Chapter 1

1. Introduction

Rice (*Oryza sativa* L.) is the major cereal crop and a staple food in South East Asia and is consumed by over half of the world population [40]. Although, widely consumed as white rice, there are many special cultivars of pigmented rice grain varied from deep-purple (black) to brown-reddish (red) in their covering layers which is due to the accumulation of natural pigments called ‘anthocyanin’. Anthocyanin, water-soluble flavonoids, is one of the primary functional components of pigmented rice, derived from the 2- phenyl benzopyrilium or flavylium ion [9]. Approximately about 400 naturally occurring anthocyanins are present in plant materials [1]. These anthocyanins, polyphenols are also helpful for heart health [19]. Such pigmented rice is known as ‘Chak-hao’ in Manipur, India, means ‘delicious rice’ (Chak = rice and ahaoba or hao = delicious) [27]. Chakhao Poreiton (Purple) and Chakhao Amubi (Black) are two main aromatic rice varieties grown in Manipur. It has a typical nutty flavour, aroma which turns into deep dark purple when cooked. It is stickier than the white rice when cooked and takes longer time to cook. The major contributor of aroma is due to the presence of volatile compound 2-acetyl-1-pyrroline which was first isolated and identified by Buttery et al., [4]. Consumption of a single cereal may cause deficiency diseases because of the lower concentration of essential amino acids, minerals and vitamins [3].

The rice varieties have great diversity in its composition, genetic background, granule size, cooking, gelatinization, and textural properties [32]. Again, the starch proportion (amylose and amylopectin) are mainly responsible for the varietal differences in physico-chemical and cooking properties of the rice kernel. Categorization of rice has been reported on the basis of high, intermediate, low amylose and waxy rice types [2]. The amylose content in rice is known to be the principal determinant of the quality of rice. Pigmented rice is consumed as a sweet delight in the form of steamed rice during traditional feasts in Manipur. Customarily, it is used for the preparation of sweets and very few locally available ready-to-eat products. Studies have reported on physicochemical, textural, rheological properties of rice and its flour and their antioxidant properties [24, 33, 35]. However, less study has been reported on the phytochemical properties of pigmented rice [10].

Pigmented rice is reported as robust source of various biologically active compounds such as phenolic acid, anthocyanin (ANCs), flavones, isoflavones, gallic, hydroxybenzoic, sinapic acid, etc. [28]. These bioactive compounds provide a potential role in preventing many diseases which are anti-inflammatory properties, anti-diabetic, anti-tumour, anti-allergic, etc. [29]. Due to their multiple biological activities, there has been an increase demand for black rice [5]. Burgeoning studies have reported its importance in terms of nutritional and medicinal aspects [33, 35].

Again, phenolic acids are present in both free and bound form. The extract from black rice bran has shown to inhibit effects on *in vitro* allergic reactions [7]. Pigmented rice bran (black and red) extracts inhibit α- amylase and α -glucosidase activity which also help in delaying of digestion and absorption of carbohydrates leading to suppression of postprandial hyperglycaemia in the diabetic person. Nutrient content of these rice varieties has gained consumers interests for utilization in nutraceuticals and has enticed the food industries. Although the phytochemicals available in whole rice grains are minute quantity, they provide various health benefits in minimizing the risks of many diseases such as heart and cardiovascular diseases, type II diabetes, obesity, and some types of cancer [31]. Despite limitations in productivity, the full potential of pigmented rice remains to be explored in terms of its applications, functional foods and nutraceuticals by direct use of its bioactive compounds. Thus, attention is currently been received on pigmented rice due to their nutritional significance for inclusion in our diet.

The pigmented rice bran is an underutilized by-product from the milling process of whole rice and currently faces limited use except used for animal feed [13, 15, 39]. Generally, commercial rice bran available is a mixture of rice bran layer and rice germ [22]. They are rich source of various bioactive compounds that includes flavonoids, phenolic acids, vitamin E and γ-oryzanol [20]. Wanyo et al., [39] has also identified bioactive compounds in rice bran and rice husk. Presence of very high content of vitamin E (γ, α and δ-tocopherol), γ-oryzanol, phenolic acids (vanilic acid, gallic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, chlorogenic acid, ferulic acid, p-coumaric acid, etc.) has been identified in rice bran. Compared to the bran from brown rice, the pigmented rice bran has been reported to possess much higher antioxidant activities which also depends on the structural properties, types, contents of the phenolic compounds [23]. Antioxidants are of great importance as they are considered to provide significance to enhance memory and immune system strength. They are also known for the compounds ‘cyanidin 3-

glucoside, peonidin and peonidin 3-glucoside and other major anthocyanins of black rice as the preventive properties of invasion of cancer cell [6]. Ichikawa et al., [12] has also described that black rice is two-fold stronger and more efficient with respect to blueberries antioxidant activities. During the last decades, research conducted has shown that it contains a complex unique naturally occurring antioxidant compounds [21]. Nowadays, attention is being given to the anti-oxidative and radical scavenging properties of pigmented rice as their consumption encourages and promotes human health by minimizing the reactive oxygen species and free radicals concentration. Even though there is high production of pigmented rice, and are also known for their antioxidant properties that provide many health benefits, 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.

Efficient and sensitive extraction and isolation procedures are mandatory for successful collection of these bioactives from rice bran. Extraction is the primary step to recover the bioactive compounds from the rice bran. Different types of extraction techniques and methods have been employed from the rice by-products that includes traditional, solvent extraction such as far-infrared radiation (FIR) and soxhlet extraction, and novel technology (such as supercritical carbon dioxide extraction, ultrasonic-assisted extraction, ohmic heating-assisted extraction, and microwave-assisted extraction) [25]. In addition, the factors such as polarity and concentration of solvent should be carefully selected for the effective extraction of these bioactive compounds. Solvents such as methanol, ethanol, and acetone have been universally used for the extraction of phenolic compounds in plant [17]. Efficient and environmentally friendly extraction techniques need to be considered for separation or isolation of these bioactive compounds from agricultural by-products. Again, soxhlet extraction, traditional method, consumes time and have very low extraction efficiency due to hydrolysis, oxidation, etc. [8]. In order to obtain efficient extraction efficiency, emerging technologies such as ultrasound-assisted extraction, microwave-assisted extraction, etc. have been used [11, 13, 18, 34, 36, 38, 42]. In this context, there has been a growing interest in bioactive compounds extraction and utilization of such extracts in food formulation.

Additionally, the extracted bioactive compounds (ANCs), which are mainly coloured materials, are recognized for their antioxidant activity. Regardless, when utilized in food operations, have low chemical stability under processing conditions and harsh environments of food matrices. One alternative for prolonging the stability of this bioactive compounds is microencapsulation, entrapping the active material inside a coating material. It offers protection of the active compounds (core particles or material) from different environmental and chemical conditions, such as of pH, light, temperature, moisture, enzymatic activities, metal ions and oxygen by developing modified wall materials, such as proteins and polysaccharides into micron structures [26, 41]. In order to stabilize this, emulsion techniques are also applied. Emulsion has been given a novel perspective to encapsulation of nutrients and other components in development of functional food formulations. Technically, an emulsion-based encapsulation system has the hydrophilic core particle (such as ANCs) dissolved in internal water droplets, several of which are trapped in bigger oil particles. As the literature has shown that anthocyanins are hydrophilic compounds, primary emulsion preparation of water-in-oil and then water-in-oil-in-water (double emulsion) has been proposed for enabling the utilization of this technique [16]. There was also a study on double emulsion technique followed by coacervation process, applied by gum and gelatin as encapsulating materials after preparing the water/oil emulsion to overcome the instability of anthocyanin [30]. Complex coacervation is an associative phase separation process that produces a colloid-rich phase called coacervates by modifying the environment under controlled conditions such as ionic strength, pH, temperature, solubility, and so on [26]. The driving force accompanying this process is the electrostatic interaction among the macromolecules charged containing in the reaction medium [37]. There is a need for promotion of this bioactive compounds from pigmented rice as functional foods because of its therapeutic benefits.

The research work 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.

Objectives of the current study

1. To study the physico-chemical and phytochemical properties of different pigmented rice
2. To standardize the ultrasound-assisted extraction of anthocyanin-rich extract from pigmented rice bran
3. To encapsulate anthocyanin-rich extract using double emulsion complex coacervation technique and its characterization
4. To develop functional coconut water jelly by incorporating anthocyanin microcapsules and its characterization

Justification

Less study has been reported on the pigments isolated from rice bran on its functional properties with a perspective to value addition. Limited studies have been done on extraction of phytochemicals on pigmented rice bran with different combination of ultrasonic power and extraction time. No studies on anthocyanin-rich extract from pigmented rice bran incorporated in a food model. Based on this, the objectives are set for this research work.

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