EXTRACTION AND MICROENCAPSULATION OF ANTHOCYANIN-RICH EXTRACT FROM PIGMENTED RICE BRAN USING DOUBLE EMULSION COMPLEX COACERVATION AND ITS APPLICATION IN FOOD MODEL

Abstract

Pigmented rice is considered a robust source of various biologically active compounds which provide a potential role in preventing many diseases. Even though there is high production of pigmented rice, there is limited study in terms of extraction, isolation and their utilization in food product development, fortification, formulation and value addition within a food matrix in order to utilize its medicinal, nutritional values in a better way for human health. In this context, there has been a growing interest in bioactive compounds extraction and utilization of such extracts in food formulation. The present investigation was conducted to make use of the pigmented rice bran by utilizing the natural bioactive compounds present in it, especially anthocyanin. The present study aimed to evaluate the physico-chemical, textural, pasting and phytochemical properties of black, red, purple and white rice. The study also includes the evaluation of the effects of extraction conditions on phytochemicals from black rice bran using ultrasound-assisted extraction (UAE). Microencapsulation of this bioactive compounds extract was carried out using double emulsion prior to complex coacervation process. Finally, these microcapsules were utilized for production of stable nutraceuticals by formulating it in food product.

The thesis is divided into seven chapters that are discussed below:

Chapter 1 includes the general introduction of the overall work of present research considering the major details about pigmented rice and its bran. The health beneficial properties and its therapeutic benefits are also discussed in this chapter. The potential of the phytochemical compounds and their antioxidant activity of the pigmented rice and bran are also briefly elaborated in this chapter.

Chapter 2 presents the review of literature cited relating to fulfil the present research objectives. It discusses the details work carried out on the investigation of physicochemical, textural, sensory and antioxidative attributes of various rice cultivars (pigmented and non-pigmented rice) and their findings. Research conducted in last five decades has shown that rice bran contains a unique complex of naturally occurring

antioxidant compounds. It also deals with various research carried out on the black rice and its bran, its extraction methods and techniques, utilization, value addition, product developments, etc. As the bioactive compounds have low stability and can be degraded in the external environment such as light, pH, temperature, oxygen, etc. They need an environmentally friendly processing conditions within a system in a food matrix. Many new technologies have been addressed regarding this context. Microencapsulation is one process which entraps this active compound using the wall materials. The different encapsulating agents being used for the process and their effects are also discussed in this chapter. Literatures has provided that the double emulsion (W/O/W) prior to complex coacervation provides more efficient protecting walls to their active core as compared to the single emulsion or encapsulation techniques. A detailed review based on the emulsion preparation and related published literatures are being discussed here. The microcapsules thus prepared can be utilized in the form of stable nutraceuticals in terms of fortification, formulation and value addition of a food product.

Chapter 3 deals with the physicochemical, textural, pasting, phytochemicals content of three pigmented and a non-pigmented rice variety. Quality parameters such as grain shape and size, grain appearance, cooking and eating qualities play a critical role in consumer preference as rice is consumed as a whole grain. Grain quality comprises a number of traits such as physical appearance, cooking, eating, sensory properties and nutritional value. Rice properties were investigated and compared with other published literature. Protein and fat contents were found maximum for purple rice. From the analysis of research work, categorization of pigmented rice varieties from very low amylose content (black rice) to waxy rice (purple and red) varieties is made. There were significant differences among the different rice varieties for both physicochemical and thermal properties ($p<0.01$). Phytochemicals of pigmented rice have a potential of a bioactive compound. Polyphenols, flavonoids contents were observed higher in colored rice varieties as compared to non-pigmented rice. The content of anthocyanin was observed highest in the black rice variety. A similarity trend was also observed for phytochemical content, antioxidant activity and anthocyanin content of all the rice bran varieties. The colored rice varieties also had high antioxidant activity. Hence, there should be encouragement of consuming traditional foods of black rice, purple rice and red rice to accomplish the requirements of nutrient and for preservation of the existing food culture of the society. Moreover, colored rice can be a source of nutraceuticals as

they could modify to incorporate in the formulation of therapeutic diets that improves the utilization of pigmented rice in food and non-food industries. The research work concluded that the colored rice variety has numerous medicinal and nutritional properties over white rice.

Chapter 4 summarizes the optimization of extraction process of phenolics, flavonoids, monomeric anthocyanin and antioxidants from black rice bran using Ultrasound-assisted extraction. The independent variables for the extraction process were extraction time and ultrasound power. Response surface methodology (RSM) was employed for optimization of extraction process. Acidified ethanol (1 M HCl) was used as extraction solvent and ultrasound power (100-300 W) and extraction time (20-60 min) were used as independent variables. Face centered composite design (FCCD) data was successfully opted and developed a 2^{nd} order polynomial equation with R^2 value of 0.94 for total phenolic content (TPC), 0.95 for total flavonoid content (TFC), 0.87 for 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 0.98 for total anthocyanin content (ANCs) respectively. The effects of ultrasound power and extraction time on the responses were evaluated and their mutual correlations were determined with empirical mathematical explanation. The optimum conditions in term of ultrasound power and extraction time were evaluated for maximum extraction of phenolics, flavonoids, anthocyanin and antioxidant activity and found to be 33.78 min and 234.01 W. Kinetic model study for extraction of anthocyanin was compared to conventional extraction process. The stability study of the UAE extract under different temperature and pH were also discussed. However, the results revealed that prolonged treatment of extraction time and power led to decomposition of anthocyanins. And quantitative determination of phenolic acids, flavonoids and anthocyanins was performed using HPLC analysis.

Chapter 5 deals with the development of anthocyanin-rich microcapsules using double emulsion complex coacervation technique. In this chapter, the anthocyanin-rich extract was concentrated using magnetic stirrer maintaining the temperature at 40 ˚C. Nine formulations of microcapsules were developed using different concentration of the wall materials: gelatin and acacia gum and core solution (concentrated anthocyanin) at ratios of 1:1:0.5, 1:1:0.75 and 1:1:1 respectively. The concentration of the encapsulating agents used were 2.5, 5 and 7.5 % w/v. The primary emulsion (W/O) was first prepared using 20 % core solution and 80 % soybean oil in presence of lipophilic emulsifier [PGPR 3 % (v/v)]. The solution was homogenized at 15,500 rpm for 4 min using Ultraturrax

homogenizer. To obtain double emulsion (W/O/W) process, gelatin solutions containing a hydrophilic emulsifier (1 % Tween 80) was added to the primary emulsion. The double emulsions were prepared at 13,500 rpm for 3 min using an Ultraturrax homogenizer. Visual observations and optical microscopy were used to evaluate the stabilities of both primary and secondary emulsions. Subsequently, the coacervated microcapsules were obtained at different pH (3, 3.5 and 4), freeze-dried and evaluated for their physicochemical properties, morphology, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction pattern (XRD), thermal behaviour and stability of anthocyanin. The results obtained for encapsulation efficiency of anthocyanin with high values indicated that the encapsulation process was effective. The morphology of the microcapsule powder was analyzed and exhibited round, hard, agglomerated structures and relatively smooth surface. The thermal degradation behaviour of microcapsules displayed endothermic reaction confirming the thermostability of the microcapsules where the peak ranged from 83.7 to 97.6 ˚C. The stability studies in terms of retention of total anthocyanin content were observed at different storage conditions; both under refrigerated condition (7 ˚C) and at room temperature (37 ˚C). The results indicated that the microcapsules obtained through coacervation can be an alternative source to develop stable nutraceuticals.

Chapter 6 deals with the utilization of the anthocyanin-rich microcapsules in food model. For this, coconut water jelly is developed using sugar and coconut water as the major ingredients and pectin and citric acid as minor ingredients. Four coconut water jelly loaded with anthocyanin microcapsules were prepared. Anthocyanin microcapsules (2.71 mg/100g) were obtained using double emulsion complex coacervation. The four jelly samples viz., J_c, the control (coconut water jelly), J_1, with 1 % anthocyanin microparticles, J_2, with 2 % anthocyanin microparticles, J_3, with 3 % anthocyanin microparticles obtained by double emulsion coacervation were produced. Determination of physico-chemical properties, texture, color, sensory and microbial analysis of the developed coconut water jelly loaded with anthocyanin were performed. The total phenolics (TPC), anthocyanin content (TANCs) and antioxidant activity of the developed jelly were also determined in this chapter. From the characterization, the jelly produced with the highest percentage of anthocyanin microcapsules showed higher retention of TPC, TANCs and provided the best antioxidant activity. The microbial analysis showed that visible moulds were not observed for the sample within the storage period of 30

days. It can be concluded that the jelly thus developed with loaded anthocyanin can be a source stable nutraceutical. The application has also shown to be technically feasible, providing the product with color and functionality.

Chapter 7 summarizes the conclusion, summary and future scope of the present research study. The research work concluded that the colored rice variety has numerous medicinal and nutritional properties over white rice. The study involved the novel extraction technique (Ultrasound-assisted extraction) of bioactive compounds (anthocyanin-rich) from black rice bran. The effect of ultrasound power and the extraction time on total phenolics, flavonoids, anthocyanin and antioxidant activity were also analyzed and regression model were established. Microencapsulation of the anthocyanin-rich bioactive compounds was carried out using double emulsion complex coacervation technique with gelatin and acacia gum as the encapsulating agents. The microcapsules thus developed showed good physicochemical properties in terms of hygroscopicity, moisture content, solubility and encapsulation efficiency. And finally, the best microcapsules were successfully incorporated in food model which also act as functional food. The research study concluded that value addition of black rice bran encapsulates as a functional component was successfully carried out for the development of value-added food product.

Promotion of the black rice bran in terms of functional foods was successfully performed in this research objective. Among the bioactive compounds, anthocyanins from pigmented rice are known to content a prominent functional component which are responsible for many biological activities and provides therapeutic benefits. In recent years, progress has been achieved tremendously in extraction of bioactive compounds and biological activities of active components in pigmented rice. Additionally, attention should be addressed to minor components in pigmented rice as special pharmacodynamic effects could be found from them. Special pharmacodynamic effects such as antiinfluenza, antiobesity and antidiabetic activities need to be explored and studied more effectively. The structural diversities and pronounced biological activities of compounds in pigmented rice indicate that pigmented rice is worthy of further studies that may lead to new functional constituent identification. In brief, pigmented rice and its by-products provide great potential in the field of food and pharmaceutical application and extraction and encapsulation could be of wonderful technique for these applications as functional ingredients.