



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI  
SHORT ABSTRACT OF THESIS

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Thesis Title: Separation, Purification and Application of Bioactive Compounds from Pigmented Rice Bran

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**SHORT ABSTRACT**

The investigation stated in the thesis was aimed to separate the anthocyanin from black and purple rice bran and used as a functional ingredient and pH indicator in the food system. In the initial study, to separate the anthocyanin from black and purple rice bran, the conventional extraction process was followed by the ultrasound assisted extraction process. In the conventional extraction process, acetone, ethanol, and water were used as extractants and optimized using four-factor rotatable central composite design (RCCD). The ethanol was found to be the most effective solvent to extract total phenolic content (TPC) from rice bran. The purple rice bran extract showed a promisingly higher amount of phytochemical content and antioxidant activity than the black rice bran extract. After the conventional extraction, the ultrasound-assisted extraction (UAE) process was used with a mixture of ethanol and water as extractant to enhance the extraction yield in terms of TPC and anthocyanin. The UAE of TPC and anthocyanin from black and purple rice bran was optimized using RCCD in terms of temperature, pH, extraction time and solvent concentration. The comparative analysis of the purple and black rice bran extract revealed higher anthocyanin (34.9 mg C3G/L) and TPC (2232 mg GAE/100 g) in the purple rice bran extractives than black rice bran (31.9 mg C3G/L and 1978.8 mg GAE/100 g). The HPLC analysis of purple rice bran extract showed the higher amount of  $\alpha$ -tocopherol, cyanidin-3-glucoside, 4-hydroxybenzoic acid than black rice bran. As the purple rice bran extract indicated higher anthocyanin contain, thus in the further study only purple rice bran extract was used.

After extraction, the adsorption and desorption process was used as a non-thermal process to separate and concentrate anthocyanin from the crude extract of purple rice bran. The adsorption and desorption behavior of anthocyanin was investigated using amberlite XAD2, XAD4, XAD7, activated charcoal, and bentonite as adsorbents. Amberlite XAD7 showed higher adsorption/desorption capacities (1.9 mg/g and 1.8 mg/g) and recovery (41.5 %) of anthocyanin from bran extract over other adsorbents. The adsorption behavior of anthocyanin was better explained by pseudo-first order kinetics than pseudo-second order kinetics model. Adsorption isotherm behavior of anthocyanin on adsorbents was found to be homogeneous and more suitable for Langmuir, and Redlich–Peterson isotherm model. The adsorption mechanisms of anthocyanin on adsorbents followed the intra-particle diffusion model and indicated that XAD7 has a higher diffusion rate than the other adsorbents. The phytochemical profiling of the separated and concentrated bran extract was investigated in terms of anthocyanins, cyanidin-3-glucoside (C3G), peonidin-3-glucoside (P3G) and phenolic acid content. After separation, the degradation behavior of anthocyanin, C3G, and P3G was investigated at various pH and temperature. The kinetics was investigated at a pH range of 2 to 8 and temperatures ranging from 60 to 90 °C. At pH 2 and 6, the anthocyanin, C3G, and P3G showed the lowest and highest degradation rate, respectively. During thermal degradation, the lowest rate of degradation for anthocyanin ( $3.46 \times 10^{-4}$  /min), C3G ( $1.75 \times 10^{-4}$  /min), and P3G ( $1.99 \times 10^{-4}$  /min) was observed at 60 °C and the highest rate of degradation were observed at 90 °C. The effect of hydrocolloids such as carboxymethyl cellulose, xanthan gum, modified starch, and gum arabic on the stability of anthocyanin, C3G, and P3G was significant ( $p \leq 0.05$ ). The modified starch showed the highest stability effect on anthocyanins and antioxidant activity in the aqueous medium.

After the degradation study, the thesis work aims to provide a stable anthocyanin powder through microencapsulation using modified rice starch as wall material. The microencapsulation was carried out in a spray dryer, and the process was optimized using RCCD. The optimum values of spray drying process parameters were 6.01 % of starch concentration, 168.8 °C inlet air temperature, and 4.96 MPa atomizer pressure. The thermal, crystallinity, and surface properties of the microcapsules were also comprehensively studied using DSC, XRD, FTIR, and scanning electron microscopy. The storage stability of microencapsulated anthocyanin showed higher stability at 4 °C than at 25 °C for 90 days. The release behavior of anthocyanin was also investigated at 2 and 7 pH. In addition, the effect of microencapsulate on the steady-shear and dynamic oscillatory rheology of the rice dough was investigated.

The developed microencapsulated anthocyanin was used in the food product as a functional ingredient. The rice-based extrudate was developed using microencapsulated anthocyanin and the storage study was investigated in terms of moisture sorption isotherm. The RCCD was used to optimize the extrusion process, and the optimized conditions were: screw speed 121 rpm (12.7 rad/s); barrel temperature 91.9 °C, and moisture content, 22.03 %. The antioxidant activity, solubility, and cooking properties of the extrudate were determined and compared with the native rice extrudate. The storage study of the extrudate was found to follow type III isotherm behavior according to the BET classification. The sorption isotherm was analyzed using several models, and the Caurie and Peleg models showed the best fit with the extrudate isotherm data.

In the end, a paper base pH-sensitive indicator was developed using anthocyanin. The efficiency of the indicator was studied with different buffer solutions of pH range 2 - 10. The indicator made with 150 mg/L anthocyanin concentration showed a distinct L, a, and b values of color. Further, the indicator was tested in different liquid foods and indicating a distinct color with respect to pH values. The degradation behavior of a-value (redness) of the indicator showed first-order kinetics behavior. At high-temperature storage conditions, the degradation rate constant of a-value was higher than the low-temperature storage condition.