Chapter 2

2. Review of literature

2.1. Pigmented rice and its nutritional values

Rice (*Oryza sativa* L.) is a staple food, consumed since a long time in Asian region, especially Japan, China, Korea and other countries in Southeast Asia [10]. Pigmented rice has been consumed since a long time comprising several other varieties in Southeast Asian countries [46]. Black rice also comes in a number of short grains, long grains and glutinous varieties similar to brown rice. The black color of the rice is due to the grain's naturally occurring pigment present in the bran layer called 'anthocyanin'. The whole nutritious rice grain is high in antioxidants, fiber, iron vitamin B and E, magnesium, thiamine, phosphorous, niacin, etc. [48]. Pigmented rice is a good source of various bioactive compounds and provides many health beneficial properties such as anti-atherosclerosis activities, anti-allergic activities, anti-diabetic activities, anti-inflammatory effects, anti-cancer activity, anti-tumour activities, alleviating gallstones, internal rejuvenation, other bioactivities, etc. [88]. Table 2.1 indicated bioactivities of pigmented rice cultivars.

Table 2.1: Pigmented rice and its bioactivities

Sl.no.	Rice variety	Bioactivities and their method	References
1	Black rice and its bran	Antioxidant activities, Antiatherosclerosis activity (Oxygen radical absorbance capacity, HPLC analysis) Hypoglycemic activities (*In vivo*)	[11], [30], [35], [96], [105]
		Antitumor activity	[12], [36], [87]
		Antiallergic activities	[14], [60]
		Inhibition against tyrosinase activity	[62]
2	Red rice	Antioxidant capacity, anti-inflamatory (*In vitro* and *In vivo*)	[68], [71]
3	Purple rice	Antioxidant activities, Inhibitory activities (Xanthine oxidase inhibition (%), Cytotoxic activity against tumor cells	[37], [38], [64]

Many scientific studies have showed that this rice powder has provided well balanced excellent food and the potentiality are truly remarkable. The demand for black rice has been increasing recently due to their various biological activities because of the presence of phenolics and antioxidative compounds, which can improve human health and immunity [10, 18]. Demonstration of the activity of antioxidants (radical scavenging ability) including both *in vitro* and *in vivo* models of the pigmented rice and its extract have also been reported in many studies [35, 38, 71, 101]. Moreover, the high antioxidant activity and capacity of the antioxidative enzyme of black rice can be correlated to the content of anthocyanin, the outer layer fraction [54, 61]. This can also be observed from the scavenging and quenching activity of pigmented rice bran for reactive oxygen species related to the polyphenols and trace content of minerals [44]. Research on rice phytochemicals has identified that pigmented rice bran comprises a very large content of phytochemicals and fiber, such as tocopherols, oryzanols, tocotrienols, vitamin B complex and other phenolic compounds. Pigmented rice extracts also provide biological effects such as antimutagenic, antiallergic activities, anticarcinogenic activities, etc. [13, 64]. Black rice is considered as the enriched rice as it has been acting as medicine traditionally for allergic treatment such as bronchitis and dermatitis [45]. Additionally, the phenolic compounds extracted and isolated from such rice reported predominant inhibition activity against aldose reductase in decreasing order such as cyanidin-3-glucoside > quercetin > ferulic acid > peonidin-3-glucoside > tocopherol [106]. In brief, attention is given to the bioactivities of pigmented rice and its bran to their main component anthocyanins [23].

2.2. Physico-chemical and phytochemical properties

Pigmented rice provides an impact on health due to its predominant antioxidant properties. However, there has been a diverse physiographic and agroclimatic landscapes, is considered as the most biodiversified rice-rich region. Most of the rice grown in Manipur are underutilized. Moreover, rice is also categorized based on the amylose content. The starch proportion (amylose and amylopectin) is the main component accounting for the varietal differences in the properties of the rice kernel. Categorization of the rice has also been reported on the basis of high, intermediate, low amylose and waxy rice types [4]. The amylose content is known to be the principal determinant of the quality of rice. The rice cultivars have great diversity in its composition, genetic background, cooking properties, granule size, gelatinization, etc. [94].

Many studies on physicochemical, texural and rheological properties rice and its flour have been determined by Chanu et al., [10], Pal et al.,[73], Sompong et al., [96], Tangsrianugul et al., [99]. Physical, thermal, pasting properties and the mineral profiles of pigmented and non-pigmented rice varieties were evaluated by measuring their physical characterization viz. axial dimension, angle of repose, etc. [87]. Similarly, Pal et al., [73] has also studied the different properties attributed to rice products (raw milled rice, popped rice, beaten rice, boiled rice and puffed rice) obtained from colored rice considering a white rice as control (Swarna Sub-1). Various differences were observed in the properties significantly about 50.67 % head rice recovery, 17.6% of amylose content, 1.87 elongation ratio and 10.10 mm kernel length after cooking and were satisfactory. However, popped rice provided the highest elongation ratio and kernel length. Highest antioxidant activity was observed in both popped and boiled rice compared to others. Singh et al., [94] also studied different properties of rice varieties and their correlation were evaluated using Pearson correlation. 1000 kernels weight, L/B ratio, content of amylose provided a variation different from other literatures. The time of cooking showed to be increase with decrease in amylose content however, increase with increase in bulk density. Investigation of antioxidant activities of black, red, and green rice bran were also carried out using DPPH radical assay and phenolic contents by HPLC method [43]. Moreover, determination of phytochemical compounds and antioxidant properties were also carried out from both pigmented and non-pigmented rice varieties [34]. FTIR spectra and HPLC analysis of the phenolic compounds were analysed and compared to the non-pigmented rice. Isolation of starch has also been performed and investigated the molecular structure and other properties such as crystallinity, solubility, swelling power, etc.

2.3. Extraction of Phytochemicals

Pigmented rice bran has a rich and potential source of bioactive compounds and their utilization on value addition has been gradually increasing in recent years [17]. The bioactive compounds can be separated and isolated from this agricultural by-product or other plant materials using environmentally friendly and efficient extraction techniques. Boiling, a traditional method, soxhlet and extraction by refluxing are time taking and have lower extraction efficiency. Oxidation, hydrolysis, and ionization could be the reason for low efficiency. These difficulties can be replaced by emerging technologies which includes ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid *extraction,* etc. Organic solvents such as methanol, acetone, ethanol, butanol and water in certain proportions are used in conventional extraction technique [32, 40]. The study on the extraction of anthocyanins, total flavonoids, and phenolics, and the antioxidant activity of Oryza sativa (black rice) were performed by Pedro et al., [74] and Tabaraki et al., [98]. Temperature variation of 10 to 50 ˚C, time-20 to 80 min, and ratio of solid to solvent-1:15–1:45 were evaluated using response surface methodology. The optimized parameters obtained a temperature of 34.7 ˚C for 80 min with solid/solvent ratio of 1:30 provided an extract of flavonoids (51.26 mg/100 g), anthocyanins (116.58 mg/100 g) and phenolics (520.17 mg/100 g) and antioxidant inhibition of 46.50 % using radical scavenging activity. Black rice phytochemicals are characterized by the presence of anthocyanins that are grouped of purple or reddish flavonoids existed in different rice cultivars. More than 95 % of predominant anthocyanin were present in black rice that represents 2.8 mg/g of cyanidin 3-O-glucoside and 0.5 mg/g of peonidin-3-O-glucoside followed by 0.5 mg/g of flavones and flavonols and 0.3 mg/g of flavan-3-ols [75]. Moreover, the total anthocyanin content in black rice cultivars varied significantly from one another. Higher content of anthocyanin was found in Chinese black-purple rice where 6.3 mg/g of cyanidin 3-O-glucoside and 3.6 mg/g of peonidin 3-O-glucoside were reported to present [105].

HPLC analysis of the two major anthocyanin presents in black rice varieties of Manipur were also performed [3]. It was reported that four main anthocyanins viz. cyanidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside and delphinidin 3-galactoside were identified in Poireiton Chakhao variety. However, three main anthocyanins viz. cyanidin 3-galactoside, delphinidin 3-arabinoside and delphinidin 3-galactoside were identified in Amubi Chakhao variety. Again, antioxidant activities, phenolic compounds were investigated for pigmented rice. Jun et al., [43]. has also determined the antioxidant activity using ABTS radical cation assay, DPPH radical assay, reducing power, chelating ability, etc. HPLC analysis of the phenolic acids were also investigated. Extraction was carried out from pigmented rice using the aqueous solutions of ethanol, methanol and acetone for determination of the solvent efficiency. And from the analysis, the best solvent mixture provided the highest antioxidant contents was acetone–water solution with 40:60 v/v.

2.4. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) of bioactive compounds or other targeted compounds from various plant materials employes ultrasound energy and solvents for the extraction process [21]. Frequency (> 20 kHz) higher than the range of human hearing audible frequency (20 Hz to 20 kHz) are used for mechanical waves in ultrasound. The main mechanism associated in the UAE is the acoustic cavitation [47]. The sound waves and the collapsing cavitation bubbles induce fragmentation, pore formation, that occur increased in absorption and swelling index in the plant cellular matrix. Solubilization of the targeted compound occur in the solvent due to this rapid fragmentation leading to particle size reduction, surface area increased and higher mass transfer rates in the boundary layer in the solid matrix [49, 80]. The bioactive compounds recovery of extraction method carried out using solvent extraction needs large solvent quantity, mechanical extraction produces lower yield, supercritical fluid extraction needs large capital and microwave assisted extraction requires an aqueous phase [86]. However, UAE requires lesser time, lesser energy requirement, low extraction temperature leading to quality retention of the extract. Ultrasounds are extensively used for phenolic compounds, carotenoids, polysaccharides, aromas extraction from the plant matrices. Precise control of frequency, power, temperature, solvent type, extraction time, duty cycle, solvent to solid ratio is required for obtaining optimal extraction [47].

Extraction of polyphenolic compounds and anthocyanin from purple and black rice bran has been modelled and optimized by Das et al.,[17]. Optimization of the extraction process was performed using a four variables RCCD design (rotatable central composite design) to obtain an improved TPC and anthocyanin content (monomeric) by RSM (response surface methodology). The temperature, pH, concentration of the solvent and time of extraction effects were analyzed on the response (extraction yield). Extract was analyzed using GC-MS and found that 4 and 9 compounds were present in black and purple rice bran extract respectively. Moreover, ultrasound-assisted extraction of phenolics and antioxidants were carried out from rice bran using ethanol as solvent (food grade) [98]. RSM was employed for the optimization of the extraction process.

Nowadays, exploration regarding the variation in technologies such as pre-treatment using UAE and sequential extraction are needed. The utilization of combined UAE with other non-thermal method of extraction such as microwave assisted extraction, pulse electric assisted extraction and enzyme assisted extraction for the extraction of bioactive compounds from plant source are limited and required further exploration. The demand for UAE extraction of naturally occurring compounds has been increasing as it showed better equipped to extract the compound without damaging and degrading the volatile bioactive compound structure.

2.5. Double emulsion

Despite the high potential of antioxidant compounds, they have low stability and can degrade under various environmental and processing conditions (temperature, pH, light, oxygen). Due to poor chemical stability, the applications of this bioactive compound in food matrix are restricted [6, 108]. An alternative study for prolonging this chemical stability is 'microencapsulation' process that aims to entrap the active material or component inside a wall or coating material. It ensures component stability and physicochemical characteristics from external environmental conditions by developing modified wall materials, such as proteins and polysaccharides into micron structures [81].

A double emulsion is a microencapsulation process that is used to trap hydrophilic active materials and slow their release. Double emulsions (multiple emulsions) are called the emulsion of an emulsion consisting of two emulsion type: water-in-oil-in-water emulsions ($W_1/O/W_2$) and oil-in-water-in-oil emulsions ($O_1/W/O_2$) [50], depending on their core material or loaded active agents [33]. Double emulsions are very promising and are mostly applied in industrial fields such as food industries and cosmetic and pharmaceutical industries. They provide a protective wall material or encapsulation to the bioactive substances that include both the hydrophilic and hydrophobic as internal droplets where controlled release of the bioactive ingredients is necessary [50]. The emulsion properties, stability and its formation is dependent on numerous aspects such as water solubility, density, polarity, viscosity, water solubility, etc. of the oil phase and the concentration and type of component present in the aqueous phase [67]. Moreover, the emulsion stability can also be employed using numerous methods. These can be divided into membrane emulsification and high shear devices. High shear mixer (Ultraturrax homogenizer), high pressure homogenizer (HPH), ultrasonicator and microfluidizer are the high shear devices employed for preparation of double emulsion. The principle of these high shear devices are collision, cavitation and turbulence causing breakdown of the droplets and uniform dispersion of the dispersed phase. The formation of the primary emulsion required device with high shear rate and the dispersion of primary emulsion required device with low shear rate in order not to break the structure of the double emulsion. The particle size produced by the ultraturrax device is in the range of micrometers while nano sized double emulsions using HPH, sonicator and microfluidizer. Regardless of the extensive potential, there is also limitations for utilization of double emulsions in food systems. Intrinsic instability which means occurrence of encapsulant leakage attributed from the inner aqueous phase. Additionally, improvement of the double emulsion stability can be obtained by using the different types of emulsifiers and the osmotic gradient controlling between the outer and inner phase.

Major applications of double emulsion include many research that includes: encapsulation of catechin and curcumin prepared using W/O/W emulsions [2]. Encapsulation of catechin has been performed in an internal water phase consisting of gelatin, NaCl, ascorbic acid and milli-Q water and curcumin in oil phase consisting of olive oil and PGPR; a lipophilic emulsifier. Research was also conducted on W/O/W emulsions system for encapsulation of *trans*-resveratrol using high pressure homogenization [102]. Microencapsulation of active ingredients in the field of pharmaceuticals [72], targeted drugs, antigens and vaccines delivery [5, 72], oral administration of vaccines [31], peptides and hormones like insulin delivery [16], etc.

Despite lots of published research, only a few products have successfully been marketed due to immense stability problems. Emulsifiers to be used is an important factor as it must be able to create, stabilize the droplets of dispersed phase and also to be nontoxic and biodegradable [55]. Literatures has shown studies on encapsulation techniques and polyphenols delivery recently [25, 56]. For the utilization of this bioactivity studies or the use of functional foods, encapsulating the active components in micron sized has been one of the possibilities.

2.6. Complex Coacervation

Coacervation denotes the associative phase separation technique generated by modifying the environmental media such as ionic strength, solubility, pH, temperature in controlled conditions. The phase separation where the colloids rich phase is termed as coacervates phase whereas, the little content of colloid is the equilibrium phase [63]. Coacervation process are of two types: simple and complex coacervation. The main difference of the processes is that in simple coacervation, a single polymer is used and formation of coacervate takes place due to water loss or dehydration mechanism which can be occurred due to addition of coacervation agents (CA) or inducing agent (IA) (salt or desolvation liquid) in the reaction medium [100]. And in the complex coacervation process, interactions of the ionic charges take place between opposite charged polyelectrolytes in the aqueous form, which can be protein and polysaccharides [57, 66]. The main driving force occurred in complex coacervation is the interaction of the electrostatic forces between the charged macromolecules contain in the reaction medium. During the process of encapsulation by coacervation technique, the polymer rich in colloids (coacervates) is settled around the active (core) ingredients leading to settling down of the encapsulated core.

Complex coacervation technique is highly promising microencapsulation technique which is widely used in food, pharmaceutical, textile and agricultural industries. It is well known for its simplicity, lower cost, reproducibility, etc. in the encapsulation of food components that yielding enhanced efficiency of encapsulation (up to 99%) [24, 97, 104]. Various biopolymers are used in this process, gelatin combined with other polysaccharides remains the most extensively used source of protein due to its outstanding functional and structural properties. Commercially, gelatin is obtained by partial collagen hydrolysis which are derived from the white connective tissues, skin and animal bones. Being obtained from the wastes from meat processing industries, its cost is relatively low as compared to other proteins. The solubility is very high at high temperature (> 40 ºC). It is also reported that proteins derived from animal are more allergenic than plant proteins [42, 52, 53]. Therefore, increasing interest has been observed in the last five decades with great functional and structural attribute which can replace gelatin [65, 103]. Biopolymer's interactions play significant role in structure, stability and texture control of the coacervates and the entrapped materials. For better coacervation, understanding the influencing factors in the process is critical and necessary. Moreover, the formation of coacervates depends not only on the pH adjustment between the protein and polysaccharide pairs [89, 95] but also the temperature needs to be controlled during the process [7, 82].

Many studies have been carried out on the complex coacervation process where the majority research is available based on pharmaceutical applications [51, 59, 70]. These include complex coacervations of core materials black raspberry anthocyanin and aspartame using the encapsulating agents: gelatin and gum arabic carried out for improvement of the stability of the dual emulsion along with freeze drying [78, 91]. Spray dried encapsulation have also been developed, characterized and evaluated for their kinetic degradation and color stability of bioactive compounds [8, 9, 26. 39]. The high value of encapsulation efficiency of ascorbic acid was observed in microencapsulation using double emulsion technique [15]. Microencapsulation of lycopene aimed to protect and control release has also been reported by Silva et al., [93]. Encapsulation of propolis extract by complex coacervation was also carried out using pectin and soy protein isolates as encapsulating materials [69]. For decades, this process has been carried out for its control release and stability enhancement in the pharmaceutical industries.

2.7. Application in food model

New product development (NPD), "ready to eat" (RTE) for consumption, fortified with functional ingredients, are increasing its popularity in recent times. Food industries are in search for alternatives to this new technology that are enriched in natural antioxidants [20]. Great efforts have been made by confectionary industry in recent years on innovation of technology based on confectionary processing by replacing some artificial ingredients present in products with healthier alternatives [1, 77]. Natural antioxidants are known to possess potential nutraceuticals that provide many health benefits and physiological functions which includes prevention of many diseases. Again, the important natural components from plant source relevant to the industries of foods and pharmaceuticals are the polysaccharides, organic acids, polyphenols, anthocyanins and flavonoids. In addition, great challenges have also been seen for the new products development of with added nutritional quality, convenience, sensorial acceptance, naturalness and attractive costs. Nowadays, new interests and attentions has been observed in food industries for new product development with aided natural antioxidants or ingredients in the form of particles or microcapsules or any other encapsulates for the control release of these bioactives in the human system (gastrointestinal tract).

There has been considerable interest in developing food colorant using color pigments from various food crops. Black rice having dark purple-colored is enriched rice with medicinal effects. Besides, its purple pigment is widely used as colorants in foods when processing bread, ice creams and liquor [84]. Recently, anthocyanin has been only considered and regarded as active substances biologically as well as food colorants and has been playing a significant role in food industries as they provide an attractive natural source of color associated with universally flavorful fruits.

As alternatives to produce food products based on enriched natural antioxidants, a fundamental to prevent many diseases, few literatures have shown to incorporate the natural antioxidants and develop food products. This food products includes jelly candy developed using encapsulates prepared from the hibiscus extract [20]. Some food products developed from natural antioxidants are given in Table 2.2.

Table 2.2. Food products developed with various natural antioxidants (encapsulates)

Food products developed	**Natural antioxidants**	**Materials or encapsulating agents used**	**Methods incorporated**	**References**
Jelly candy	Hibiscus extract, rapseed oil and pectin	Calcium chloride	Ionic gelation, double emulsion (dripping-extrusion and atomization)	[20]
Gummy jelly	Lamduan anthocyanin-rich extract	Maltodextrin dextrose equivalent 20, arabic gum	Encapsulation: double drum dryer	[85]
Yoghurt	b-carotene, Palm oil, carotenoids	Chitosan-sodium tripolyphosphate, chitosan-CMC (carboxymethyl cellulose)	Nanoencapsulation using complex coacervation	[83]
Beverage	Curcumin and catechin	Olive oil, sunflower oil, soybean oils, MCT with 5% PGPR	Double emulsion (W/O/W)	[2]
Jelly powders	Barberry extract	Maltodextrin and Gum Arabic (MD+GA), gelatin and maltodextrin (GE+MD) and maltodextrin (MD)	Spray drying technique	[59]
Glassy food model	alpha-tocopherol	Maltodextrin and gelatin	Emulsion: Quick frozen and freeze-drying	[27]

Yogurt	Lactic acid bacteria and Bifidobacteria	Nanofiber mats, Corn starch and sodium alginate	Nanoencapsulation (electrospinning method)	[29]
Gummy candies	*Bougainvillea glabra* bracts extract	Sodium alginate and inulin, Calcium chloride	Ionic gelation	[92]
Curcumin-loaded milk	Curcumin	Skim, low-fat and whole milk	pH driven method	[28]
Oil, sugar or protein models	Catechin	β− cyclodextrin	Encapsulation: vacuum dried	[34]

2.8. Coconut water

Coconut water has become a well-known drink and has also been gaining popularity in food and beverage industry due to its high nutritional characteristics [22, 76, 90]. It has also been demanded for its pleasant, sweet scent and smell from certain parts of fruits; coconut meat and coconut water [41]. The total soluble solids of the coconut water are about 5 to 8 %, where the major content of sugars is 3 to 7 % [58]. Remarkable content of various nutritional properties like minerals and salts such as sodium, potassium, chloride, magnesium and also sugars. The coconut water drink provides a great potential for health and rehydration [107]. Various important compounds are also known to be contained in the coconut water which includes free amino acids (tyrosine, glycine, serine, phenylalanine, histidine, leucine and isoleucine), B-complex vitamins and enzymes (catalase, phosphatase, peroxidase, diastase, dehydrogenase, polyphenol oxidase, RNA polymerases [79]. The antioxidant activity of coconut water has been analyzed and identification of phenolic compounds were also performed [58]. Preservation process of coconut water has also been studied using green technology like ultrasound, thermosonication, high pressure homogenization from activity of enzymes ensuring the microbial quality and maintain the original characteristics of sensory [19]. Even though the thermal process kills the microbial loads, some of the native enzymes are highly resisted and required a severity process which can also leads to drawbacks in sensorial and nutritional values [79].

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