

***DEDICATED***

***TO***

***PAPA AND MAA***

*For endless love, support, and encouragement*

## DECLARATION

I hereby declare that the thesis entitled “Development and evaluation of an optimized passion fruit based-beverage enriched with Pickering nanoemulsion of fibre and oil extracted from its byproducts” submitted to the School of Engineering, Tezpur University in partial fulfilment of the requirements for the award of the Doctor of Philosophy in Department of Food Engineering and Technology is a record of original research work carried out by me. Any text, figures, theories, results or designs that are not of my own devising are appropriately referenced in order to give credit to the original author(s). All the sources of assistance have been assigned due acknowledgement. I also declare that neither this work as a whole nor a part of it has been submitted to any other university or institute for any degree, diploma, associateship, fellowship or any other similar title or recognition.

Place : Tezpur University

Date : 09-11-2023



(Hemanta Chutia)

Registration No. TZ203788 of 2021



**DEPARTMENT OF FOOD ENGINEERING & TECHNOLOGY  
TEZPUR UNIVERSITY**

(A Central University established by an Act of Parliament)

**Napaam, Tezpur-784028, Assam**

Dr. Charu Lata Mahanta  
Professor

Email: [charu@tezu.ernet.in](mailto:charu@tezu.ernet.in)  
Tel: +91-03712-275702

---

**CERTIFICATE OF THE SUPERVISOR**

This is to certify that the report entitled “Development and evaluation of an optimized passion fruit based-beverage enriched with Pickering nanoemulsion of fibre and oil extracted from its byproducts” submitted to the School of Engineering, Tezpur University in partial fulfilment of the requirements for the award of the Doctor of Philosophy in Department of Food Engineering and Technology, is a record of original research work carried out by Mr. Hemanta Chutia under my supervision and guidance.

All help received by him from various sources have been duly acknowledged.

No part of the thesis has been submitted elsewhere for the award of any other degree.

Place: Tezpur University

Date: 09-11-2023

(Prof. C. L. Mahanta)

## ACKNOWLEDGEMENT

---

*The journey towards earning a Ph.D. has been learning experience and it has been crucial step in achieving my life dreams and goals. I will take opportunity to thank all those who provided me support, assistance and guidance.*

*First and foremost, I would like to sincerely thanks to my respected supervisor, Prof. Charu Lata Mahanta for all the help, time, and academic guidance throughout this PhD. Her support has truly been crucial, from the start of this research work up to the submission of my thesis. Thank you is a very small word to describe her contributions towards developing in me the necessary attitude for research and teaching. Her dedication towards her wok has always motivated me and I hopeful that I would carry forward the knowledge and dedication for future.*

*Another important person I would like to extended my gratitude is Dr. Suvendu Bhattacharya, Former scientist at Central Food Technological Research Institute, Mysore for his kind support. I admire his way of taking up explaining things which has helped me a lot in my research work and future endeavours. I also want to thank Prof. Munundra Kakoti, former Vice chancellor Assam Rajiv Gandhi University of Cooperative Management, Sivasagar and Prof. Keshavan Niranjana, University of Reading, United Kingdom for their constant support.*

*I would like to acknowledge the technical and institutional support from Tezpur University, Assam. I would also like to thank all members of my Doctoral Committee; and faculty members and staff in the Department of Food Engineering and Technology, Tezpur University: Prof. Sankar Chandra Deka, Prof. Manuj Kumar Hazarika, Prof. Brijesh Srivastava, Prof. Nandan Sit, Prof. Laxmikant Shivnath Badwaik, Prof. Poonam Mishra, Dr. Dipankar Kalita, Dr. Arup Jyoti Das, Mr. Labadeep Kalita, Mr. Bhaskar Kalita and all the non-technical staff for supporting me throughout my research and making my time in the laboratories enjoyable.*

*Also special thanks to Prof. Debendra Chandra Baruah (Faculty Energy, TU), Prof. Pritam Deb (Physics, TU) and Dr. Rupak Mukhopadhyay (MBBT, TU), Prof. Manabendra Mandal (MBBT, TU) for their constant support and facilities. I would also like to thank Dibrugarh University particularly Chemical Science and Pharmaceutical Science department for valuable support during my Ph.D.*

*My sincere thanks to the Department of Science and Technology, Government of India for financial assistance (DST-INSPIRE Fellowship) for my work. And also, Commonwealth Commission for the selection.*

*I would like to express my gratitude to my seniors and friends: Dr. Himjyoti Dutta, Dr. Nikhil Kumar Mahnot, Dr. Pallab Kumar Borah, Dr. Mainao Alina Gayary, Manas Jyoti Das, Manoj Sharma (MBBT), Mayuri Bora (Physics), Munmi ba (MBBT), Subham, Fogila, Beatrice, Awanish, Diti, Madhusmita and all my well wishers for their great support, best efforts and listening ears.*

*And of course, none of this would have been possible without my family members: Mr. Puneswar Chutia (father), Mrs. Bulumoni Bhuyan Chutia (mother), Mr. Mintu Chutia (brother), Mr. Jintu Chutia (brother), who gave me the courage to do a Ph.D. as well as provided countless suggestions, guidance, moral support, and patience during this critical time.*

*Lastly, I am extremely thankful towards God, the Almighty, who helped me become a stronger and better man throughout the years.*

## LIST OF TABLES

Table No	Caption	Page
Table 1.1	Different parts of yellow passion and its composition	3
Table 2.1.	Nutritional characterization (mean values) of passion fruit and its co-products	18
Table 3.1	Real and coded values of the variables of the UAE and MAE processes	57
Table 3.2	ANOVA table for UAE of carotenoids using oils as solvent	68
Table 3.3	Optimization table for carotenoids extraction by UAE using oils as solvent	69
Table 3.4	ANOVA table for MAE of carotenoids using OO as solvent	71
Table 3.5	Quality parameters of UOO and CEOO	75
Table 3.6	Pseudo-second-order model kinetics parameters for the UAE and CE of carotenoids from PFP at different extraction temperatures	77
Table 3.7	Parameters of phenomenological model for extraction of carotenoids	79
Table 3.8	Parameters of pseudo-first order model and model based on instantaneous washing followed by diffusion for extraction of carotenoids from PFP.	80
Table 3.9	Effective diffusion coefficient, mass transfer coefficient, and mass transfer at different temperatures during UAE and CE of carotenoids from PFP	82
Table 3.10	Thermodynamic parameters for UAE and CE of total carotenoids content from PFP.	84
Table 4.1	Flowability classification of powders based on several indices	96
Table 4.2	Real and coded values of the independent parameters for development of StNPs	101
Table 4.3	$g(\vartheta)$ and $f(\vartheta)$ Algebraic expressions functions for Kinetics modelling mechanism of the Coats–Redfern (modified)	103
Table 4.4	Colour value of StNPs	106
Table 4.5	Flow properties of StNPs	106

Table 4.6	Linear fit of plots for kinetics modelling of Coast–Redfern (modified)	117
Table 4.7	Estimation of the kinetic and thermodynamic parameters of StNPs	118
Table 4.8	Hydroxyl groups and physiosorbed water determination on the surface of starches	122
Table 4.9	ANOVA table of nanoemulsion preparation process	126
Table 4.10	Optimization conditions of carotenoid-enriched nanoemulsion	126
Table 4.11	Biochemical properties of carotenoids enriched starch nanoemulsion	127
Table 4.12	Oxidative stability of nanoemulsion	129
Table 5A.1	Proximate analysis of passion fruit seeds	147
Table 5A.2	Phenomenological model parameters for extraction of oil from passion fruit seeds	153
Table 5A.3	Parameters of pseudo-first order model and model based on instantaneous washing followed by diffusion for extraction of oil from passion fruit seeds	153
Table 5A.4	Physicochemical properties of passion fruit seed oil	154
Table 5A.5	Fatty acid composition of UASEO	156
Table 5B.1	Real and coded values of the variables	167
Table 5B.2	Physicochemical composition of the raw and defatted passion fruit seed	168
Table 5B.3	ANOVA table for yield of DFs extraction using ultrasonication	170
Table 5B.4	FTIR transmittance peaks of the constituents of passion fruit seed fibres	173
Table 5B.5	Relative crystallinity and physicochemical and functional properties of DFs	175
Table 6A.1	Physicochemical properties of SPFJ.	194
Table 6A.2.	Relative influence of the independent variables on the responses	199

---

Table 6A.3	Response variables at optimized condition using ANN/GA model	199
Table 6A.4	The parameters of the Baranyi equation for total microorganisms (TPC)	201
Table 6A.5	Parameters and statistical analysis of the square root type model for the effect of temperature on the growth rate of TPC	202
Table 6A.6	Shelf-life determination using mathematical equation and sensory analysis	202
Table 6A.7	The activation energy ( $E_a$ ) at reference temperature and $Q_{10}$	203
Table 6B.1	Sum of sensory scores for the quality attributes of blended beverage samples	216
Table 6B.2	Preferences to the importance of quality attributes of blended beverage	219
Table 6B.3	Similarity values for blended beverage samples	223
Table 6C.1	Real and coded values of the independent parameters for the development of fibre-based Pickering emulsion	227
Table 6C.2	ANOVA table of fibre-based Pickering nanoemulsion optimization	230
Table 6C.3	Optimization table of fibre-based Pickering nanoemulsion	233
Table 6D.1	Chemical composition of artificial saliva	242
Table 6D.2	Changes in quality parameters after the treatments	245
Table 6D.3	Changes in particle size and bio-accessibility after the treatments	260

---

## LISTS OF FIGURES

Figure No	Caption	Page
Fig. 2.1	Images of passion fruits (yellow) of (a) Flower, (b) Tree with flower, (c) Whole passion fruit, (d) Inside passion fruit (cut), (e) Different parts of passion fruit	16
Fig. 2.2	A schematic image of size reduction by ultrasonication (probe)	24
Fig. 2.3	A schematic image of size reduction by high pressure homogenization (Two stage)	25
Fig. 2.4	A schematic image of nanoemulsion development using biomaterials (Fibre) as emulsifier/stabilizer	28
Fig. 3.1	Response surface plots showing the effects of parameters on extraction of carotenoids in UAE process using OO as solvent. Parameters in (a) Time and temperature, (b) Time and solid to oil ratio, (c) Temperature and solid to oil ratio, and (d) Deviation from reference point	66
Fig. 3.2	Response surface plots representing the effects of input parameters on extraction of carotenoids in UAE process using sunflower oil as solvent. Parameters in (A) Time and Temperature, (B) Time and Solid to oil ratio, (C) Temperature and Solid to oil ratio, and (D) Deviation from reference point	67
Fig. 3.3	Response surface graphs showing the effects of extraction of carotenoids of MAE treatment, using olive oil as solvent by the parameters (a) microwave power and treatment time, (b) microwave power and solid to oil ratio, (c) treatment time and solid to oil ratio, and (d) Deviation from reference point.	70
Fig. 3.4	Pseudo second order kinetics and experimental graphs of extraction of carotenoids by (a) UAE, and (b) CE extraction.	77
Fig. 3.5	Phenomenological kinetics of carotenoids extraction from PFP by SO at different temperatures: (A) UAE, (B) CE.	78
Fig. 4.1	(a) Effect of ultasonication treatment time on particle size and PDI value; Particle size distribution of: (b) NS, (c) US-StNP, and (d) HP-US-StNP	104



Fig. 4.2	Powder of (a) NS, (b) US-StNP, (c) HP-US-StNP, (d) C-HP-US-StNP	105
Fig. 4.3	(a) Swelling power of StNPs, (b) Solubility (%) of StNPs	107
Fig. 4.4	(a) FTIR absorbance spectra of starches samples; (b) Relation between 1047 and 1022 $\text{cm}^{-1}$ bands, (c) Relation between 1022 and 995 $\text{cm}^{-1}$ bands.	109
Fig. 4.5	X-ray powder diffraction curve of different starches	110
Fig. 4.6	Morphological structure of different starches: (a) NS (150X), (b) NS (500X), (c) US-StNP starch (150X), (d) US-StNP (500X), (e) HP-US-StNP (150X), (f) HP-US-StNP (500X), (g) C-HP-US-StNP (500X) and (h) Carotenoids starch (double oil amount) (500X).	111
Fig. 4.7	Static rheological properties of 1 % and 5 % starch sample suspensions	112
Fig. 4.8	DSC thermograms of starch particles	114
Fig. 4.9	Possible thermal degradation pathway of starch nanoparticles	115
Fig. 4.10	TGA thermograms of starch particles	116
Fig. 4.11	Plot $\ln\left(\frac{g(\alpha)}{T-2}\right)$ vs $\frac{1}{T}$ of (a) NS, (b) US-StNP, (c) HP-US-StNP, and (d) C-HP-	120
Fig. 4.12	Response surface plots showing the effects of parameters on particle size in nano emulsion preparation process. Parameters in (a) 2D contour plot between pressure and oil, (b) 3D contour plot between pressure and oil, (c) 2D contour plot between pressure and surfactant, (d) 2D contour plot between pressure and surfactant, (e) 2D contour plot between pressure and oil, (f) 3D contour plot between pressure and oil, (g) Deviation from reference point, and (h) Predicted vs. actual data	124 - 125
Fig. 4.13	Starch nanoparticles after 24 h storage at room temperature, (a) Native starch, (b) HPH starch nanoparticles, (c) Nanoemulsion without HPH, (d) HPH treated nanoemulsion.	127
Fig. 4.14	Emulsion stability in (A) Fresh emulsion, (B) after heat treatment, and (C) after freeze-thaw treatment.	128
Fig. 4.15	Protective effect of nanoemulsion on antioxidant activity	129

Fig. 4.16	Storage stability of emulsion after 35 days: (a) Storage at 25 °C with HPH treatment, (b) Storage at 25 °C without HPH treatment, (c) Storage at 6 °C with HPH treatment, and (d) Storage at 6 °C without HPH treatment	130
Fig. 4.17	Stability of nanoemulsions stored at 6 and 25 °C.	130
Fig. 5A.1	Effect of extraction parameters on extraction yield of passion fruit seed oil: (a) Solid-solvent ratio, (b) Temperature, and (c) Time	149
Fig. 5A.2	UAE, Soxhlet and UAES of oil: (a) Extraction oil yield, (b) Phenomenological modeling kinetics, (c) Predicted and Actual value of yield oil	152
Fig. 5B.1	Extraction yield of dietary fibre from defatted passion fruit seed as effect ed by (a) Time and power; (b) Time and solid-liquid; (c) Solid-liquid ratio and power ratio and (d) Perturbation (deviation from reference point).	169
Fig. 5B.2	SEM analysis of (a) ADF(500X), (b) ADF(1500X), (c) AUDF(500X), (d) AUDF(1500X), (e) UDF(500X) and (f) UDF(1500X) sample.	172
Fig. 5B.3	FTIR analysis of ADF, AUDF, and UDF	173
Fig. 5B.4	XRD graphs of ADF, AUDF and UDF	174
Fig. 5B.5	TGA analysis of ADF, AUDF and UDF	176
Fig. 5B.6	Cytotoxicity assay (MTT) of ADF, AUDF, and UDF	179
Fig. 6A.1	ANN structure of the TS treated SPFJ	192
Fig. 6A.2	Effect of temperature and time on: (a) Total plate count (3D), (b) Total plate count (3D scatter), (c) Phenolic content, (3D), (d) Phenolic content (3D scatter), (e) $\beta$ -carotene (3D), and (f) $\beta$ -carotene (3D scatter).	196
Fig. 6A.3	R value between Target and Output values of the selected ANN structure for Training set, Validation set, Testing set and Overall performance	198
Fig. 6A.4	The variation of total microorganism population during storage as a function of storage temperature	201

Fig. 6A.5	Changes in the quality parameters of SPFJ and OPFJUS during refrigerated storage: (a) TSS, (b) pH, (c) Total titratable acidity, (d) Ascorbic acid, (e) Phenolic content, (f) DPPH free radical scavenging activity, (g) $\beta$ -carotene and (h) Radar graph for sensory analysis.	204 - 205
Fig. 6B.1	Representation of triangular membership function distribution pattern of the sensory scale	214
Fig. 6B.2	Standard Fuzzy Scale Used For Ranking Of The Blended Beverage Samples	215
Fig. 6B.3	Graphical view of overall sensory score as triangle ABC and triplet ABC	215
Fig. 6C.1	. Effect of (a) Oil and pressure, (b) Fibre and pressure, (c) Fibre and oil, on particles size and (d) Actual vs predicted.	229
Fig. 6D.1	HPLC analysis of phenolics (a) SPFJU, (b) SPFJT, (c) BSPFJU, (d) BSPFJT, (e) BSPFJTT and (f) BNESPFJT	250
Fig. 6D.2	HPLC analysis of carotenoids: (a) SPFJU, (b) SPFJT, (c) BSPFJU, (d) BSPFJT, (e) BSPFJTT, and (f) BNESPFJT	252
Fig. 6D.3	Images of juice after 1 h of storage at room temperature:(a) SPFJU, (b) BSPFJU, (c) BSPFJT, (d) BNESPFJT, and (e) BSPFJTT.	256
Fig. 6D.4	Sensory radar chart of SPFJU, SPFJT, BSPFJU, BSPFJT, BSPFJTT and BNESPFJT	262
Fig. 6D.5	Cytotoxicity MTTs assay of SPFJU, BSPFJU, BSPFJT, BSPFJTT and BNESPFJT	264
Fig. 6D.6	Storage study of BSPFJT, BSPFJTT and BNESPFJT storage at 5 and 25 °C of (a) pH, (b) TTA, (c) Total sugar, (d) TSS, (e) Ascorbic acid, (f) Total phenolic, (g) Total carotenoids , (h) Antioxidant activity (DPPH %) , (i) Antioxidant activity (ABTS %), (j) Color L*, (k) Color a*, (l) Color c*, (m) Cloud stability, (n) Microbial load (TPC).	267 - 268

## LIST OF ABBREVIATIONS

Abbreviations	Full form
$CC_r$	Ratio of extracted content that did not diffuse out
$\rho_{\text{aerated}}$	Aerated bulk density
$\rho_{\text{tapped}}$	Tapped bulk density
AA	Ascorbic acid
AAI	Antioxidant activity index
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ADF	Alkaline extracted dietary fibre
ANN	Artificial neural network
ANOVA	Analysis of variance
atm	Atmosphere
AUDF	Combination of alkaline-ultrasonication extracted dietary fibre
AV	Acid value
BBD	Box–Behnken design
BET	Braunauer-Emmett-Teller
BNESPFJT	Passion fruit seed fibre and oil based nanoemulsion added passion fruit based HPH treated blended beverage
BSPFJT	Sensory optimized passion fruit based HPH treated blended beverage
BSPFJTT	Sensory optimized passion fruit based heat treated blended beverage
BSPFJU	Sensory optimized passion fruit based untreated blended beverage
CAC	Codex Alimentarius Commission
CDV	Conjugated diene value
CE	Conventional extraction
CEOO	Carotenoids enriched olive oil
C-HP-US-StNP	Carotenoids Ultrasonicated followed by high pressure homogenized starch nanoparticles
d.w.	Dry weight
DF	Dietary fibre
DLS	Dynamic light scattering
DPPH	2,2-diphenyl-1-picrylhydrazyl
DSC	Differential scanning calorimetry
EC	Emulsion capacity

---

ESI	Emulsion stability Index
FAO	Food and Agricultural Organization
FCCD	Face-centred central composite design
FCR	Folin–Ciocalteu Reagents
FF	Free-flowing
FFF	Fairly free flowing
FTIR	Fourier transform-infrared spectroscopy
f.w.	Fresh weight
GA	Genetic algorithm
GAC	Glucose-adsorption capacity
GAE	Gallic acid equivalents
GAE	Gallic acid equivalents
GRAS	Generally recognised as safe
HPH	High pressure homogenization
HPLC	High performance liquid chromatography
HP-US-StNP	Ultrasonicated followed by high pressure homogenized starch nanoparticles
HR	Hausner ratio
IC50	50% effective radical inhibition concentration
IDF	Insoluble dietary fibre
IDF	Insoluble dietary fibre
LSD	Least significant difference
MAE	Microwave-assisted extraction
min	Minute
NS	Native starch
OD	Over all desirability
OHC	Oil holding capacity
OO	Olive oil
OPFJUS	Optimized thermosonicated treated sugar added passion fruit juice
PDI	Polydispersity index
PFJ	Passion fruit juice
PFP	Passion fruit peel
PFS	Passion fruit seed

---

---

PFSDF	Passion fruit seed dietary fibre
PFSO	Passion fruit seed oil
PV	Peroxide value
p-value	Probability value
RSM	Response surface methodology
RSM	Response Surface Methodology
S/L	Solid-to-liquid ratio
S/L	Solid to liquid
SDF	Soluble dietary fibre
SDF	Soluble dietary fibre
SEM	Scanning electron microscope
SGF	Simulated gastric fluid
SO	Sunflower oil
SPFJ	Sensory optimized passion fruit juice
SPFJT	Sensory optimized sugar added HPH treated passion fruit juice
SPFJU	Sensory optimized sugar added untreated passion fruit juice
SPSS	Statistical Package for the Social Sciences
SSF	Simulated saliva fluid
TCC	Total carotenoids content
TEAC	Trolox Equivalent Antioxidant Capacity
TGA	Thermogravimetric analysis
TPC	Total plate count
TS	Thermosonication
TTA	Total titrable acidity
UAE	Ultrasonic assisted extraction
UDF	Ultrasonication extracted dietary fibre
UOO	Untreated olive oil
US	Ultrasonication
US-StNP	Ultrasonicated starch nano-particles
UV	Ultraviolet
Vis	Visible
WHC	Water Holding Capacity
XRD	X-ray diffraction

---

## LIST OF SYMBOLS

Symbols	Full form
$C_{sa}$	Carotenoids concentration at saturation conditions
$h_0$	Initial extraction rate
$k_s$	Extraction rate constant of the Pseudo second-order model.
$B_i$	Biot number
$CC_r$	Ratio of extracted content that did not diffuse out
$C_p$	Specific heat
$D_e$	Effective diffusion coefficient
$D_p$	Particles Size
$E_{1\text{ cm}}^{1\%}$	Extinction coefficient
$E_a$	Activation energy
$E_v$	Energy density
$K_{eq}$	Equilibrium constant
$P_m$	Power per unit mass
$R^2$	Coefficient of determination
$k_0$	Initial extraction rate constant
$k_1$	Washing rate constant
$k_2$	Unhindered diffusion constant
$k_3$	Hindered diffusion constant
$k_{mt}$	Coefficient for mass transfer
$k_s$	Pseudo second order extraction rate constant
$^{\circ}\text{K}$	Kelvin
$\mu\text{g}$	Micro gram ( $10^{-6}\text{kg}$ )
$^{\circ}\text{C}$	Degree Celsius
CV	Coefficient of variance
D	Dilution factor
f	Fraction of extractable materials located at external surfaces,
g	Grams
h	Hour
Kg	Kilogram
KOH	Potassium hydroxide

---

L	Litre
m	Mass
mg	Milligram
min	Minutes
mL	Millilitre
P	Power
R	Gas constant
r	Radius of particle
RMSE	Root mean squared error
S	Solid
sec	Seconds
SSE	Sum square error
SSR	Sum square of regressions
SSTO	Total sum square errors
t	Time
T	Temperature
V	Volume
W	Watt
$\Delta G$	Gibbs free energy
$\Delta H$	Change in enthalpy
$\Delta S$	Entropy change
$\alpha$	Repose angle

---