efficiency. From the study, it was affirmed that the needle with 22 G size indicated higher encapsulation efficiency (72.62 ± 0.23) in comparison to needle of other sizes. For the needles of other sizes, the encapsulation efficiency varied from 61 – 69.3%.

Additionally, in preliminary experiments, the alternate concentrations of pectin were utilised to achieve variegated blends with the sodium alginate. The effectiveness of each blend was evaluated for its capacity to entrap the maximum total TP content from the PL extract. In the encapsulation process, the pectin and sodium alginate served as a filler and wall material respectively. Both pectin and alginate belong to natural ionic polysaccharides that undergo chain-chain association and create hydrogels through the addition of divalent cations such as Ca^{2+} (Najafi-Soulari et al., 2016). The blend with 0.5 % (w/v) pectin was chosen as it demonstrated the best encapsulation performance in terms of encapsulation efficiency and maintained good sphericity characteristics of the beads. Beyond this concentration of pectin, the beads with irregular size and shape were obtained. All samples were prepared at room temperature. Thereby, no effect has been observed on the stability of TP molecules or coating materials in the ionic gelation process.

Furthermore, the encapsulation efficiency of the beads was analysed to be dependent on the cross liking solution. The preliminary experiments (1.5 % (w/v) sodium alginate, 3 % (w/v) $CaCl_2$, 0.5 % (w/v) pectin and 2 mL/min flow rate of syringe pump using 22G stainless steel needle) indicated that the resultant Ca-Al pectin beads in $CaCl_2$ solutions indicated lower encapsulation efficiency (63.3 ± 0.11 %) in comparison with those beads gelled in solution containing the PL extract (72.62 ± 0.23 %). The reduction in encapsulation efficiency can be attributed to the considerable diffusion of TP molecules into the $CaCl_2$ solution. This has been indicated through the proportion of non-encapsulated TP molecules being recovered in the gelling bath after removing the beads (Sampaio et al., 2019). These findings indicated that the PL extract that got leaked into the aqueous $CaCl_2$ solution during

encapsulation and its proportion was critically influenced through the formulation of the alginate blend (Mozafari et al., 2008). A comparable diffusional pattern was previously documented (Arriola et al., 2016) and also reported elsewhere (Singh et al., 2018). Moreover, this study supports the notion that as a collecting solution, the PL extract can considerably improve the TP content of the beads and thereby significantly enhance the encapsulation efficiency (Zhang et al., 2021). Additionally, irrespective of the blend constitution, the two developed PL loaded beads demonstrated significant encapsulation efficiency (EE) of the TP components (> 60%). In comparison with the reported findings on the alginate based encapsulation of plant TPs with ionic gelation method, the beads gelled in solutions containing PL extract has very high EE. Thus, the studies illustrated that both blend compositions and the gelling circumstances had profound influence on the ion gelation based encapsulation efficiency of the PL extract in alginate blends. Based on the higher encapsulation efficiency of beads obtained from above mentioned methodologies, the method with maximum encapsulation efficiency (beads gelled in solution containing the PL extract) was followed throughout the work.

Table 7.1: FCD based RSM experimental values for encapsulation efficiency of papaya leaf extract in calcium alginate beads loaded with pectin.

S.No	Calcium chloride concentration (A) (%)	Sodium alginate concentration (B) (%)	Flow rate (mL/min) (C)	Encapsulation efficiency (%)
1.	6.00	2.00	2.00	77.4 ± 0.29
2.	9.00	1.00	3.50	46.82 ± 0.18
3.	6.00	2.00	2.00	78.55 ± 0.39
4.	6.00	2.00	2.00	75.59 ± 0.22
5.	9.00	3.00	3.50	54.32 ± 0.77
6.	6.00	3.00	2.00	55.87 ± 0.26
7.	9.00	2.00	2.00	72.5 ± 0.42
8.	6.00	2.00	3.50	85.6 ± 0.16
9.	3.00	3.00	3.50	43.69 ± 0.37
10.	3.00	3.00	0.50	35.97 ± 0.04
11.	6.00	2.00	2.00	76.06 ± 0.30
12.	3.00	2.00	2.00	64.11 ± 0.25
13.	3.00	1.00	3.50	44.34 ± 0.74
14.	6.00	2.00	2.00	77.74 ± 0.78
15.	9.00	3.00	0.50	47.56 ± 0.41
16.	6.00	1.00	2.00	51.0 ± 0.14
17.	6.00	2.00	2.00	78.5 ± 0.12
18.	3.00	1.00	0.50	32.52 ± 0.85
19.	9.00	1.00	0.50	34.41 ± 0.72
20.	6.00	2.00	0.50	72.21 ± 0.67

7.3 ANOVA Data Summary

ANOVA was used to evaluate the significance of the independent variables' influence on the response variable (Table 7.2). The ANOVA of the quadratic regression model illustrated that the model was significant. This was based on the high F-test value (309.89) and lower p value ($p < 0.0001$). A satisfactory value of the coefficient of determination (R^2) and as 0.9964, further supported the model's fitness. The adjusted coefficient of determination (R^2adjusted) was calculated to be 0.962, and thereby affirmed that the model is highly significant. Further, the lower value of the coefficient of variation (2.38%) indicated greater accuracy and reliability of the model. The non-significant lack of fit value of

1.67 provided additional support for the fitness of the quadratic model. In the best fit model, linear terms (A, B and C), bilinear terms (AB and BC), and quadratic terms (A^2 and B^2) were significant with larger F and lower p values. The mathematical equation for the relationship between encapsulation efficiency and process variables can be represented as follows:

$$EE = 77.82 + 3.50A + 2.83B + 5.21C + 2.23AB - 0.046AC - 1.22\,BC - 10.30\,A^2 - 25.17B^2 + 0.28\,C^2 \quad (7.1)$$

Where A, B and C represents, calcium chloride concentration, sodium alginate concentration and flow rate of a syringe pump, respectively.

Table 7.2: ANOVA data summary for encapsulation efficiency of papaya leaf extract in calcium alginate beads loaded with pectin for all independent variables.

Components	**Encapsulation Efficiency**	
	***F* value**	***P* value**
Model	309.89 (Quadratic)	< 0.0001 (Quadratic)
A	59.70	< 0.0001
B	39.13	< 0.0001
C	132.24	< 0.0001
AB	19.43	0.0013
AC	8.350E-003	0.9290
BC	5.80	0.0368
A^2	142.22	< 0.0001
B^2	849.75	< 0.0001
C^2	0.11	0.7485
Lack of Fit	1.67	0.2930
R squared		0.9964
Adequate Precision		50.614

7.4 Response Surface Characteristics

The 3D response surface plots are graphical representations of the regression equation that can be used to assess upon the sensitivity of encapsulation efficiency with respect to the varied process variables.

These graphs are generated as a function of the first two parameters, with the third variable held constant at its mean value (Wani & Uppaluri, 2022). Fig. 7.1 illustrates the response surface plots of the encapsulation efficiency with respect to any two combinations of $CaCl_2$ concentration (% w/v), sodium alginate concentration (w/v %) and flow rate (mL/min). The response plots indicated nonlinear and quadratic dependence of the variable response surface. Also, the 3D surface response plot (Fig.7.1) demonstrated the profound influence of flow rate on the response variable. Thereafter, the critical influence of sodium alginate and $CaCl_2$ concertation were apparent. For all cases, an enhancement in any two process variable values facilitated an increase in the encapsulation efficiency. From response surface plot, it can be observed that the encapsulation efficiency increased with an enhancement in both sodium alginate (1– 3 % (w/v)) and $CaCl_2$ (3–9 % (w/v)) concentrations and only upto a certain point. Thereafter, a further increase in both concentrations fostered a reduction in the encapsulation efficiency. This is due to the fact that with increasing alginate concentration, the number of biopolymer molecules per unit volume enhance and thereby increase the number of calcium ion binding sites (Romanini et al., 2021) As a result, a denser gel structure is formed and thereby encapsulates more TP molecules. In a relevant prior art, it has been opined that the pectin being used to encapsulate a variety of bioactive components does enhance the density of the gel and effectiveness of encapsulation (Ćorković et al., 2021).

For a fixed choice of flow rate (2mL/min), the encapsulation efficiency increased nonlinearly from 51–72.5% for an alteration in sodium alginate concentration (% w/v) and $CaCl_2$ concentration (% w/v) from 6 – 9 % and 1 – 2 % respectively. On the other hand, for a fixed choice of sodium alginate concentration (2% w/v), the encapsulation efficiency increased non-linearly from 64.11– 85.6 % with respect variation in $CaCl_2$ concentration from 3 – 6 % w/v but linearly with respect to the alterations in flow rate from 2 – 2.5 mL/min.

Further, at fixed concentration of $CaCl_2$ (6% w/v) and for variation in sodium alginate concentration from 1–2 % w/v, the encapsulation efficiency varied nonlinearly from 51– 85.6 %. However, with respect to the variation in flow rate from 2– 3.5 mL/min, the encapsulation efficiency varied almost linearly.

Thus, in summary, it can be concluded that the interaction of any two process variables translated into a significant variation in the encapsulation efficiency of Ca-Al pectin beads. Based on the RSM based experimental data, the highest encapsulation efficacy of 85.6 % was obtained for the process-parametric combination of 6 % w/v $CaCl_2$ concentration, 2% w/v sodium alginate concentration and 3.5 mL/min flow rate.

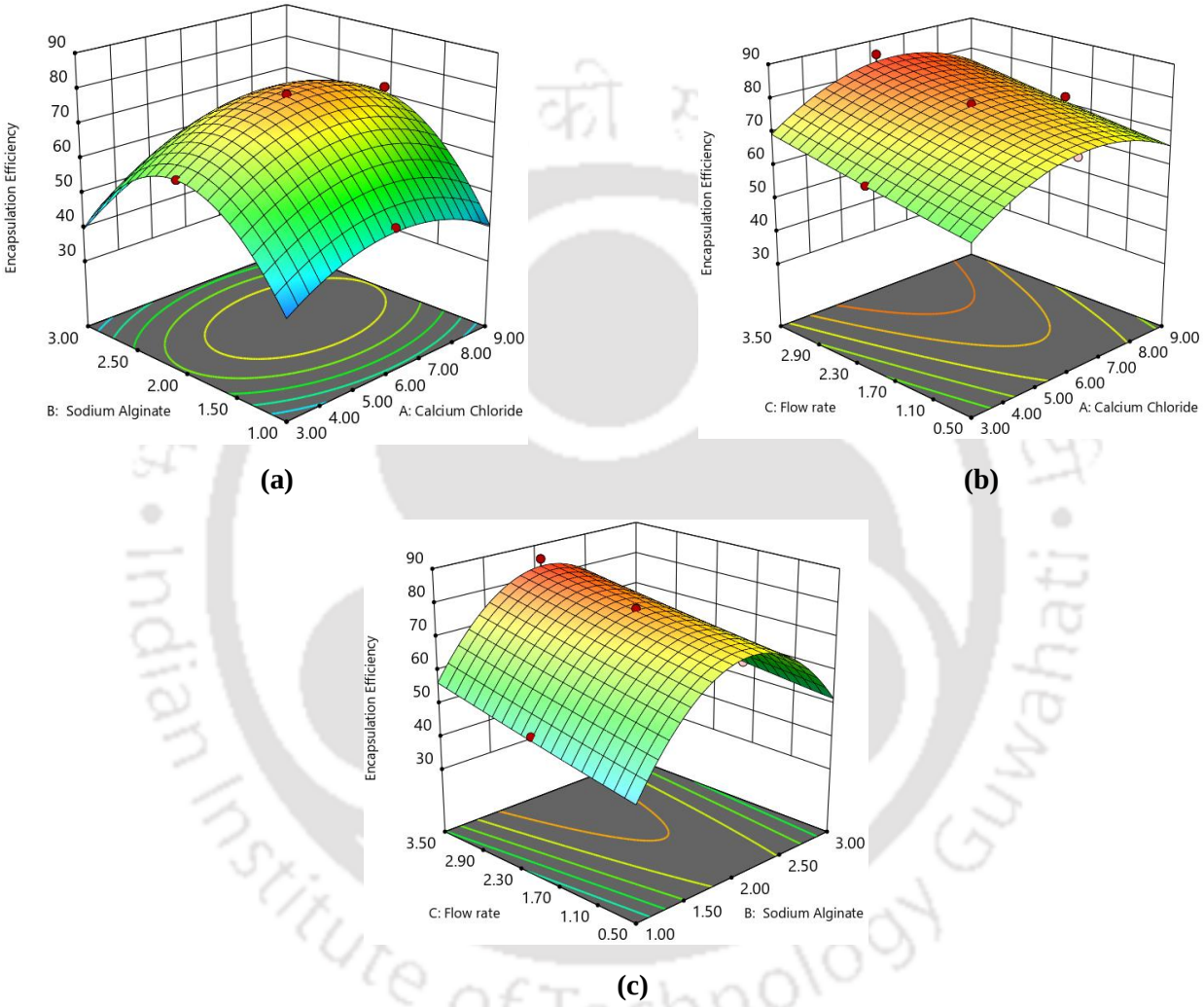


Figure 7.1: Response surface plots of encapsulation efficiency for encapsulated papaya leaf extract calcium alginate beads loaded with pectin.

7.5 Optimality of Independent Variables

The optimum value of the process variables such as sodium alginate concentration, $CaCl_2$ concentration and flow rate have been predicted numerically to obtain the desired encapsulation efficiency. The optimum values were obtained as 2% (w/v) sodium alginate concentration, 6.61 % (w/v) $CaCl_2$ concentration and 3.5 mL/min flow rate. Under these optimum conditions, the predicted optimum value for encapsulation efficiency was 86.7 % which is comparable to the optimal experimental encapsulation efficiency of 85.6 %. These findings indicate that the regression equation has been an adequate model to explicate upon the relationship between process parameters and encapsulation efficiency of Ca-Al pectin beads. Furthermore, alginate concentrations above 3% (w/v) might have resulted in enhanced encapsulation efficiency. However, processing problems do arise. Beyond 3% (w/v), the alginate solution employed in this investigation became extremely viscous and was difficult to pump.

7.6 Encapsulation Efficiency and Antioxidant Activity of Dried and Wet Calcium Alginate (Ca-Al) Pectin Beads

Based on the optimum results, the Ca-Al encapsulated PL extract beads loaded with pectin were prepared and part of these beads were dried at oven 40 ºC temperature for 12 h. Thereby, the encapsulation efficiency and antioxidant activity of dry and wet beads were evaluated. From the findings, it can be observed that the encapsulation efficiency of wet beads (85.56 ± 0.16 %) was almost similar to that of the dry beads (85.06 ± 0.43 %). With respect to antioxidant activity, dried beads (73.78 ± 0.14 %) indicated marginally higher antioxidant activity than that obtained for the wet beads (72.64 ± 0.72 %). Drying at a higher temperature might cause a reduction in encapsulation efficiency and subsequent marginal reduction in antioxidant activity of dried beads due to degradation of polyphenols of PL extract (Zhang et al., 2021). Further, the study indicated that the encapsulated PL extract wet beads loaded with pectin had higher encapsulation efficiency and antioxidant activity in comparison to beads without pectin (79.14 ± 0.33 % and 69 ± 0.29 % respectively). Additionally, drying of beads result in moisture loss and transforms the beads into stable concentrated encapsulated form in comparison to the wet beads. Hence, they are better suited as a medium for food and pharmacological

systems. These findings are consistent with the relevant prior art that demonstrated alginate based encapsulation of plant phenols (Zhang et al., 2021).

7.7 Size of the Beads

The diameters of the dried beads (encapsulated and non-encapsulated with and without pectin) were measured using a Delsa Nano instrument. The encapsulated dried alginate beads loaded with pectin possessed higher diameter of 3.66 ± 0.03 µm in comparison to the non-encapsulated beads and encapsulated beads without pectin 3.05 ± 0.07µm and 3.15 ± 0.03 µm respectively. It must be noted that many factors can influence the shape and size of the beads. These include needle's diameter, solid content, crosslinking solution, the distance between the needle and the surface of the crosslinking solution and surface tension (Sun et al., 2020).

7.8 Moisture Content of Dried Beads

The moisture content of the encapsulated beads loaded with or without pectin was compared with those of the non-encapsulated alginate beads. From the findings, it can be analysed that the moisture content of the beads varied from 3.10% – 4.45 % for respective beads. The encapsulated Ca-Al beads loaded with pectin and PL extract indicated lower moisture content (3.10 ± 0.08 %) in comparison to beads without pectin (3.85 ± 0.12 %) and non-encapsulated alginate beads (4.45 ± 0.18 %). Thereby, it can be concluded that Ca-Al beads loaded with pectin possessed lower moisture content in comparison to non-encapsulated beads. Hence, these beads could be stored for an extended period of time. Similar findings have been reported by Sing et al. (2018) for alginate/pectin based encapsulation of α-tocopherol.

7.9 Stability Study of Calcium Alginate Pectin Beads in Refrigerated and Room Temperature Storage Condition

In this study, two types of beads were stored under different conditions. The beads stored with PL extract was named as B_1 beads. Similarly, the beads stored without PL extract was named as B_0 bead. These two types of beads samples were stored at refrigerated conditions (B_{1REF} and B_{0REF}) and at room temperature conditions (B_{1RT} and B_{0RT}). Fig 7.2 depicts the TPC alterations in the beads with storage

time (30 days). From the figure, it can be observed that the TPC remained relatively constant in the beads (B_{1REF} and $B_{0REF)}$ stored under refrigerated conditions. This might be due to the protective effect of Ca-Al pectin system on the TP molecules (Arriola et al., 2016). During the first 5 days of storage, the TPC of the beads stored in refrigerator (B_{0REF}) indicated marginal loss in the TPC value in comparison to the beads stored at room temperature (B_{0RT}). For the B_{1REF} beads, the TPC value indicated marginal variation between 5 – 10 days and reduced for an extended time of 20 days. Thereafter, the TPC in beads remained stable for a further extended time of 30 days. On the other hand, the B_{0RT} beads stored at room temperature indicated significant reduction in the TPC value during the first 10 days of storage. Thereafter, the pattern was similar to that of beads stored in refrigeration and for 20 days of storage period. Then afterwards, the TPC remained nearly constant from 20 – 30 days of storage. Furthermore, it was found that there was substantial degradation of non-encapsulated TPC of PL extract that was stored at the refrigeration condition for 30 days of storage. The PL extract had developed a foul smell and its colour has changed to pale brown. Incidentally, a drastic reduction in its TPC has been evaluated and from 133 (for encapsulated Ca-Al pectin beads) to 8.42 mg GAE/g. Similar findings have been reported by Zhang et al. (2021). The authors inferred that the degradation of polyphenols in grape pomace extract was significantly reduced after its encapsulation in malto-dextrin and milk proteins.

Additionally, the TPC of the beads stored in the PL extract at both refrigeration and room temperature conditions significantly increased during the first 10 days of storage period. Thereafter, it declined gradually during the next 20 days, and eventually remained fairly stable for an extended storage period of 30 days. A similar pattern was observed in alginate beads encapsulated with stevia extract (Arriola et al., 2016). The authors analysed that the enhanced TPC during storage could be due to the polyphenol formation being attributed to several pertinent phenomena such as condensation reactions between anthocyanin's and other TP molecules and residual enzymatic activity.

In summary, it can be concluded that the beads stored at refrigeration conditions indicated better stability in comparison to those beads stored at room temperature conditions. Additionally, the encapsulated beads indicated more stability than the PL extract.

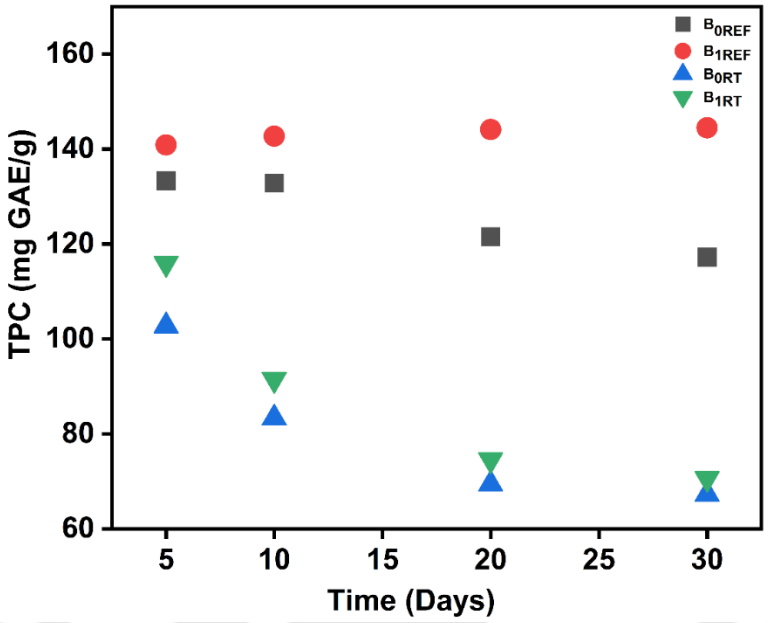


Figure 7.2: Storage stability of encapsulated papaya leaf extract beads loaded with pectin under refrigeration (4 °C) and room temperature during 30 days of storage. B_{1REF} and B_{1RT} refer to beads stored in papaya leaf extract at refrigeration and room temperature conditions. B_{0REF} and B_{0RT} refer to beads stored without papaya leaf extract at refrigeration and room temperature conditions.

7.10 Characterization of Beads

7.10.1 FESEM Study

FESEM study was conducted to obtain needful details upon the shape and surface characteristics of the beads. Thereby, useful insights can be obtained with respect to the influence of pectin on these parameters (Fig. 7.3). Three types of beads were subjected to FESEM analysis. These were non-encapsulated Ca-Al beads and beads with and without pectin. The beads surface is the first to contact its fluid, solid, or gaseous environment and thereby, along with the internal structure of the bead, its surface affects the bead functionality. A rough and almost non uniformly shaped structure with depressions was apparent in the non-encapsulated Ca-Al beads. In addition, Ca-Al pectin PL extract encapsulated beads had round and smoother surface in comparison to the beads prepared without pectin. Further, the beads loaded with pectin were bigger in size in comparison to the beads prepared without

pectin. This confirmed that the incorporation of pectin as a filler had a significant effect on the shape and size of the of the Ca-Al beads. Numerous factors do influence upon the shape and size of the beads. These include the distance between the needle and the surface of the crosslinking solution, the diameter of the needle, the surface tension solid content and the crosslinking solution (Sun et al., 2018). It is well known that when porous Ca-cross-linked alginate beads are freeze dried, artefacts form due to water leakage from the particles. Consequently, the weakening of the matrix's gel structure occurs and causes collapse of the pores' walls and shrinkage of the beads (Romanini et al., 2021). The existence of porous and collapsed structures in ionic cross-linked alginate-based microbeads after drying has been reported previously (Stojanovic et al., 2012). Thereby, it was inferred that the shape retention of dried microbeads can be achieved using internal gelation microencapsulation in calcium cross-linked alginate and pectin. The prevention of shape distortion and preservation of the spherical microbeads shape could due to a better association of the alginate and pectin polymer chains and smaller size of such particles that ensured easier shape maintenance in comparison to the large dried beads. Several studies using tapioca starch (Lozano-Vazquez et al., 2015), whey proteins, and bovine serum albumin have reported with similar filler effects in due course of biopolymers addition to the alginate for microencapsulation (Đorđević et al., 2015).

According to the FESEM micrographs, the use of pectin as a filler appears to be significant from the perspective of maintaining the sphericity of the beads. Furthermore, these findings indicate that the oven temperature drying method can be a possible method for the preservation Ca-Al pectin beads. This is one of the most important findings of this work and its criticality in conjunction with the desired commercial applications.

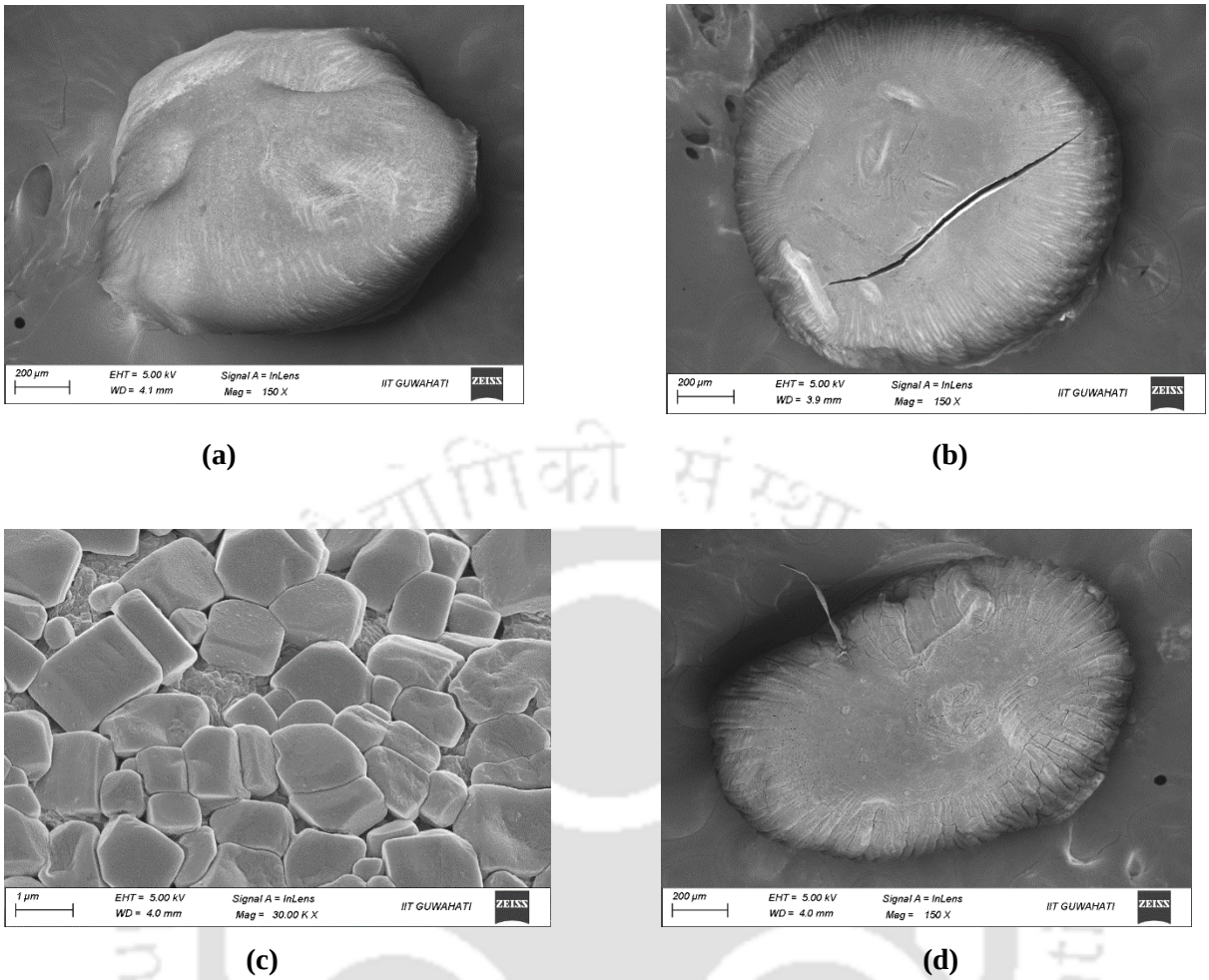


Figure 7.3: Morphology of beads (a) non-encapsulated Ca-Al beads, (b and c) encapsulated Ca-Al beads loaded with pectin and (c) encapsulated Ca-Al beads without pectin

7.10.2 FTIR Spectra based Structural Analysis

The pertinent functional groups for all prepared bead samples (EALB, ALEXB, ALEXPB, EALPB), PL and ALPOW and possible yet potential interactions between the molecules in the extract and the matrix have been analysed using FTIR technique (Fig. 7.4 (a) and (b)). The vibration bands seen in encapsulated ALEXPB beads and PL extract have been almost similar to those found in the EALB, EALPB and ALPOW. From the FTIR spectra, it can be observed that there has been a spreading of the absorption line for all cases in the wave number range of 3000-3600 cm^{-1}. This is believed to be due to the stretching and flexion of OH groups and especially for the ALEXPB, ALEXB and ENALB case (Belščak-Cvitanović et al., 2016). Also, it is evident from the FTIR graph that the addition of pectin, possessing several OH groups lead to the increased peak intensity (1591 cm^{-1}) in the ALEXPB beads.

This is attributed to the fact that the addition of pectin to the encapsulated Ca-Al beads results in the hydrogen bond formation between pectin and alginate (Đorđević et al., 2015).

Further in the ALEXPB beads, the bands near 1,024 cm^{-1} represent a C-O-C vibration, a characteristic of the sodium alginate polysaccharide structure. The bands at 1594 cm^{-1} and 1,412 cm^{-1} have been attributed to asymmetric and symmetric vibrations peaks of carboxylate salt (COO–) groups (Vallejo-Castillo et al., 2020).

For the case of pectin spectra, the stretching of ester carbonyl (C = O) groups was attributed to an absorption band that appears at 1734 cm^{-1} in the pectin spectra and a moderate peak at 2,922 cm^{-1} that corroborates with the stretching of the methyl C-H group (Ćujić et al., 2016). For the PL extract, the band at 1,040 cm^{-1} can be attributed to the -C-O stretching of the alcoholic group. Belak-Cvitanovi et al. (2017) have reported similar type of bands in the extracts of *P. sarmentosum* and green tea respectively.

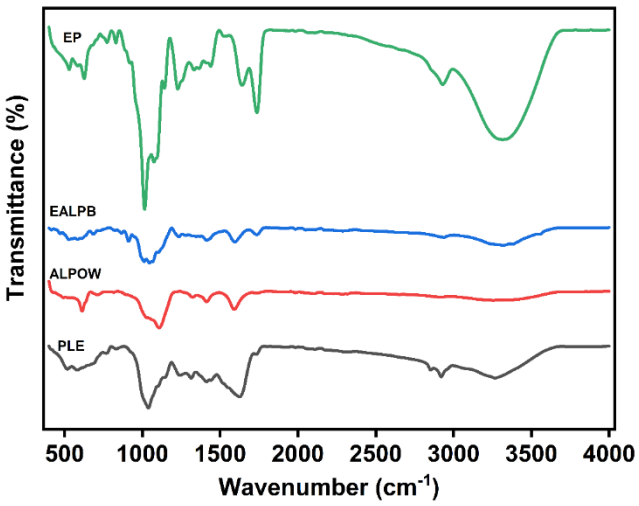
Further, bands in the spectra of PL extract and EP have been similar to ALEXPB bands (3000- 3500 cm^{-1}). These can be attributed to O-H stretching and hydrogen bonds. The bands in ALEXPB have been more significant at 1600 and 1018 cm^{-1} and respectively corresponds to the C = O and C-O of the carboxyl group (Ćorković et al., 2021).

Additionally, modifications in the alginate-pectin spectrum's region of the carbohydrate fingerprint (1000 – 1600 cm^{-1}) have been observed. These could indicate the existence of pectin galacturonic acid moieties. Consequently, it can be concluded that the Ca-Al pectin combination could be a suitable material to encapsulate the bioactive components that are found in plant extracts (Vallejo- Castillo et al., 2020).

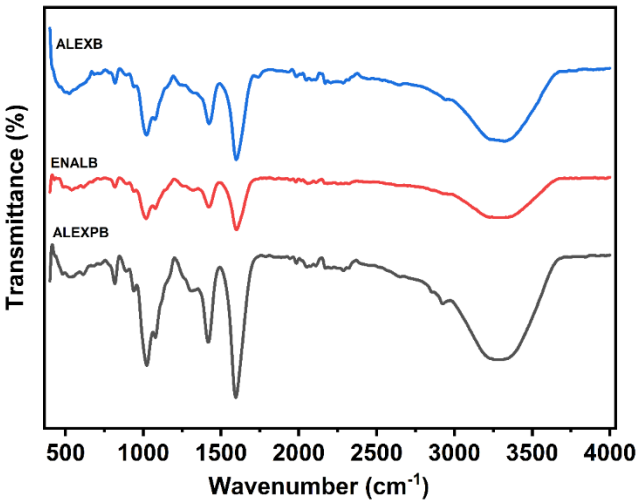
The spectra for the ALEXB beads have been similar to those obtained for the ALEXPB beads. However, the latter had more intense peaks at 1022, 1417 and 1600 cm^{-1}. Thereby, they suggest that there has not been any chemical interaction between the polyphenols of PL extract and the encapsulating materials (pectin and calcium alginate) (Sampaio et al., 2019). This is due to the reason that the spectrum does not illustrate any new bands or significant band alterations in the region between 1,700 and 1000 cm^{-1} of the spectrum of the ALEXPB beads. However, ALEXPB display strong intense peaks in comparison to ALEXB beads.

Also, the variations observed in the region (1200-1050 cm $^{-1}$) refer to the C-OH stretch and corresponding groups of alcohols, polyphenols, and polysaccharides. These findings suggest that a potential interaction between the encapsulated PL extract and the encapsulation materials might have occurred (Shi et al., 2021).

The peaks for non-encapsulated alginate beads and Ca-Al beads with and without pectin respectively, were observed at wave numbers 3289 cm^{-1}, 3290 cm^{-1} and 3284 cm^{-1}. The small peaks in the wavelength range of 2921 – 2940 cm^{-1} may have originated due to CH_2 groups (C–H stretching vibrations) (Arriola et al., 2019). The intensive absorption peaks from 1594 – 1602 cm^{-1} and from 1417 –1426 cm^{-1} correspond to carboxylate anions (COO–). Peaks in the region of 1016 – 1030 cm^{-1} can be attributed to the alcoholic and cyclic ether groups from polyphenols. These might have existed due to the addition of PL extract to the Ca-Al pectin beads (Fig. 7.4 (a)) (da Silva Carvalho et al., 2019). Thereby, from the findings of FTIR spectra, it can be concluded that significant modification did not occur in the Ca-Al pectin beads after encapsulation. Thus, the Ca-Al pectin beads were suitable for the encapsulation of PL extract. These findings have been consistent with the studies conducted by Stojanovic et al. (2012) and Istenic et al. (2015) on similar encapsulation system.



(a)



(b)

Figure 7.4: FTIR spectrum of (a):- non-encapsulated Ca-Al beads (EALB), alginate powder (ALPOW), papaya leaf extract (PL extract), non-encapsulated Ca-Al-pectin beads (EALPB), extracted pectin (EP), (b):- non-encapsulated Ca-Al beads (ENALB), Ca-Al-pectin beads with PL extract (ALEXPB), and Ca-Al beads with PL extract (ALEXB).

7.10.3 Differential Scanning Calorimetry (DSC) Study

The thermal behaviour of all prepared beads (EALB, EALPB, ALEXB and ALEXPB) along with thermograms of PL extract, ALPOW and EP was analysed by the DSC. These have been shown in Fig. 7.5. The thermograms for ALPOW and EP confirm an initial endothermic peak at 70.97°C and 95.97°C, respectively, followed by an exothermic peak at 290.97 °C and 240.0 °C that indicated water loss and decomposition respectively. Two endothermic peaks associated to water loss and melting have been apparent on the thermogram for PL extract at 75.97°C and 165.97°C, respectively. Similar type of thermograms have been reported for the cocoa and jabuticaba exocarp extracts (Lupo et al., 2015). The thermogram for non-encapsulated Ca-Al beads (EALB) demonstrates one decomposition endothermic peak at 175°C and two exothermic peaks at 240 °C and 295°C. On the other hand, the thermograms of non-encapsulated Ca-Al pectin beads (EALPB) and ALEXB confirm one endothermic peak and one exothermic peak at 190.2°C and 290.5°C respectively. These decomposition peaks have been absent in both EP and ALPOW system. Thus, these observations confirm upon the formation of the "egg-box"

crosslinking pattern between alginate and pectin (Pasukamonset et al., 2016). Thereby, homogenous crosslinking between the two polysaccharides has been confirmed. Thereby, it can be concluded that addition of pectin in the alginate matrix enhanced its peak temperatures values.

Additionally, in the thermogram of beads loaded with pectin and PL extract (ALEXPB), one endothermic and one exothermic peak can be observed at 200 °C and 295 °C. These can be attributed to addition of pectin in the Ca-Al matrix. For the Ca-Al beads loaded with PL extract and pectin, the decomposition endothermic peak reached a higher temperature of 200 ºC. Thereby, the interaction between PL extract and the alginate-pectin system in the beads led to higher thermal stability of these beads in comparison to beads without pectin and PL extract. These findings imply that the alginate might retain the chemical instability of the PL extract during the encapsulation process. In addition to the thermal stability, the chemical stability has been corroborated to the development of new chemical entities with altered thermal and absorption capabilities. Similar findings have been reported for the encapsulation of lycopene from water melon concentrate (Sampaio et al.,2019).

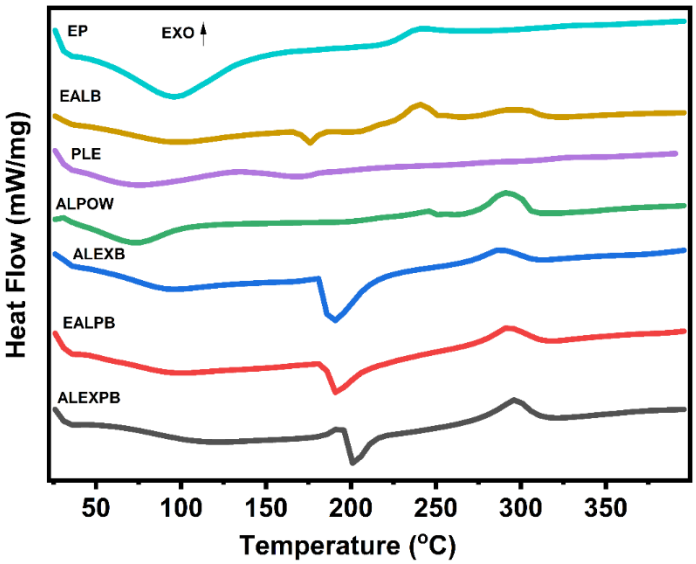


Figure. 7.5: DSC thermograms of non-encapsulated Ca-Al beads (EALB), alginate powder (ALPOW), PL extract (PL extract), non-encapsulated Ca-Al pectin beads (EALPB), extracted pectin (EP), Ca-Al pectin beads with PL extract (ALEXPB), and Ca-Al beads with PL extract (ALEXB).

7.11 Release Kinetic Study of TP Molecules from Encapsulated Beads in Water

The TP release characteristics from wet and dried ALEXPB samples is shown in Fig.7.6. To do so, both beads were dissolved in DI water and the TP of the dissolution medium was determined. It was analysed that both samples demonstrated similar pattern of the TP release profiles. The pattern involved quicker release of TPs during the initial and first phase of 0-40 min and subsequent slower release in the second phase (50- 200 min). During the first 40 min, about 51.75% and 56.42 % of TP molecules respectively got released from the wet beads and dry beads. However, for both cases, a similar stabilized TP release has been apparent during the second phase. The initial rapid release of the TP molecules could be due to the fast dissolution of TP molecules on the surface of beads. Thereby, as time passed, the release of TP molecules progressively shifted from the surface to the interior of the beads. This phenomenon leads to a reduction in the mass transport and thereby the release rate reduced (Zhang et al., 2021). Thereby, the release rate got reduced. The declining release characteristics could be attributed to the interaction hydrophilic Ca-Al with water molecules that resulted in a viscous gel structure. Such a structure might have blocked the surface micropores of the beads and delayed upon the release of TP molecules for a prolonged period of time (Lamoudi et al., 2016). Thus, both types of beads demonstrated an early burst of TP molecules and followed an attenuating release and time bound constrained release of the TP molecules. Similar findings have been reported by Zhang et al. (2021) for the encapsulation of mulberry pomace extract in Ca-Al beads.

Additionally, the dried beads released more TP molecules than the wet beads. This phenomenon has been attributed by the authors to the drying process. During drying, water migrates from interior region of the beads and reaches to the beads surface (Rassis et al., 2002). Thereby, it induces the encapsulated TP molecules to reach onto the surface. Thereby, a larger proportion of dissolved TP molecules could disperse into the water through the convection process. This resulted in an irregular distribution of TP molecules and increased proportion of TP molecules on the surface of the beads (Voo et al., 2016). Therefore, the accumulation of TP compounds on the surface of the beads driven by the drying process could account for the rapid TP release during the first 10 min of the kinetic profile of the dried beads. In the wet samples, the lower TP release rate can be corroborated to the difference in the water uptake

profile of the beads in aqueous medium. In due course of the release of encapsulated TP molecules, the mobility and migration of bioactive molecules are often characterised by an initial chain plasticization (involving a reduction in the attractive forces between chains), followed by a combination of expansion and relaxation (Song et al., 2020).

Alternate kinetic models, namely the zero-order, Higuchi, Korsmeyer-Peppas and Peppas-Sahlin models have been evaluated for the fitness with the measured dissolution data of TP molecules in water. For all models, corresponding kinetic parameters and coefficient of determination (R^2) values have been summarised in Table 7.3. The R^2 value of the models were used to assess the goodness of fit. Both Peppas-Sahlin and Korsmeyer-Peppas models affirmed large R^2 (0.97, 0.94 and 0.99,0.99 respectively) values than other models for both types of beads. Hence, these two models have been selected for further investigations. Also, the research reported by Zhang et al. (2021) inferred that the Peppas-Sahlin and Korsmeyer-Peppas models possessed good fitness index for the representation of the TP molecule release from Ca-Al beads.

In the Korsmeyer-Peppas model, the exponent value n can be used to define the kind of diffusion. Thereby, a value of n less than or equal to 0.45 denotes a Fickian diffusion process (case I). In the case, the diffusion rate is substantially lower than the rate of sponge relaxation and the release is regulated by diffusion. On the other hand, a value of n greater or equal to 0.89 refers to a case II transport. In this case, relaxation rate largely surpasses diffusion rate and carrier relaxation controls the TP release. The intermediate value of n ($0.45 < n < 0.89$) confirms a non-Fickian diffusional mechanism (Zhang et al., 2021).

In the conducted studies, the n values for wet (0.39) and dried beads (0.31) were both lower than 0.45. Thereby, it can be concluded that the TPs release was governed by the diffusion process. In the case of Peppas and Sahlin model, the Fickian diffusional contribution is represented with the first term in the expression. Further, case-II relaxation contribution is represented by the second term in the mathematical expression. As conveyed in the Table 7.3, $k_d > k_r$ suggested that the TP release was controlled by the diffusion process. Similar findings have been reported by Zhang et al. (2021) for the release of TP molecules from Ca-Al beads.

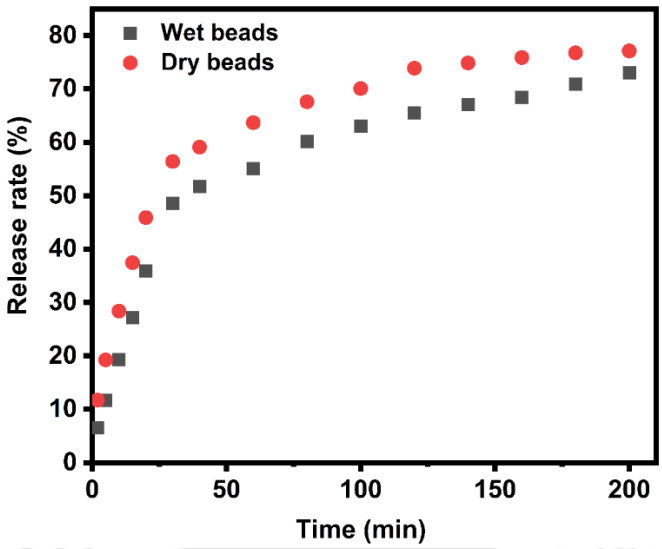


Figure 7.6: Release kinetics of total polyphenol molecules in water at 25 °C from the wet and dried Ca-Al pectin beads encapsulating papaya leaf extract.

Table 7.3: Kinetic parameters of various models obtained from release kinetics data of total polyphenol molecules from wet and dried beads.

S.No	Release model	Model parameters	Wet beads	Dry beads
1.	Zero-order	k_0	0.44	0.54
		R^2	0.34	- 0.21
2.	Higuchi	k_H	5.33	6.72
		R^2	0.93	0.77
3.	Korsmyer-Peppas	k_P	8.91	15.93
		n	0.39	0.31
		R^2	0.97	0.94
4.	Peppas-Sahlin	k_d	5.04	8.88
		k_r	-0.098	-0.25
		m	0.62	0.57
		R^2	0.99	0.99

k_0: zero order release constant; k_H: Higuchi model rate constant; k_p: Korsmeyer-Peppas model kinetic constant; n: the value of the release exponent; k_d: Peppas-Sahlin diffusion kinetic constant; k_r: Peppas-Sahlin relaxation kinetic constant; m: the value of Fickian diffusion exponent; R^2: the coefficient of determination.

7.12 Comparative Analysis with Prior Art

The encapsulation efficiency of ALEXP beads have been compared with those reported in the relevant prior art data (Table 7.4). The encapsulation efficiency of ALEXP beads being reported in this work have been better than those reported in the relevant prior art (Singh et al., 2018). Comparatively, for the encapsulation of α-tocopherol using alginate-pectin mixture, Singh et al. (2018) reported 31 % lower encapsulation efficiency with respect to the optimised efficiency reported in this work. In addition, Corkovic et al. (2021) performed encapsulation of chokeberry polyphenols and volatiles using alginate and pectin as wall materials. The authors reported 37 % reduced encapsulation efficiency in comparison with the optimal data set of this work. These variations have also been corroborated to variations in the initial content of polyphenols present in the plant extract, loading ratio used for the extract preparation, and drying conditions.

The encapsulation efficacy of beads without pectin has been lower than that obtained for the beads loaded with pectin. However, it is higher than that presented in the literature. Sun et al. (2020) reported significantly higher encapsulation efficiency (76%) with pectin beads in comparison to those prepared without pectin. Similar findings have been reported in the earlier studies of Rassis et al. (2002). Further, in the relevant prior art of Corkovic et al. (2021), the pectin has been found to be more effective to encapsulate chlorogenic acid. The authors inferred that the addition of the polysaccharides (pectin, cellulose derivatives, and chitosan) or fillers (carob powder or cocoa) into alginate system did improve TP encapsulation. This could be achieved through an improvement in the alginate porous structure to customize encapsulation of substances with lower molecular weight.

In general, encapsulation efficiency is affected by a variety of parameters. These are polymer hydrophilicity, porosity, crosslinking, and the interaction of polymer and extract components in few cases. The possible reason for enhanced encapsulation efficiency for beads loaded with pectin could be attributed to several factors such as higher concentrations of sodium alginates (2% w/v), incorporation of filler polymer such as pectin, altered needle size and varied syringe pump flow rate (not considered in the mentioned prior art) (Vallejo- Castillo et al., 2020). As mentioned previously, encapsulation efficiency enhanced for either sodium alginate and $CaCl_2$ concentration or both (Corkovic et al., 2021).

In this regard, it shall be noted that sodium alginate concentration above 3% (w/v) reduced the syringe pump flow rate. Also increasing $CaCl_2$ concentration beyond 9% (w/v) did not significantly enhance the encapsulation efficiency. Also, for this case, beads with a soft surface and an irregular shape were achieved.

In summary, it can be concluded that encapsulation efficiency of the Ca-Al beads could be improved through (a) either enhancing the concentration of sodium alginate or $CaCl_2$ or both, (b) adding a suitable filler such as pectin whose hydroxyl groups interact with the polar parts of polyphenol groups and (c) enhancing the syringe pump flow rate.

Table 7.4: Literature and optimal data summary of the encapsulation efficiency with ion gelation method.

Bioactive component	Sodium alginate concentration (%)	Calcium chloride concentration (%)	Pectin concentration (%)	Needle size (G)	Flow rate (mL/min)	Encapsulation efficiency (%)	Literature
Chlorogenic acid	2	7	1	NA	NA	50	Corkovic et al. (2021)
α-tocopherol	1.5	5	2	25	NA	59.91	Singh et al. (2018)
TP	2	6.61	0.5	22	3.5	86.7	This work
TP	2	6.61	NA	22	3.5	79.14	This work

7.13 Summary

In this work, ion gelation technique has been deployed successfully to encapsulate TP molecules from PL extract in Ca-Al beads loaded with pectin. Through face centered response surface methodology, the influence of interaction between the process variables such as sodium alginate, $CaCl_2$ concentration and flow rate of a syringe pump on the encapsulation efficiency as the response variable have been investigated.

The significant findings of the carried out research can be listed as follows:

- The optimum conditions for maximum encapsulations efficiency of beads loaded with pectin corresponds to 2% w/v sodium alginate concentration, 6.61% w/v $CaCl_2$ concentration and 3.50 mL/min syringe pump flow rate. Under these conditions, the encapsulation efficiency of beads loaded with pectin was 86.7%.
- Moreover, studies confirmed that the beads loaded with pectin indicated higher encapsulation efficiency than the beads without pectin and the effect of flow rate on the response variable was dominant followed by concentrations of $CaCl_2$ and sodium alginate.
- The study also suggested that a low alginate concentration may result in a paucity of carboxyl groups, which are essential to form the beads with calcium ions.
- Further, based on the antioxidant activity data, it can be inferred that the addition of pectin increased AA for beads loaded with pectin in comparison to the achieved beads without pectin.
- FESEM of beads demonstrated that the final characteristics of the beads were enhanced through the addition of pectin, and FTIR spectroscopy demonstrated good interaction between the polyphenols from PL extract and bead matrix.
- Further, the dry beads affirmed the extended storage and stability under refrigerated storage. Also, the in-vitro dissolution of Ca-Al beads in water indicated a long-lasting and diffusion-controlled release of TP molecules.

In summary, it can be concluded that the Ca-Al beads can provide protective effect on the encapsulated TP molecules. Also, these findings suggested that a combination of alginate and pectin could be

employed as a wall material for the encapsulation of polyphenols from PL extract to improve encapsulation efficiency. Also the findings of this study indicate that ionic gelation could be a promising alternative technique for the encapsulation of TP from PL extract.

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Chapter 8:

Conclusions and Future Work

Chapter 8

Conclusions and Future Work

The chapter elaborates upon important subjective and objective inferences being deduced from the experimental investigations carried out in the Ph.D. thesis. These have been outlined in various subsections that devoted to the fulfilment of the set alternative objectives in the Ph.D. thesis. Following this, section 8.2 summarizes the possible scope for future work in the chosen field of research.

8.1 Conclusions

In the Ph.D. thesis, the potential of guava leaves, papaya leaves, papaya pulp, and papaya peel as sources of bioactive compounds has been investigated. The conducted research enabled maximum yield based identification of best source of these compounds. After a careful analysis of each source and its ability to ensure greater bioactive compounds yield in the aqueous extract, the most effective source was identified. Also, the Ph.D. thesis addressed very useful investigations in the field of UAE based pectin extraction from pomelo peel using FCD based RSM and characterization of sodium alginate – pectin beads encapsulation with the papaya leaf extract.

Since the findings in the thesis involved generalized characterization in terms of TPC, TFC and AA etc., the choice among any one of the mentioned plant sources could not be rationally explored. The biological influence of the extract can better serve such a rational perspective. This is beyond the scope of targeted perspectives. Such an objective is time consuming and hence recommended for the future work.

From the findings, it was inferred that the papaya leaf extract constituted maximum amount of bioactive compounds in terms of TPC, TFC and AA. Thus, the thesis involved the realization of the five major objectives and needful engagement towards possibilities for bioactives encapsulated product development. In the following subsections, the most important objective and subjective findings of the

mentioned five major objectives of the Ph.D. thesis have been delineated as the conclusion of the carried research work.

8.1.1 RSM based Ultrasound-Assisted Extraction of Bioactive Constituents from Guava Leaves

a) Objective Conclusions

- For the NUAE process and in the aqueous guava leaf extract, the optimized value for TPC, TFC, % AA and VITC have been obtained as 67.95 mg GAE/g, 264.47 mg QE/g, 77.50 % and 21.56 mg/100g respectively for the optimal process parameters of 62.19 °C ET, 14.94 min ST, and 0.19 g/mL LR.
- For the PUAE process and in the aqueous guava leaf extract, the best values have been achieved for the TPC, TFC, % AA, and VITC as 72.62 mg GAE/g, 288.13 mg QE/g, 86.07 %, and 18.83 mg/100g, respectively. Corresponding optimal process characteristics have been 66.21 °C ET, 14.31 min ST, and 0.18 g/mL LR.
- Incidentally, the best process-product characteristics for guava leaf HWE have been 43.7 mg GAE/g TPC, 184.3 mg QE/g TFC, 57.87 % AA and 8.15 mg/100g VITC for the PUAE based optimal process parameter choice of 62.19 °C ET, 14.94 min ST and 0.19 g/mL SLR.

b) Subjective Conclusions

- Based on its very efficient performance, the PUAE can be deployed as a beneficial and promising low cost environmentally friendly technology for the effective extraction of bioactive constituents from guava leaves.
- The criticality of the reported best findings in this work have been due to the consideration of LR as an additional degree of freedom and shade but not oven drying of fresh guava leaves. Compared to literature reported methodology, process performance has been analyzed to be critically improved with shade drying in comparison with the oven drying of fresh guava leaves.

- Except for the VITC content, the PUAE performed better in comparison with the NUAE and HWE process for the evaluated bioactives namely TPC, TFC and % AA.
- Both NUAE and PUAE processes out performed HWE for the extraction of bioactives from shade dried guava leaves.
- The non-linear effect of the LR has been comparatively higher than the ET and ST in achieving the maximum extraction of the mentioned bioactive component characteristics from shade dried guava leaves.
- Non-linearities of each process parameter can be inferred from the response surface plots to be as per the hierarchy or order of LR > ST > ET for the NUAE/ PUAE based guava leaf aqueous extraction process.
- Non-linearities associated to the binary process parametric interaction can be inferred from the response surface plots and as per the hierarchy or order of LR – ET > LR – ST > ET – ST for the investigated NUAE/PUAE based aqueous guava leaf extraction process.

8.1.2 Comparative Efficacy of Hot Water and Ultrasound-Assisted Extraction of Bioactives from Papaya Leaf System

a) Objective Conclusions

- For the aqueous papaya leaf extract system, HWE, NUAE and PUAE, the PUAE provided the best response and parametric choice of 160.54 mg GAE/g of TPC, 548.32 mg QE /g of TFC, and 89.87 % of AA, 69.92°C ET, 19.92 min ST, and 0.2 g/mL SLR.
- In the NUAE process based papaya leaf extract system, the optimal TPC, TFC and % AA have been obtained as 149.12 mg GAE/g, 533.46 mg QE /g, and 86.71 % respectively for an optimal process parametric choice of 62.84 ºC ET, 19.98 min ST and 0.2 g/mL SLR.
- For a process parametric choice of 70 ºC ET, 20 min ST and 0.2 g/mL LR, the HWE of papaya leaf system affirmed 122.52 mg GAE/g TPC, 305.0 mg QE/g TFC and 85.5 % AA. These values

have been comparatively yet moderately lower than those achieved with the PUAE and NUAE processes.

b) Subjective Conclusions

- Compared to literature findings that involved the deployment of oven dried papaya leaves during aqueous UAE, the thesis inferred that better bioactive extraction with HWE, NUAE and PUAE processes can be achieved with shade dried papaya leaves at 25 ºC and for 24h.
- Compared to the NUAE, the PUAE process affirmed marginal improvement in responses and marginal alterations in process conditions during aqueous UAE of papaya leaf system.
- Also, compared to the HWE, the PUAE process ensured promising performance with moderately higher enhancement of 31.03%, 91.58% and 5.1% enhancement in the TPC, TFC and AA respectively.
- In the both PUAE and NUAE of aqueous papaya leaf extraction, the nonlinear effect of LR has been the dominant factor followed by the sonication temperature and time for the maximum yield of bioactives.

8.1.3 Ultrasound-Assisted Extraction of Bioactives from Papaya Pulp and Papaya Peel

a) Objective Conclusions

- The aqueous NUAE of PPU system witnessed optimal values of TPC, VITC and % AA as 148.82 mg GAE/g, 148.81 mg /100 g and 87.47 % respectively. To do so, optimal process conditions have been 60.33 ℃ ET, 19.82 min ST and 0.40 g/mL LR.
- The aqueous PUAE of PPU system witnessed optimal values of TPC, VITC and % AA as 169.11 mg GAE/g, 162.13 mg mg/100g, and 92.29 % respectively. To do so, optimal process conditions have been 58.59 ℃ ET, 18.82 min ST, and 0.40 g/mL LR.

- The aqueous NUAE of PPE system witnessed optimal values of TPC, TFC and % AA respectively were 138.58 mg GAE/g, 312.20 mg QE/g and 81.10 % for optimum process condition of 66.60 °C ET, 18.53 min ST and 0.40 g/ mL LR.
- The aqueous PUAE of PPE system witnessed optimal values of TPC, TFC, and % AA respectively were 146.40 mg GAE/g, 301.28 mg QE/g, and 87.88% for the optimal process conditions of 70 °C ET, 17.67 min ST, and 0.40 g/mL LR.
- During aqueous HWE of PPU system being conducted at optimal PUAE process condition (58.59 °C ET, 18.82 min ST, and 0.40 g/mL LR), the TPC, VITC and % AA have been obtained as 105 mg GAE/g, 89.4 mg/100 g, and 76.50 % respectively.
- Likewise, during aqueous HWE of PPE system being conducted at optimal PUAE process condition (58.59 °C ET, 18.82 min ST, and 0.40 g/mL LR), the TPC, TFC and % AA respectively have been obtained as 88.5 mg GAE/g, 145 mg QE/g, and 71.5 %.

b) Subjective Conclusions

- With respect to relevant prior art, the thesis affirmed comparative efficacy of shade dried papaya peel and freshly cut papaya pulp but not oven dried pulp and peel system for the best extraction of bioactives during aqueous HWE, NUAE and PUAE.
- Compared to the HWE, both NUAE and PUAE have been efficient green extraction technologies for the aqueous extraction of the bioactives from, PPU and PPE systems.
- For both PPU and PPE systems, the aqueous PUAE process witnessed marginally better performance and marginally altered optimal conditions with respect to those indicated by the aqueous NUAE process.
- During aqueous NUAE and PUAE of PPU and PPE, LR followed by ET and ST have been significant to achieve maximum bioactives extraction in the aqueous media.
- Among PPU and PPE and for both aqueous PUAE AND NUAE, the PPU ensured higher TPC and AA in the aqueous extract with respect to the PPE.

8.1.4 Continuous and Pulsed Ultrasound-Assisted Extraction of Pectin from Pomelo Fruit Peel Using Citric Acid

a) Objective Conclusions

- During PUAE of pectin from pomelo peel with CA, the optimal conditions are 1.70 pH and 16.11min ST for a maximum PY and DE of 40.41% and 58.95 % respectively.
- During NUAE of pectin from pomelo peel with CA, the optimal conditions are 1.72 pH and 22.33 ST for a maximum PY and DE of 33.76 % and 56.44 %, respectively.
- During AHWE process being operated at optimal PUAE process condition, the PY and DE of pectin from CA based pomelo peel are 14. 8 % and 51.5 % respectively.

b) Subjective Conclusions

- Among PUAE, NUAE and AHWE, the best process has been the PUAE in terms of highest PY and DE values and reduced process time.
- Among PUAE, NUAE and AHWE process parameters, the pH has been most significant process parameter to influence the PY and DE of pectin.
- Among PUAE, NUAE and AHWE processes and among many conventional non-organic acids, the CA has a profound influence on PY and DE of pectin.
- With its green and food grade utility, the CA can serve as a better extraction agent and can further reduce pectin purification needs for food grade pectin production in the food industry. Thereby, better utilization of raw or minimally purified pectin can be targeted using NUAE or PUAE.
- FTIR spectrum analyses affirmed similar band pattern for both PUAE and AHWE based pectin samples. However, the PUAE based pectin has been found to have more intense peaks in comparison to the AHWE based pectin sample.
- Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) affirmed comparatively higher thermal stability for the pectin obtained with the PUAE process.

- X-ray diffraction (XRD) analysis further confirmed that the pectin obtained from both PUAE and AHWE processes possessed amorphous structure. The XRD analysis of pectin extracted with PUAE process also affirmed retention of native pectin structure during UAE based pectin extraction.
- The FESEM images revealed that the pectin extracted with the PUAE possessed a smoother and flatter surface in comparison to the AHWE based pectin. Further, pectin macrographs affirmed light brown colored pectin powder sample with the PUAE process.

In summary, it can be concluded that in comparison to AHWE process, the PUAE and NUAE are promising processes with short extraction time, moderate operating temperature and more efficient recovery of pectin and with better properties such as improved thermal stability and smoother structure with finer particle size distribution. Thereby, NUAE or PUAE is recommended for the recovery of pectin from pomelo peel powder for further application as a thickening agent, stabilizer, or as a food additive.

8.1.5 Efficacy of Ionic-Gelation Based Encapsulation of Bioactives from Papaya Leaf Extract

a) Objective Conclusions

- The optimum conditions for maximum encapsulation efficiency of beads loaded with pectin correspond to 2% (w/v) sodium alginate concentration, 6.61% (w/v) calcium chloride concentration and 3.50 mL/min flow rate. For the optimal condition, the encapsulation efficiency of beads loaded with pectin was 85.57%.
- The encapsulation efficiency of wet beads (85.56 ± 0.16 %) has been similar to that of the dry beads (85.06 ± 0.43 %).
- Dried beads (73.78 ± 14 %) affirmed marginally higher antioxidant activity than the value obtained for wet beads (72.64 ± 0.72 %).

- The encapsulated dried Ca-Al pectin beads possessed a higher diameter of 3.66 ± 0.03 µm in comparison to the non-encapsulated and encapsulated PL extract Ca-Al beads loaded without pectin (3.05 ± 0.07µm and 3.15± 0.03 µm respectively).

- The Ca-Al beads loaded with pectin and PL extract possessed lower moisture content (3.10 ± 0.08 %) in comparison to the beads without pectin (3.85 ± 0.12 %) and non-encapsulated Ca-Al beads (4.45 ± 0.18 %).

b) Subjective Conclusions

- The Ca-Al beads loaded with pectin affirmed higher encapsulation efficiency and antioxidant activity with respect to the Ca-Al beads prepared without pectin. Also, the effect of flow rate on the response variable has been dominant followed by solution concentrations of calcium chloride and sodium alginate.

- A low alginate concentration can result in a paucity of carboxyl groups, which is essential for capsule formation.

- Based on the antioxidant activity data, it can be inferred that the pectin addition increased AA for Ca-Al beads loaded with pectin in comparison to the Ca-Al beads prepared without pectin.

- FESEM of beads demonstrated that the morphological features of the Ca-Al beads were enhanced with pectin addition.

- FTIR spectroscopy demonstrated good interaction between the polyphenols from PL extract and Ca-Al bead matrix.

- DSC findings affirmed a higher thermal stability of Ca-Al beads loaded with pectin and PL extract in comparison to the Ca-Al beads loaded with PL extract only.

- The dry Ca-Al beads affirmed the extended storage and stability under refrigerated storage condition.

- The in-vitro dissolution of Ca-Al beads in water indicated a long-lasting and diffusion-controlled release of TP molecules.

In summary, it can be concluded that the Ca-Al beads can provide protective effect on the encapsulated TP molecules. Also, these research findings suggested that a combination of alginate and pectin could be employed as a wall material for encapsulation of polyphenols from PL extract to improve encapsulation efficiency. Dried Ca-Al beads possess promising potential as a useful carrier for TP molecules in pharmaceutical or functional food product development.

8.2 Future Work

Based on the quantum of work addressed in the Ph.D. thesis, the following areas of work have been identified for needful consideration in the near future to further encourage customized subjective research in the vast field of UAE of bioactives and their encapsulation with sodium alginate pectin system for various food and pharmaceutical applications:

- The carried out investigations need to be extended in the near future in the context of further characterization, purification and identification of bioactive constituents from guava leaves, papaya leaves, papaya pulp and papaya peel.
- Additional classification of bioactives using high performance liquid chromatography (HPLC), in-vitro biological activity of the extract obtained under ideal conditions and the utilization of the optimally extracted bioactive aqueous systems in the food, herbal, and pharmaceutical industries to create sustainable functional products can be envisaged in the near future.
- Further, future research also needs to focus on optimization, predictive modelling, and scale-up integrating energy efficacy for the affirming of the commercialization of the NUAE or PUAE processes.

- The combinatorial criticality of LR and alternate solvents needs to be targeted along with shelf life storage and toxicological studies.
- Coupling of UAE with other novel technologies such as microwave extraction for the maximum extraction of bioactives compounds can also be targeted. Also, comparative extraction efficiency of probe sonicator with respect to the bath sonicator can be as well explored.
- The UAE based papaya leaf aqueous extract can be explored for the development and utility of commercial herbal dry supplements. Similar efforts can be extended for other non-conventional leafy system based extracts.
- The NUAE or PUAE process efficacy with respect to LR, sonication frequency and ET as other degrees of freedom can be targeted along with additional characterization of extracted pectin. Also, the purity of extracted pectin can be considered as an additional response variable.
- Future research can target the complexity of calcium-alginate pectin mixtures in achieving higher encapsulation efficiency and controlled release of bioactive extracts. Eventually, the effectiveness of such systems should be assessed for the oral administration of loaded Ca-Al-pectin beads for controlled release of polyphenols through the in-vivo studies. Additionally, the effect of other filler materials such as chitosan, starch etc., on the encapsulation efficiency of sodium alginate system should be explored. Studies such as in-vitro biological activity, swelling index and stability of encapsulated beads at different temperatures and pH can also be targeted.

Publications

List of Publications

Published articles in international refereed journals

[1] **Wani, K. M**., & Uppaluri, R. (2022). Efficacy of ultrasound-assisted extraction of bioactive constituents from *Psidium guajava* leaves. **Applied Food Research, 2(1), 100096**.

[2] **Wani, K. M**., & Uppaluri, R. (2022). Pulsed ultrasound-assisted extraction of bioactive compounds from papaya pulp and papaya peel using response surface methodology: Optimization and comparison with hot water extraction. **Applied Food Research, 2(2), 100178.**

[3] **Wani, K. M**., & Uppaluri, R. (2022). Continuous and pulsed ultrasound-assisted extraction of pectin from pomelo fruit peel using citric acid. **Biomass Conversion and Bio-refinery (2022)**

[4] **Wani, K. M.,** & Uppaluri, R. V. (2022). Continuous and pulsed ultrasound-assisted extraction of pectin from pomelo fruit peel using citric acid. **Biomass Conversion and Biorefinery, 1-16.**

[5] **Wani, K. M**., & Uppaluri, R. V. (2023). Recovery of Bioactive Constituents from Unripe Papaya Peel and Pulp Using Ultrasound Assisted Extraction: Optimization of Process Parameters. **In Agro and Food Processing Technologies: Proceedings of NERC 2022 (pp. 119-139). Singapore: Springer Nature Singapore.**

Under Decision

[1] **Wani, K. M.,** & Uppaluri, R. (2022). Comparative efficacy of ultrasound assisted and hot water extraction of papaya leaf system. **Journal of Herbal Medicine.**

Articles to be submitted

[1] **Wani, K. M.,** & Uppaluri, R. (2022). Characterization of pectin extracted from pomelo peel using UAE and AHWE process.

Conference Presentations (National and International)

[1] Wani, K. M., & Uppaluri, R. V. (2023). Recovery of Bioactive Constituents from Unripe Papaya Peel and Pulp Using Ultrasound Assisted Extraction: Optimization of Process Parameters. In Agro and Food Processing Technologies: Proceedings of NERC 2022 (pp. 119-139). Singapore: Springer Nature Singapore.

Appendix

Appendix A: Calibration Curve for the Determination of Total Polyphenolic Content (TPC)

Tharasena & Lawan. (2014) method was deployed to determine the TPC of the samples (guava leaves, papaya leaves, papaya pulp and peel and pectin) used in this Ph.D. thesis work. To do so, 1 mL of FCR was mixed with 1 mL of aqueous extracts and the mixture was vortexed. After 5 minutes, 10 mL of Na_2CO_3 (7% w/v) was added to the aforementioned solution, and the system was set aside for 90 mins. The sample's absorbance was measured at 750 nm using a UV spectrophotometer (UV-2600, Shimadzu, Singapore). Using a calibration curve generated with standard gallic acid, the data was finally represented in gallic acid equivalents (GAE, mg gallic acid/g of sample). The obtained calibration curve is depicted in **Fig. A1**.

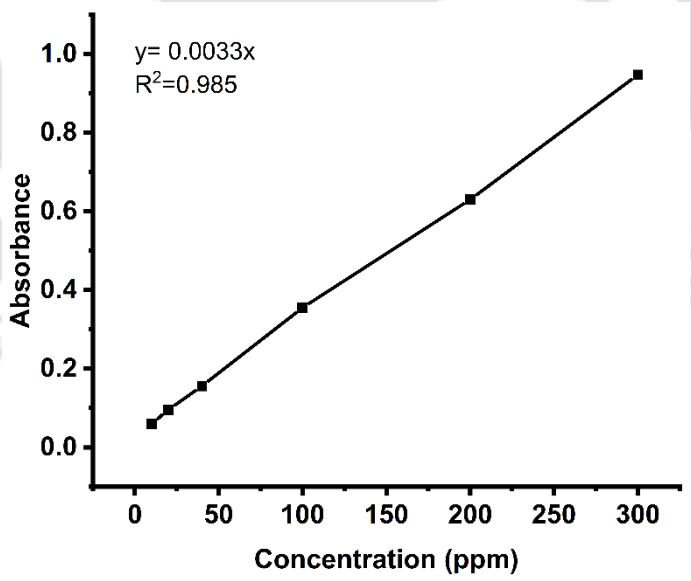


Figure. A1: Calibration curve for the determination of total polyphenolic content.

Appendix C: Calibration Curve for The Determination of Soluble Protein Content

Bradford (1990) method was deployed to determine the soluble protein content of the pectin extracted from the pomelo fruit peel. The methodology involves calibration curve preparation through the dilution of standard bovine serum albumin solution (BSA) (100 ppm). Thereafter, the diluted solutions with 20, 40, 60, 80 and 100 ppm solutions were prepared. Subsequently, 2 mL of Bradford reagent was added to each of these solutions and after thorough mixing, their absorbance was measured a UV-visible spectrophotometer (Model No.: UV-2600, Make: Shimadzu, Singapore). Thereby, the absorbance versus concentration plot was prepared as a calibration curve to express the soluble protein content of analyzed sample as an equivalent of the BSA. The obtained calibration curve is depicted in **Fig. C1**.

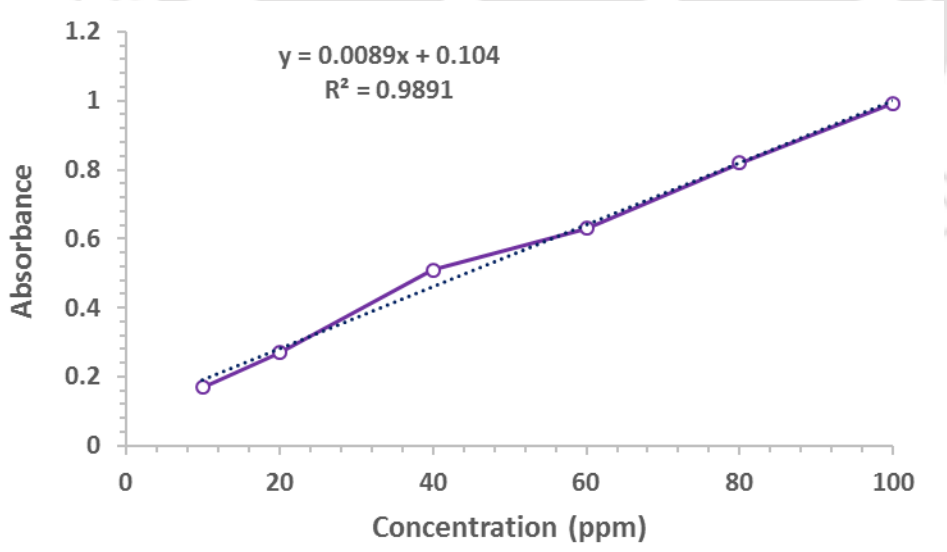


Figure. C1: Calibration curve for the determination of soluble protein.

Appendix B: Calibration Curve for the Determination of Total Flavonoids Content (TFC)

The TFC content the samples (guava leaves, papaya leaves, papaya pulp and peel and pectin) deployed in this Ph.D. thesis work was determined using the $AlCl_3$ method (Tharasena & Lawan, 2014). Briefly, 1 mL of filtered extracts were diluted with 4 mL DI water and was vortexed. After that, 0.3 mL of a 5% (w/v) $NaNO_2$ solution was added, and the mixture was vortexed for another 5 minutes. Thereafter, 0.3 mL of 10% (w/v) $AlCl_3$ was added to the mixture, which was vortexed for 6 minutes. Thereafter, 2 mL of 1M NaOH was added to the solution and vortexed. The resulting solution, which had a volume of 10 mL, was stored for 30 minutes at ambient temperature in a dark environment. The absorbance of the mixture was then measured at 517 nm. The TFC of the sample was calculated in terms of mg of Quercetin equivalents (QE) /g fresh weight of sample (mg/g sample) using a calibration curve prepared with Quercetin. The obtained calibration curve is depicted in **Fig. B1**.

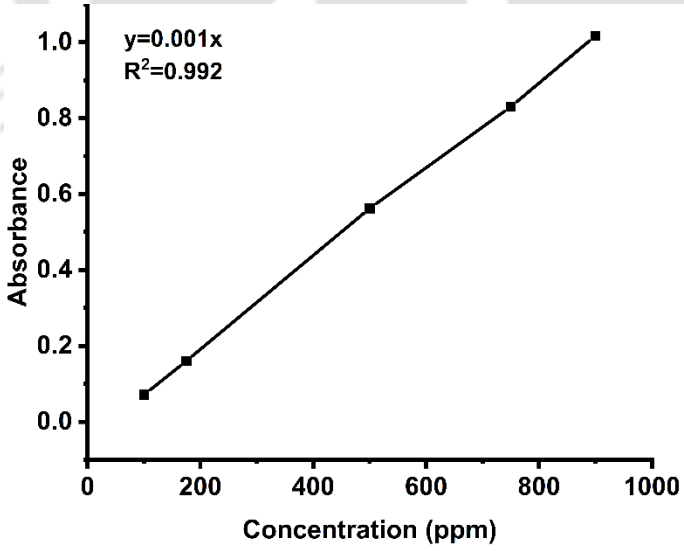


Figure. B1: Calibration curve for the determination of total flavonoid content.

Appendix D: Images of Alternate Samples Studied in the Ph.D. Thesis

(a)

Guava Leaves

Papaya Leaves

Papaya Fruit

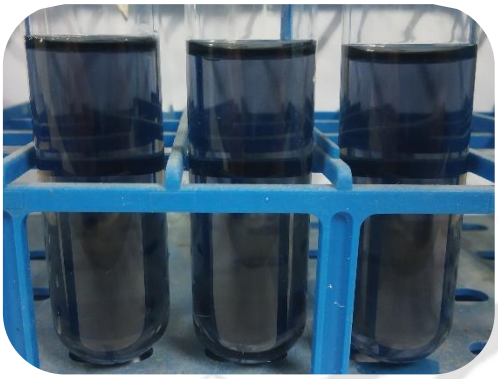
Papaya Peel

Papaya Pulp

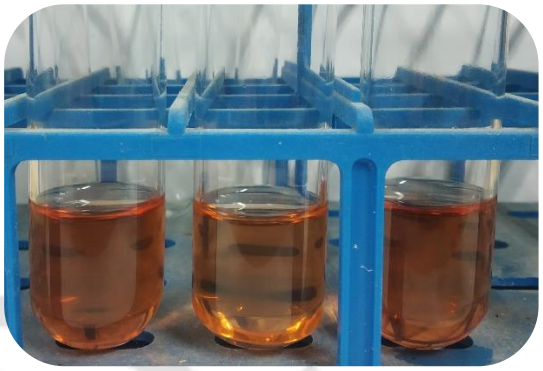
Pomelo Fruit

Pomelo Peel (Internal Portion)

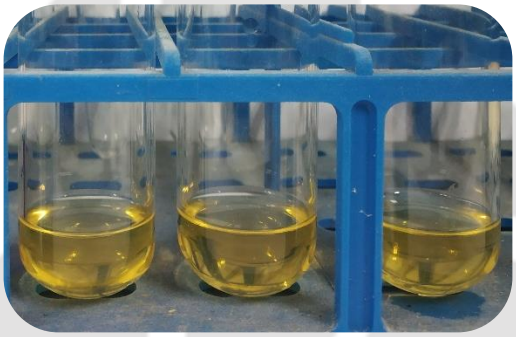
(b)



TPC Assay

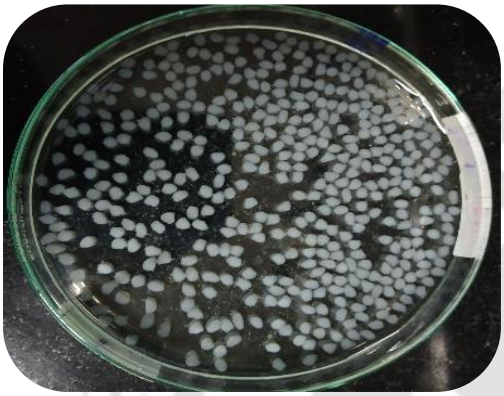


TFC Assay

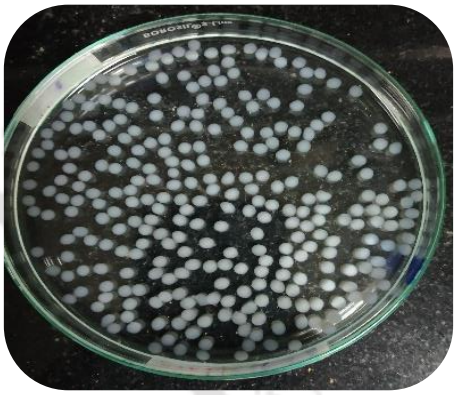


AA Assay

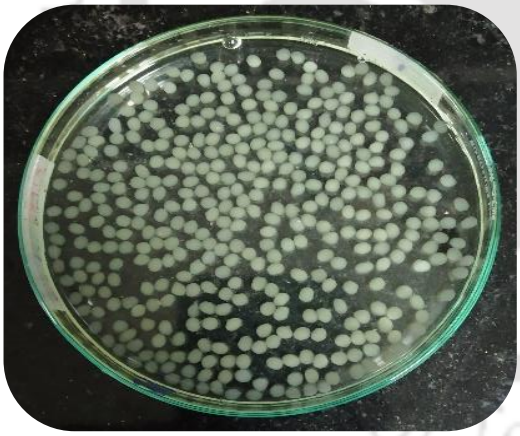
(c)



Encapsulated Ca-Al Beads



Non Encapsulated Ca-Al Beads with Pectin



Encapsulated Ca-Al Beads with Pectin

Drained Encapsulated Ca-Al Beads with Pectin

Wet Extracted Pectin

Dried Pectin Powder