SUPERCRITICAL FLUID EXTRACTION AND ULTRASOUND ASSISTED EXTRACTION OF PHYTOCHEMICALS FROM UNDERUTILIZED BHIMKOL (*MUSA BALBISIANA*) BANANA BLOSSOM, ITS ANTIDIABETIC PROPERTY AND APPLICATION

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By

SANGITA MUCHAHARY

Registration No.: TZ189881 of 2018

DEPARTMENT OF FOOD ENGINEERING AND TECHNOLOGY

TEZPUR UNIVERSITY,

NAPAAM- 784028, ASSAM, INDIA

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Chapter 8

Conclusions

8.1 Conclusions

In the present study, the investigation on underutilized agricultural by-product bhimkol blossom was conducted with six clearly focused objectives. It primarily focuses on the development of phytochemical enriched ready to cook soup mix from bhimkol blossom with antidiabetic property. Extraction of major phytochemicals by using different extraction techniques from bhimkol blossom and optimization by using heuristic functions and their antioxidant properties were performed. The investigations of nutritional components, biochemical, phytochemicals, and volatile phytochemicals were conducted along with study of its antioxidant and antibacterial properties and then nutrition-rich nacho from bhimkol blossom was developed. Major phytochemical extracted were isolated, characterized and then microencapsulated to target its bioactivities in intestinal cells. Microbeads obtained by microencapsulation of major phytochemicals were incorporated in ready to cook soup mix developed from bhimkol blossom powder after conducting an *in vivo* acute toxicity study. Sensory analyses were conducted to investigate the customer satisfaction and market sustainability of the phytochemical enriched ready to cook soup mix from bhimkol blossom. Finally, thorough *in vitro* and *in vivo* investigations of antidiabetic property of bhimkol blossom extract were performed on Wister rats.

Additionally, during experimental optimization in phytochemical extraction, nacho development, and encapsulation, various models and heuristic functions were used such as, RSM-CCD, ANN-PSO, ANN-ACO, respectively to obtain a best set of experimental parameter and output from the set of problem.

The salient findings of the thesis are summarized below:

- Bhimkol banana blossom (BB) is found to be highly rich in nutrition and phytochemicals.
- Phytochemical extraction by SCFE and UAE was found to be the more efficient method compared to the conventional extraction methods and evinced better recovery of TPC and antioxidant activity.

- Moreover, UAE was the more feasible and convenient with minimal operation cost and extraction time.

- Therefore, UAE can be adopted for large scale of phytochemical extraction for less mid heat sensitive samples, whereas SCFE can be used for the more sophisticated extraction and for the heat sensitive samples.

- Significant phytochemical compounds were detected in each optimized extracts of SCFE and UAE and better extraction of it in the former than the latter.

- Optimized condition for SCFE was obtained at 60^{o}C, 210 bar, 40 min, and 5 g/min CO_2 flow-rate in both dependent responses (TPC and antioxidant activity).

- Optimized condition for UAE was obtained at 60^{o}C temperature, 35% amplitude, and 20 min of extraction time in both dependent responses (TPC and antioxidant activity)

- Highest TPC (2750.37 mg GAE/100g) was obtained in WB by the SCFE method vis-à-vis the antioxidant activity (79.41%).

- The phytochemicals present in the bhimkol blossom are the main factors for antioxidant activities and they may be responsible for many other medicinal properties.

- The content of TPC, flavonoids, tannin, phytate, alkaloids, syringic acid, tannic acid, ferulic acid, caffeic acid, rutin, quercetin, gallic acid, coumarin of bhimkol blossom further accentuate the age old belief of medicinal compounds present in it.

- Nutritional content in bhimkol blossom have the potential to supply day-to-day human healthy nutrition diet.

- Rich dietary fiber content of BB indicates to beneficial in gastrointestinal absorption, stool bulking, also helps in gastric emptying duration, and delays nutrients absorption.

- Bhimkol blossom exhibited high antioxidant property, which have tendency to treat cell damage, reduce inflammations, lipid oxidation reactions, etc.

- It also showed some antibacterial properties over some food borne pathogens, that will help in extend of help life of the food product and may contribute as defense mechanism against pathogenic bacteria in human body.

- Nachos prepared from BB with good overall acceptability from the sensory evaluation were obtained after optimizing the formulation of processing parameters using an effective metaheuristic approach (OMD-ANN-PSO).

- After the effective training of the network, optimized BB nachos were obtained from ANN-PSO with less RMSE, MAPE, and MAE.

- The modeling of nachos by particle swarm optimization resulted optimum set of parameters corn starch, wheat flour, bhimkol blossom, refined oil, and black pepper at 0.13, 0.39, 0.33, 0.66, and 0.26 g, respectively.

- Overall acceptability was positively affected by increased wheat flour exhibiting high fracturability and crispiness.

- A satisfactory reading of sensory quality was obtained for BB nachos to control and commercial nachos.

- The nutrition-rich nachos have immense potential for commercialization on a small to large profitable industrial scale.

- Among the phytochemicals detected in bhimkol blossom, quercetin was found to be major content, which have tremendous health beneficial properties according the literature.
- Therefore, quercetin isolation and purification from bhimkol blossom successfully for various applications.
- Isolated quercetin rich fraction (BBQ) had extraction yield at 2.35±0.08 µg/ml from RP-HPLC.
- NMR, FTIR, HPLC characterization of isolated quercetin rich fraction showed the presence of bonding characteristics of quercetin devrivatives.

- Characterized isolated quercetin rich fraction had purity of isolated quercetin rich fraction 53.12±0.31% with antioxidant activity (68±0.12%).
- Remaining percentage of purity might be occupied by other phytochemicals and some bioactive compounds.
- Isolated quercetin rich fraction may have high possibilities of medicinal properties. This can further carry for application in numerous ways (*viz*., encapsulation, incorporation in food model, etc.).
- An effective, optimized encapsulation process is developed from the thirteen experimental (obtained by Box-Behnken design) by artificial neural network coupled with ant colony optimization.
- An efficient encapsulation of isolated quercetin rich extract with chitosan-alginate polyelectrolyte complex was obtained at quercetin 0.2%, sodium alginate 4%, chitosan 0.5%, and agitation 300 rpm with 84.54% encapsulation efficiency.
- Increased encapsulation efficiency was obtained by increasing sodium alginate to a significant extent.
- Chitosan content and agitation also significantly enhanced the encapsulation efficiency of microbeads up to some extent.
- Microbeads obtained at optimal encapsulation conditions exhibited high encapsulation efficiency and good drug release characteristics.
- Encapsulated quercetin showed good pH resistance with 68.27 mg QE/g quercetin release in simulated gastric fluid at 60 min.
- At the target of our study, microbeads entrapping antioxidant (quercetin) inside will exhibit functional benefits to the cells in intestinal linings after surviving the high acid medium in the stomach.
- Microbeads average particle size of 2.71µm diameter with a diverse combination of particle ranges.
- Microbeads had 0.909 polydispersity index, indicates the widely distributed particles in the solution.
- The Ca^{+} ionic crosslinking was observed in the bonds within ligand (calcium chloride) and other molecules.
- Ligand bonded with quercetin at glucoside, with alginate by –OH bonding, and with chitosan at first –CH bond.

- Microbeads stored below 25±10^{o}C found to execute conserved activity during six months of storage study and hence, that may retain the better shelf life of quercetin in microbeads.
- Bioavailablity of quercetin in BBQM was observed higher than the quercetin standard.
- Produced microbeads containing natural quercetin rich fraction will have a vast scope and application in the food industries and pharma sectors.

- Bhimkol blossom extract had no toxicity signs on Wister rats at 500 mg/kg body weight.

- Therefore, a ready to cook soup mix from bhimkol blossom powder (BBP RTC-SM) was concluded to be prepared under a safe dose.

- Soup prepared from developed RTC-SM showed good customer satisfaction, which indicates the good scope of market demand.

- The incorporation of microcapsules had no significant effects on the sensory parameters of the soup.

- Both soups prepared from bhimkol blossom with or without the incorporation of microcapsules containing quercetin rich extract exhibited good antioxidant activities whereas, the incorporation of microcapsules in BBP RTC-SM enhanced the antioxidant activity of BBP RTC-SM.

- Developed ready to cook soup mix product from BB by incorporation of isolated quercetin rich fraction is the phytochemical enriched food product.

- After consumption of soup prepared from BBP RTC-SM with microcapsules, entrapped quercetin will mostly survive in stomach digestion and release its health beneficial bioactivities and functional activities in intestinal cells.

- The incorporation of microcapsules containing quercetin rich extract is a highly effective approach to the value addition of underutilized agricultural by-products.

- The phytochemical extract of bhimkol blossom (BBE) was found to exhibit a strong ability to treat DM-1 and DM-2.
- Phytochemicals in BBE inhibited some diabetic-enhancing enzymes (viz., α-amylase, α-glucosidase, and DPP-IV enzymes).
- The myoblast L6 cells were also found to uptake glucose effectively after the treatment with BBE, that indicates, lowering blood glucose in blood stream and increasing glucose uptake in muscle cells.
- Very excellent ability of BBE to lower blood glucose in diabetic Wister rats without any unusual effect on their body weights was observed.
- Glucose tolerance level and insulin tolerance level, BBE enhanced the glucose tolerance level of BBE treated diabetic rats (group C) with increasing insulin sensitivity of insulin receptors, which might be due to the high antioxidant properties of BBE.
- A strong antidiabetic property of BB might be due to its bioactive compounds, phytochemicals and other antioxidants.
- Therefore, the application of BBE in food industries or the pharmaceutical sector to extract the benefits from this underutilized variety of banana blossom.
- Developed microbeads by encapsulation of isolated quercetin rich fraction may possess many other medicinal properties along with antidiabetic properties.
- Developed phytochemical enriched food product is concluded to possess strong antidiabetic property along with other health beneficial properties.
- BB with high antioxidant and antidiabetic property may have many other additional health beneficial properties.

8.2 Future scope of the present investigation

- Commercialization of developed nutrition rich nachos from bhimkol bossom is encouraged
- Unknown phytochemical compounds peaks (HPLC chromatogram) present in bhimkol blossom can be explored.
- Study of bioactivities of microbeads containing isolated quercetin rich fraction in microbiota of guts.
- Applications of isolated quercetin rich extract and microbeads in many fields like, pharmaceutical, food and food packaging industries.
- *In vitro* / *in silico* / *in vivo* study of other health beneficial properties
- Study of insoluble fibers and its applications

8.3 Research publications

8.3.1 Journal publications

1. Muchahary, S. and Deka, S. C. Impact of supercritical fluid extraction, ultrasound-assisted extraction, and conventional method on the phytochemicals and antioxidant activity of bhimkol (*Musa balbisiana*) banana blossom. *Journal of Food Processing and Preservation*, *45*(7), e15639, 2021.
2. Muchahary, S., Nickhil, C., and Deka, S. C. An artificial intelligence approach for modeling nachos developed from bhimkol banana (*Musa balbisiana*) blossom. *Journal of Food Process Engineering*, e14227, 2022.

8.3.2 Communicated

1. Encapsulation of isolated quercetin rich fraction with chitosan-alginate polyelectrolyte complex and its optimization by ant colony optimization
2. Molecular binding of quercetin with chitosan-alginate complex in calcium chloride solution and its *in vitro* bioavailability

3. Study of antidiabetic property of bhimkol banana (*Musa balbisiana*) blossom extract on streptozotocin induced Wister rats

8.4 Poster and paper presented

1. Got 2nd Position on Paper Presentation on the Topic " Docking Simulation of Chitosan-Alginate Complex in Encapsulation of Quercetin Rich Fraction and its Optimization Using RSM-BBD and ANN" in International Conference "SAFETy 2021" Conducted by University of Georgia, Georgia and Tezpur University, India.
2. Poster presentation on the topic "*In vitro* study of potential α-amylase activity of bhimkol banana (*Musa balbisiana*) blossom" in 27th ICFoST 2020 at Tezpur University, India.
3. Poster presentation on the topic "Optimization of the Super Critical Fluid Extraction of phytochemical from *Musa balbisiana* blossom using Response Surface Methodology" in International Conference "Technological Innovations for Integration of Food and Health (TiiFH 2019)" organized by Tezpur University, India.

8.5 Training/ workshop attended

1. Participation in National Symposium (PFFHeM2019), organized by the Dept. of Food Engineering & Technology, Tezpur, India.
2. Participation in the Industry Academia Conclave organized by Centre for University-Industry Interface (CUII), Tezpur University, India, 2019.
3. Participation in six days Finishing School on Trends in Food and Biotechnology: Analytical Approach, organized by FET Department of Tezpur University, India, 2018.
4. Participation as volunteer in conducting AICTE-NEQIP sponsored on week Faculty Development Program, conducted by FET Department, Tezpur University, India, 2017.