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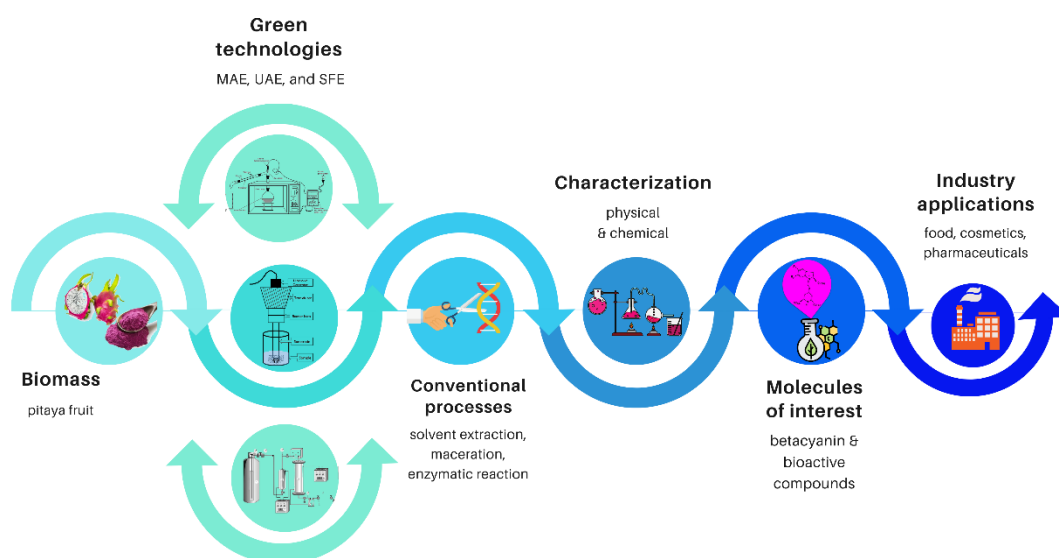
**Trends on valorization of pitaya fruit biomass through value-added and green extraction technology – A critical review of advancements and processes**

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# Trends on valorization of pitaya fruit biomass through value-added and green extraction technology – A critical review of advancements and processes

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Abstract:

Background

The increasing production of food and agricultural waste worldwide imposes a severe burden on the environment, depletion of finite landfill space and irreversible environmental problem like global warming. In order to lessen this on-going threat, recent studies focus on valorization of waste products and value-added trends. Bioactive compounds mining is one of the targeted applications. The extraction of high value molecules therefore requires the design of novel green processes that could generate high yields of target compounds with less waste of harmful and toxic solvents.

Scope and Approach

In this review three green technologies are highlighted based on the quality and amount of pitaya (*Hylocereus spp.*) extracted. The different bioactive compounds and nutritional content found in the fruit are summarized using the extraction techniques. The cultivation, maturation, and pigment formation are thoroughly discussed to understand the formation of the natural pigment betacyanin.

Key Findings and Conclusion

Cultivation of high productivity pitaya fruit is becoming popular not only due to its exotic appearance but also as a promising super fruit attributed by the richness of bioactive compounds and nutritional content it poses including phytochemicals such as polyphenols, flavonoids, triterpenoids and organic dye betacyanin which promotes anti-oxidant activity and have demonstrated potent health-promoting effects including anti-microbial, anti-cancer, anti-obesogenic, anti-diabetic, anti-inflammation, anti-aging, and whitening properties. Moreover, the presence of sugar, polysaccharide, and pectin promotes its application in food industry. Future studies are recommended to explore the different parameters of green technologies to increase yield and get a better quality of extracts.

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## Keywords (5)

food industry, fruit peel, bioactive compounds, plant-based dye, betacyanin

## 1. Introduction

The increasing production of food and agricultural waste worldwide imposes a severe burden on the environment, depletion of finite landfill space and irreversible environmental problem like global warming. The Food and Agriculture Organization reports around 14 percent of the world's food amounting \$400 billion per year is being lost after harvest and during transport to producers. This global volume of food wastage is estimated at 1.6 billion tonnes of primary product equivalents causing an economic consequence running to \$750 billion annually excluding fish and seafood (FAO – State of Food and Agriculture, 2019).

According to Sharma et al. (2021), it is estimated that food waste amounts to US\$936 billion worldwide, annually. In Europe alone, it was reported by several studies (Corrado & Sala, 2018; Stenmarck et al., 2016) that the food waste generated ranges from 158 to 298 kg/year/capita. As the population increases, it is expected to rise if no additional prevention policies and activities are in place. One of the solutions presented by Shah et al. (2021) towards the growing threat brought by accumulation of food and agricultural wastes is by the adoption of ‘Sustainable Developmental Goals’ by all the countries. Reducing the loss of food along the supply chain and valorizing agri-food wastes are the main objectives of this concept. Through this, agri-food waste valorization can become a viable concept for sustainable bioeconomy by promoting strategies for the valorization of these materials in consideration of the food manufacturing industry through the idea of green economies or eco-economies (Garcia-Garcia et al., 2019). Some of the advanced valorization strategies such as chemical, biochemical, and thermochemical conversion strategies, extraction, fermentation, and enzymes technologies are used by researchers and food producers for a sustainable and efficient waste management approach (Otles & Kartal, 2018).

Bioactive mining from various fruits and vegetables has recently advanced as the demands for natural sources of bioactive raw materials for the most promising sectors, as identified by Teigiserova et al. (2019) and Stegmann et al. (2020), comprises high-value biomass uses such as bioplastics, pharmaceuticals, food and feed additives. These strategies were developed towards the direction of a sustainable, resource-efficient valorization of biomass and for the reduction and/or elimination of the wastes along the food supply chain through transformation into value-added products that will serve as raw materials for other products (Teigiserova et al., 2019).

Valorization of fruit by-products addresses the environmental concerns of waste management by reducing food waste volumes accumulated in landfills. Among the tropical fruits, pitaya could generate up to 22-44% peels during the processing of its pulp, especially for beverage production industry (Jiang et al., 2021). Pitaya is famously known as dragon fruit or pitahaya and belongs to the *Hylocereus* genus that are herbaceous perennial and climbing cactus that are highly tolerant to drought (Arivalagan et al., 2021). It is widely cultivated due to its high productivity and high nutritional value. Recent studies suggested the potential of pitaya to become a super fruit due to the rich number of bioactive compounds (Arivalagan et al., 2021; Attar et al., 2022), antioxidant, hypocholesterolemic, and cytotoxic activities (Paško et al., 2021; Fathordoobady et al., 2019; Luo et al., 2014a) and its high nutritional content found in both the fruit and its by-products. The activity profile of pitaya fruits may be associated with the presence of flavonoids and phenolic acids (Zain et al., 2019) but especially betacyanins (Fathordoobady et al., 2019), natural food pigments rarely found in edible products. These compounds have antioxidant and anti-inflammatory properties, which may be helpful in preventing some oxidative stress-related disorders (Quiroz-González et al., 2018).

This exotic fruit has recently given much interest to the European market being known to be the largest importer of fresh fruits and vegetables in the world. Several farms, especially in Reunion Island, are trying to cultivate it for fruit pulp and by-products that it produces (Le Bellec et al., 2006). Its production has significantly expanded throughout the world however, its production and marketing data are rarely available apart from its spontaneous expansion (Sharma et al., 2021). The major producers of world’s dragon fruit averaging to 93 % are Vietnam, China, and Indonesia. Table 1 shows the overall percentage contribution of different countries producing dragon fruit in the world. Vietnam alone produces up to 51.1% of the world production over an area of 55, 419 ha with average productivity of 22–35 metric

tons (MT)/hectar (ha)/year. Production and marketability of dragon fruit are expected to rise because of high demand and limited commercial producers.

This paper aims to contribute to a better understanding of the pitaya and the valorization processes that are being recently adapted to enhance the potential of this super fruit not only from its pulp but also from its by-products. The critical review presents the complete process from cultivation and maturation of fruit, promising bioactive compounds, green extraction techniques, application both in food, pharmaceutical, and cosmetic industries, and the growing interest and market size of pitaya fruit in Europe.

## **2. Cultivation, maturation, and pigment formation of Pitaya (*Hylocereus spp.*)**

### *2.1. Botanical classification*

The botanical family of Cactaceae and genus *Hylocereus* is the origin of pitaya. The genus is characterized by lianoid plants, with aerial roots and bearing glabrous berries with leafy scales (Fournet, 2002). In warm temperate and tropical semi-desert regions of Latin America, many different cultivated species and fruits are given the generic name of pitaya making it difficult to identify their botanical classification. However, all pitaya is grouped into four main genera: *Stenocereus* Britton & Rose, *Cereus* Mill., *Selenicereus* (A. Berger) Riccob and *Hylocereus* Britton & Rose genus (Le Bellec et al, 2006). *Hylocereus* comprises sixteen species and possess similar morphological characteristics and/or environmental conditions. Table 2 summarizes all the botanical characteristics and images of the different known species of pitaya commonly used for several research studies.

The introduction of the white pitaya on Reunion Island dates back a long time, but the precise date is unknown (Le Bellec, 2003). The red pitaya, on the other hand, is much rarer and is only found in gardens. The white pitaya is naturalized in the south and in the western heights' areas of Reunion Island (Bossier et al., 1984), where all its ecological requirements are met. As of present, only two species are listed as endemic to the Island; *Hylocereus undatus* (Haw) Britton & Rose or white pitaya and *Hylocereus purpusii* (Weing) Britton & Rose or red pitaya.

### *2.2. Maturation*

Fruit maturation determines the period during which the fruit reaches physiological maturity and time for fruit harvest and quality (Chuine et al., 2003). The maturation rates and post-harvest behavior of pitaya fruits, or their quality parameters are relatively unknown. Nerd et al. (1999) suggested some features considered as an indicator for maturity and important during the harvest of the fruit: pericarp color, soluble solid content, titratable acidity, and number of days after anthesis (DAA) and post anthesis (DPA). Harvested overripe fruit is likely to show senescence which causes quantitative and qualitative losses while prematurely harvested fruit is more susceptible to physiological disorders caused by cellular disorganization, and to the rupture of cell walls (Chitarra & Chitarra, 2005).

Pitaya fruit can be harvested when the peel turns fully red, i.e. from green to red or rosy-pink color (Nerd et al., 1999) . It is about 25 to 30 days after anthesis, then four to five days later, it will reach the maximum coloration (Le Van et al., 2002). If it is harvested five to eight days after full coloration, the storage life is slightly reduced but it is said to have improved fruit sensorial property and have a sweeter taste and blackberry flavor for the specific species *H. costaricensis* (Le Bellec et al., 2006). A study conducted by Wu et al. (2020a) on two native species of pitaya found in China- Jinghonglong or white pulp pitaya and Zihonglong or red-pulp pitaya, showed the effect of fruit maturation and

pigmentation on the production of citramalic acid. Figure 1(a) depicts the comparison of pigment formation of 'Jinghonglong' white-pulp pitaya and 'Zihonglong' red-pulp pitaya during maturation stage. The fruits were harvested during 22DPA and both red and white-pulp fruits show no pigmentation. The start of pigmentation was observed for Zihonglong between day 25 to 28 post-anthesis. The intense coloration of pulp was observed on 30 DPA indicating the full maturation of the fruit. Pitaya fruit was observed to have no production peak of ethylene or CO<sub>2</sub> due to its non-climacteric nature but its physico-chemical characteristics and sensory properties decline slightly after harvest (Nerd et al., 1999).

Classification of the fruit stages gives better accuracy if more than one variable is used to characterize the stage of maturation. By correlating these characteristics, we can validate them against each other to better understand fruit ripening. The result of the study by Ortiz & Takahashi (2015) observed that physical and chemical characteristics had an influence on the maturation of pitaya fruits. They reached physiological maturity between 30 and 32 DAA with the fruit turning completely red at 30 DAA and becoming intense at 31-32 DAA. This is in comparison to published study of Martinez Chàvez (2011) which suggested that the useful stage of harvest was between 25 to 31 DAA. Timing is therefore one of the important factors when harvesting the pitaya fruit in order to achieve better fruit quality and extending shelf life. The 22<sup>nd</sup> to 30<sup>th</sup> days after anthesis are the crucial days for harvest as the fruit is in its mature stage while extending for two to three more days may result to intense coloration of the pulp and peel but increases the chance of losing some of its sensory properties.

### 2.3. Pigment formation

Color is an important parameter for producers and consumers because it indicates whether the fruit displays the ideal conditions for commercialization and consumption. However, in most cases, color does not contribute to an effective increase in the nutritional value or quality of the product (Chitarra and Chitarra, 2005). According to Nerd et al. (1999), it takes 4 to 5 days for the pericarp of pitaya fruit to become completely red after the first change in color. This is between 30 to 32 DAA while a similar study in Vietnam, specific to *H. undatus* showed that the change in color occurs in 28 to 30 DAA (Ortiz & Takahashi, 2015). The red coloration of the pericarp in pitaya fruit is the result of the accumulation of betacyanins (Wybraniec & Mizrahi, 2002; Le Bellec et al., 2006). This pigment is synthesized due to the high availability of sugar and light as the fruit reaches maturation stage.

In the study by Wu et al. (2020a), the distribution characteristic of pigment cells in the peel (Figure 1(b and c)) and pulp (Figure 1(d and e)) of matured pitaya fruit was compared and monitored from the start of pigmentation until the complete appearance of pigments at mature stage. It was observed that the red pigment in pitaya peel found in the cell forming horizontal layers beneath the epidermal cells was distributed in a linear pattern. The outermost layer of the peel was the cuticle, followed by a layer of small and closely arranged epidermal cells. As the volume of the peel cells increased gradually, the color of the pigment cells appeared lightened looking at the surface to the inside of the cell. Prior to maturation, the cells of the peel appeared to have green similar granules and no pigment cell was observed. Upon approaching maturation Figure 1(b), the cells containing the pigment start to appear and the entire pigment cell was colored red when the fruit is already in the maturation stage, Figure 1(c).

In comparison to the pigmentation pattern of pitaya fruit peel, a few pigmentation cells were already observed in the pulp during the early stage of fruit development. As the fruit reaches the maturity stage, more pigment cells are observed. Green granular tissue similar to that observed in the granular tissue of the peel was not observed in the pulp cells of 'Zihonglong' (red-pulp) at the beginning stage of maturation. The pigment emerged mainly as scattered dots at color broken stage and as it reaches the coloring stage in Figure 1(d) the entire pigment cell shows a faint pink pigmentation. During maturation, it was observed that the pigment cells of the pulp were relatively concentrated Figure 1(e), and the color of each cell did not have the same intensity as the pulp approached maturity. The characteristic of pitaya fruit manifested that there were obviously spatial and temporal difference in coloring process of different single cells, and the increase of pigment cells and the deepen of pigment in cells promoted the appearance of redness in pitaya fruit (Wu et al., 2020a).

The de-greening of the pericarp to produce the typical fruit color for the species provides an important indicator of the state of maturation of the fruit and optimal time of harvest (Chitarra & Chitarra, 2005). The increased in enzymatic degradation of chlorophyll as a result of strong absorption of light in the red and blue region as the fruit ripeness could be attributed to the color change in fruit peel (Ding et al., 2007).

The non-climacteric nature of pitaya requires knowledge of ideal cultivation and harvesting methods in order to produce export quality standards. As discussed previously in section 2.2, the number of days after the flower opening of the plant until it reaches the maturity stage is crucial for the development of physico-chemical and sensorial properties of the fruit. In addition, cell pigment formation in various species of pitaya is affected by temporal and spatial differences as shown by the varying colors of peel and pulp of the fruit in table 2.

### **3. Bioactive compounds found in pitaya**

This part of the review summarizes the nutritional content, phytochemical profile and antioxidant activities of pitaya (*Hylocereus spp.*) pulp and peel. It is said that the fruit contains several essential nutrients, like sugar, polysaccharides, pectin, lipid, amino acids, and vitamins and minerals, making it an ideal superfruit and food ingredients, such as natural food dye, dietary fiber, functional foods, active food packaging, edible coatings, and nutrition fortifiers. These macronutrients found from pitaya raise interest among researchers to explore the future trends in food application of the fruit and its waste byproducts especially in smart food packaging as we are leading towards sustainability, eco-friendly and organic food product designs. Moreover, the significant amount of polyphenols, betalain compounds, and triterpenoids present in this fruit makes it a potent free-radical inhibitor capable of producing high antioxidant activities. These substances have been shown to actively protect the cells from damaging free radicals and their abundance in pitaya gives the fruit its future prospective as food supplements offering various biological activities such as, anti-diabetic, antiproliferative, cardioactive, anti-cancer – to name a few and strong health-promoting potential. Figure 4 shows the overall summary of bioactive compounds found in pitaya making it an ideal superfruit with promising potential application, not only the fruit, but also its waste byproducts for food enhancement and industries like pharmaceuticals and cosmetics.

#### **3.1. Betalain pigment**

The pigment betalain originated from tyrosine in fruits and vegetables is a natural water-soluble pigment containing nitrogen. It is divided into two groups: betacyanin

and betaxanthin (Gandía-Herrero & García-Carmona, 2013). These compounds are hydrophilic pigments and responsible of the bright coloration of fruits and flowers, roots and leaves of plants belonging to the order Caryophyllales. Among the Caryophyllales plants, red beet roots (*Beta vulgaris*) (Hempel & Bohm, 1997) and the dragon fruits from *Hylocereus* cacti (mainly *Hylocereus polyrhizus*) are two of the most known edible sources of betacyanins and betaxanthins (Wybraniec & Mizrahi, 2002). According to Wink (1997), these compounds are synthesized in the vacuoles of the cells and mainly found in the epidermal and sub-epidermal tissues of plants because of their hydrophilic nature. The violet betacyanins absorb light at a wavelength around  $\lambda_m = 536$  nm and as a result of the glycosylation and acylglycosylation of one or two hydroxyl groups while the yellow betaxanthin, where no glycosylation has ever been reported, shows an absorbance spectrum centered at a wavelength around  $\lambda_m = 480$  nm (Gandía-Herrero et al., 2016). Both groups share betalamic acid as the structural and chromophoric unit. It is condensed with amines and amino acids in the immonium derivative betaxanthins and with cyclo-dihydroxyphenylalanine (cyclo-DOPA) in betacyanins (Gandía-Herrero & García-Carmona, 2013).

Betalains are known to have a wide range of biological activities as an antioxidant, anti-inflammatory, hypoglycemic, antiproliferative, cardioactive, radioprotective, neuroprotective, diuretic, hypolipidemic, and osteoarthritis pain reliever as discussed in the article review published by Ibrahim et al. (2018a). It was shown to have an active dose-dependent inhibition of cancer cell growth and proliferation with the studies on different cell lines. Dietary betalains have also demonstrated strong health-promoting potential by inhibiting the formation of tumors in vivo in mice. Unlike other families of plant pigments, the biosynthetic pathway of betalains remains to be fully clarified (Gandía-Herrero & García-Carmona, 2013).

### 3.2. Polyphenols

These are group of antioxidant compounds believed to have high radical scavenging ability that reduces the risk of chronic diseases. Phenolic acids, flavonoids, stilbenes, and lignin are some examples of this bioactive group (Wan et al., 2020). They are found to inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase activity together with the modulation of postprandial hyper-glycaemia due to the interaction between starch and polyphenols (Quek & Henry, 2015). The betalain pigments found in pitaya are said to be rich in polyphenols and have been reported to have high bioactive activity, and anti-inflammatory and anti-tumor cell line health benefits (Yong et al., 2017a). In the study conducted by Arivalagan et al. (2021) a total of 48.30 mg Gallic Acid Equivalent/100 g, 31.2 mg Citric Acid Equivalent/100 g and 20.40 mg Betacyanin Equivalent/100 g total phenolic, flavonoids, and betacyanin were measured, respectively.

In another study Bhagya Raj & Dash (2020), the authors were able to identify that pitaya seeds and peels possessed higher total polyphenols and betacyanins than pulps. Red pitaya seeds exhibited the highest amount of total phenolics and flavonoids, with 375.1 mg GAE/g and 264.4 mg Quercetin Equivalent/g (QE/g), followed by peels, 294.8 mg GAE/g and 193.8 mg QE/g. The use of liquid chromatography coupled with mass spectrometry or diode-array detection (MS/DAD) techniques are used to have an idea of the polyphenol profiles from pitaya

fruit. Some of the identified phytochemicals such as polyphenols, flavonoids, and organic acids are summarized in Table 4.

### 3.3. *Sugar and polysaccharides*

Polysaccharides are a group of long-chain polymeric carbohydrates composed of more than hundred monosaccharides bound with glycosidic linkages. The peels of the pitaya fruit contain polysaccharides which are valuable for food products due to their stabilizing property, gelling activity and as thickeners. Polysaccharides in red pitaya peel have been extracted and purified by various conventional and novel extraction techniques in recent studies (Ouyang et al., 2021; Wu et al., 2020). The extraction of polysaccharide is strongly affected by a high temperature, thus Castro-Enríquez et al. (2020) used an ultra-high-pressure enzymatic technique to extract polysaccharides from red pitaya peel. The results showed that the main component of polysaccharides derived from red pitaya peel was a polysaccharide with 112.94 kDa average molecular mass, 17.19% degree of esterification, 97.22% water solubility, 5.51 g/g water holding capacity and 4.52 oil holding capacity. Galacturonic acid (42.56%), galactose (22.55%) and rhamnose (16.53%) were the main compositional monosaccharides.

The contents of carbohydrates (sucrose, glucose, xylose, fructose, total sugars) were also measured by Attar et al. (2022) in fruits of two pitaya species (red and white pulp). It was noted that the sugar composition affects the perception of sweetness in pitaya fruits. They were able to measure the sugar contents of the red-fleshed specie with glucose (7.52%) found to be higher than fructose (3.70%) and xylose (0.03%). On the other hand, in the white-fleshed species, glucose content (5.22%) was lower, but fructose was higher than in the red-fleshed species. The dominant sugar in both species was found to be glucose with a high value detected in the red-fleshed species of pitaya. In addition, the total sugar content of the red-fleshed species (11.25%) was higher than in the white-fleshed one (10.24%).

### 3.4. *Pectin*

Pectin is a native high-molecular weight natural polysaccharide, accounting for up to 35% of the cell wall and middle lamellae of many land-growing plants, especially those of fruits and vegetables (Zhang et al., 2015). The sugar acid, D-galacturonic acid (GalA) - an oxidized form of D-galactose, constitutes the main monomeric unit of the rather complex pectin molecule. The peel of the red pitaya fruit is one of the most important sources of pectin used as a natural gelling and thickening agent in the food industry used in fruit juice, fermented dairy products, jams and jellies (Chen et al., 2015). In the study conducted by Thirugnanasambandham & Sivakumar (2017), the maximum yield of extracted pectin from pitaya peel using microwave-assisted methods was 7.5%. Another study by Montoya-Arroyo et al. (2014) characterized the cell wall polysaccharides of pitaya peel and revealed the total pectic fraction constituted about 50.23% of the cell wall, with the major fraction being the water-soluble pectin fraction, followed by the oxalate-soluble pectin fraction. They also noted that these products could substitute for commercial gums in food and cosmetic products. Muhammad et al. (2014) investigated the degree of esterification, molecular weight, and monosaccharide composition of extracted pectin from red pitaya peel. It was identified that the fresh inner layer of the red dragon peel appeared to be high methoxy pectin with 63.74% of the degree of esterification and  $0.88 \times 10^5$  Da molecular mass. Moreover, the predominant monosaccharide in pectin was found to be galacturonic acid averaging to 39.11%, followed by mannose (17.78%) and rhamnose (14.47%).

### 3.5. Amino acids

Amino acids are the building blocks of protein and made up of an amine group (-NH<sub>2</sub>) in one end and carboxyl group (-COOH) functional groups with a side chain that determines the specific type of amino acid (Yu & Yang, 2020). The nine essential amino acids that the human body cannot synthesize and must come from food are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. These vital amino acids are said to be abundant in some fruits. Wu et al. (2020) identified phenylalanine (183 mg/g) as the most abundant amino acid found in red pitaya (same result obtained by Arivalagan et al. (2021)) followed by arginine (126-149 mg/g), glutamic acid (101-127 mg/g), and proline (69.6-116 mg/g). The guidelines set by FAO/WHO, (2014) the chemical score calculated for essential amino acids showed that dragon fruit is a good source for essential amino acids such as histidine, lysine, methionine, and phenylalanine which had a chemical score above 100, while cystine, tryptophan, and valine had a chemical score of <100. Despite this high value, the amount of protein present in the fruit is only about 0.887 to 1.11 g/100 g, and dietary reference intake of protein for normal population is about 0.83 g protein/kg body weight, and thus the contribution of dragon fruit for essential amino acids intake in the diet could be minimal. Both studies stated that red pitaya was a good source of essential amino acids, but the protein concentration in the fruit was much lower than the required nutrient reference values for daily intake.

### 3.6. Vitamins and mineral profile

The pulp of pitaya fruit is not only rich of phytochemicals such as polyphenols and flavonoids but also in vitamins and minerals. Moreover, the edible black seeds interspersed in the pulp are said reportedly rich in soluble sugars, proteins, and minerals like potassium, magnesium, and calcium, as well as other bioactive compounds (Ibrahim et al., 2018). The mineral constituents (macro and micro) of pitaya fruits were measured by Arivalagan et al. (2021) based on 100 g of edible part of fruit, fresh weight basis. Moreover, pitaya fruits contain a considerable amount of both water- and fat-soluble vitamins. Potassium (193 mg/100 g) was found to be the highest mineral followed by magnesium (45.9 mg/100 g), then calcium (45.7 mg/100 g), and phosphorus (22.5 mg/100 g). These macro minerals were measured from different varieties of pitaya (red and white pulp). Meanwhile, micro minerals like iron content (1.55 mg/100 g) was found to be high followed by zinc (0.38 mg/100 g), while manganese and copper contents were low.

### 3.7. Triterpenoids

Another group of bioactive compounds that exhibits antioxidant and anticancer effects are triterpenoids found abundantly in fruits and vegetables. It could be used as a cytotoxic agent against tumor cells to prevent breast cancer (Bishayee et al., 2011). The mechanism of cancer prohibition focuses on triterpenoids preventing cell proliferation and migration, along with the promotion of apoptosis (a process in the human body that removes abnormal or unneeded cells), leading to the cleavage of cancer cells (Li et al., 2020). In the study of Luo et al. (2014b) using supercritical carbon dioxide to extract red pitaya, the authors were able to identify four types of triterpenoids namely:  $\beta$ -amyrin (15.87%),  $\alpha$ -amyrin (13.90%), octa-cosane (12.2%) and  $\gamma$ -sitosterol (9.35%).

#### 4. Different methods of extraction of bioactive compounds

Over the years, several methods of extraction have been developed and optimized to extract the targeted bioactive compounds and increase the percentage yield with better purity. Many studies would still use the different conventional type of extractions as it is widely studied and proven to produce better yield. Some shortcomings of using the traditional extraction technologies were observed such as high energy consumption, the use of a large amount of solvents, and long processing time for extraction. The search for novel “green” extraction techniques that would address all these issues on conventional techniques encouraged chemists to venture on potential methods that would combine lesser amount of solvent and high-power systems to achieve rapid extractions of bioactive compounds.

##### *4.1. Conventional methods of extraction*

Conventional extraction is a widely used method of extraction for a variety of plant and fruit biomass. It sometimes referred to as conventional solvent extraction techniques because it utilizes a solvent other than water to extract the bioactive compounds present in the plant sample. Some of the existing types of conventional method includes: (1) hydro-distillation which could be further classified into (i) water distillation; (ii) water and steam distillation and (iii) direct steam distillation; (2) Soxhlet extraction; (3) maceration; (4) percolation; (5) infusion, (6) decoction; and (7) hot continuous extraction, etc. These methods work with varying the plant biomass (leaves, fruits, stem/bark, or root) and size of the sample (fine powder or coarsely cut), the duration of soaking time in solvent (1 hour up to 72 hours), temperature, and type of solvent. Moreover, they require higher volume of mild or more polar solvents for extracting directly from biomass. Some of the commonly used solvents in these processes are ethanol, methanol, hexane, n-butane, petroleum ether, and water (Patra et al., 2022).

In general, the process of extraction is determined based on the amount of solvent used, the types of equipment and the compounds extracted. Solvent types play a major role in the extraction process in order to screen the analyte of interest. According to Ares et al. (2018), high polar solvents are used to extract bioactive compounds while low polar solvents such as petroleum, ether, and chloroform are used to extract lipophilic compounds and certain pigments, such as carotenoids and chlorophyll. Acidified high polar solvents are good in extracting anthocyanin and flavonoids (Okolie et al., 2019).

In the study of Ramli et al. (2014) a conventional method of extraction was used to investigate the total phenolic content and total flavonoid content, betacyanin content, and antioxidant activity using ferric ( $\text{Fe}^{3+}$ ) reducing antioxidant power and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay of pitaya peel and pulp. The solvent used was water at 1/25 (w/v) and 1/55 ratio for pulp and peel, respectively. The results showed that the highest extraction yield was obtained from the peel of red dragon fruit extracted using a conventional extraction method (95.25%). Betacyanin content of pitaya pulp (82.79 mg/100 g) was higher as compared to peel (18.67 mg/100 g) when extracted using the conventional method. These results were slightly higher than those obtained by Tang & Norziah (2007) in which the extraction was performed at room temperature (25°C) and found  $10.1 \pm 0.6$  mg/100 g dry weight and  $6.7 \pm 0.2$  mg/100 g dry weight in pulp and peel, respectively. Higher TPC was measured for both peel and pulp showing the capacity of the traditional method to extract bioactive compounds in an aqueous solution. Whereas, antioxidant activity showed that the conventional method was not an effective means of extraction compounds capable of free radical scavenging

activity in the peel of pitaya as the results showed lower values as compared to the other methods performed in the study. Interestingly, four times increase in the scavenging activity was observed from the pulp extracted for a longer period (2 hours) using the conventional method at 50°C. The effects of time and temperature during sample preparation and extraction on antioxidant capacities are unique to the specific plant parts (Ramli et al., 2014).

In general, the use of conventional method of extraction depends on the type of solvent. However, in order to reach the maximum yield of the target bioactive compounds, ideal organic solvents are widely used due to their effective extraction with varying polarities. These solvents are considered harmful for human exposure and for the environment, thus recent green-technologies are being explored to extract molecules of interest and using water as a universal solvent.

#### *4.2. Ultrasound extraction*

Power ultrasound is an intensification technique that is widely used for the extraction of bioactive compounds from natural products with applications in industries such as food and pharmaceuticals (Chaves et al., 2020). This extraction technique utilizes ultrasonic energy with high intensity as a result of the implosion of cavitation bubbles producing physical, chemical, and mechanical effects (Rojas et al., 2016). Ultrasound is generated by ultrasonic transducers that turn the electric energy into acoustic waves at the desired frequency (>20 kHz) and intensity (>1 W/cm). The accumulated energy in the bubbles reaches a critical point where they implode and releases this energy, breaking intermolecular interactions between target compounds and the sample matrix (Um et al., 2018). The mechanical energy released interact with the solvent system releasing high pressure enough to break the cell membrane of the particle, resulting in the release of bioactive compounds within a short period of time. The ultrasound assisted extraction (UAE) operates at low frequency ultrasound (20 to 100 kHz) in order to see the physical and mechanical effects on the cellular destruction of the biomass extracted. Moreover, Chemat et al. (2008) pointed out that the use of ultrasonic energy in the extraction of bioactive compounds facilitates more efficient mixing, faster energy transfer, reduced thermal gradients and extraction temperature. In general, the main advantages of UAE among other techniques in recovery of natural pigments from biomass are reduced extraction and processing time, energy, CO emissions and solvent used, enhance extraction yield, use of aqueous extraction or alternative clean and/or green solvents (Carrera et al., 2021a; Chemat et al., 2017). However, some limitations of using UAE is mainly related to the degradation of pigments under severe conditions like processing at high temperature, power, and/or amplitude for long processing times, difficulty of extrapolation of optimized parameters during scale-up and energy consumption which is highly dependent on several factors such as the US system, additional techniques associated with the UAE process, and sample matrix and composition (Linares & Rojas, 2022).

The effect of ultrasound on the betacyanin content was measured by Pérez-Loredo et al. (2017) using ultrasonic bath (Branson CPX5800H, USA) with 40 kHz frequency under the controlled temperature of 20°C and two extraction time of 5 and 35 minutes. It was reported that when the ultrasound power is increased from 0.5 W/g to 3.5 W/g, a 68.92% increase in the yield of betacyanin was observed. Using the lower power ultrasound (20 kHz) with shorter sonication time of 2.5 min gave them a 0.2014 mg/g amount of betacyanin which is similar to the results reported by other studies using power ultrasound (Phan Van et al., 2020; Chemat et al., 2008).

Another study Carrera et al. (2021a) used UP200S ultrasonic device (Hielscher Ultrasonics GmbH, Teltow, Germany) with 7mm diameter probe with 200 watts power and 24 kHz frequency. It was concluded that UAE method is a reliable, economical, and rapid technique for obtaining greater yields of betacyanin compounds from red dragon fruit. Using chemometric tools such as Box–Behnken Design (BBD) in conjunction with Response Surface Methodology (RSM), they were able to evaluate and optimize relevant UAE extraction variables such as temperature, solvent composition, sample-to-solvent ratio, power and cycling. Using real samples of red dragon fruit from three different origins and the application of optimized UAE method, they obtained a range of total betacyanin content comparable to other published values, 0.5–0.6 mg/g (Yong et al., 2017a) or 0.6–0.8 mg/g (Tenore et al., 2012), and for freeze-dried samples, 1.0–1.6 mg/g (Bhagya Raj & Dash, 2020). The optimum extraction conditions were established as 21.4% methanol in water at 54.6 °C for 5 min under 20% ultrasound power, a 0.3/s cycle and a 0.2/10 g/mL sample-to-solvent ratio. The proposed optimized method was effectively validated with adequate linearity and precision values (Carrera et al., 2021a). Tentative identification and quantification of UAE betacyanin compound extracted from pitaya peels was performed by using mass spectrometry (Roriz et al., 2022). Four compounds were tentatively identified at various retention time, namely betanin at 19.40 min ([H]<sup>+</sup> at m/z 551), isobetanin ([H]<sup>+</sup> at m/z 551) at 20.86 min, phyllocactin ([H]<sup>+</sup> at m/z 637), and iso phyllocactin ([H]<sup>+</sup> at m/z 637) that appears at 22.74 and 23.76 min, respectively. Phyllocactin (14.8 mg/g) was found to be the major compound present followed by isobetanin, isophyllocactin, and betanin. Overall, a total of  $48.3 \pm 0.8$  mg/g betacyanin was measured from the peels of pitaya. Moreover, tocopherols, organic acids, phenolics, flavonoids, and other bioactive compounds were extracted in several pitaya species using UAE as presented by several studies in Table 3. Commonly used solvent was a mixture of alcohol and water at lower concentration and US frequency ranging from 20 kHz to 100 kHz while the quantification method used is primarily by High Performance Liquid Chromatography coupled to a Diode Array Detector/Mass Spectrometry (HPLC-DAD/MS). Results obtained by various studies showed that UAE increased the yield of betacyanin and phytochemicals (phenolics and flavonoids) extracted at room temperature and shorter period of time. However, limitations of using this technology, such as energy consumption which has an economic impact and the difficulty of extrapolating optimized parameters during scale-up, can be addressed with further supplemental studies of various parameter optimization for high- and better-quality yield of target bioactive compounds.

#### *4.3. Microwave-assisted extraction*

The fundamentals of the microwave assisted extraction (MAE) process differ greatly from the conventional methods of extraction as they result from electromagnetic wave-induced changes in cell structure. Microwave energy is a non-ionizing radiation that covers a third order of magnitude scale from 300 MHz to 300 GHz (Chemat et al., 2012). A typical extraction procedure takes 15–30 min and uses small solvent volumes in the range of 10–30 ml. These volumes are about 10 times smaller than volumes used by conventional extraction techniques. The sample throughput increases as several samples can be extracted simultaneously (Eskilsson & Björklund, 2000).

The principle of microwaves heating is based on its direct effects on the molecules of the material. Electromagnetic energy is converted to heat following the mechanisms of ionic conduction and dipole rotation. According to Jain (2009), MAE involves three sequential

steps; (1) separation of the solutes from the active sites of the sample matrix under increased temperature and pressure, (2) diffusion of solvent across the sample matrix, and (3) release of the solutes from the sample matrix to the solvent. In order to achieve higher yield of target compounds using MAE, the operating condition parameters should be in their maximum efficiency namely: solvent extraction, temperature and time of the extraction, microwave power and the physicochemical characteristics of the material (Eskilsson & Björklund, 2000). MAE is a feasible green solvent extraction method as it uses water or alcohols at elevated temperature and controlled pressure conditions. The limitations of this technique would relate to the higher operating costs due to the nature of the process which requires special equipment for the microwave intensification. In the review paper conducted by Zin et al. (2020) for the recovery of phytochemicals- betalain and phenolics, MAE achieved 1.7 times higher percentage yield of phenolic compounds whilst operation time was two times shorter than UAE (Wang et al., 2010). In addition, 5 minutes of microwave irradiation could provide an equivalent amount of phenolic and flavonoid compounds with an hour of ultrasonic treatment time (Gharekhani et al., 2012). Some of the optimum conditions such as; power (100 – 600 W), exposure time (1 min to 8 mins), and solid-to-solvent ratio (0.5% -5%) within experimental variable ranges, for the extraction of betacyanin and phenolic compounds from pitaya fruit and family as well as their wastes was detailed by Zin et al. (2020) in their review article. Based on the amount of betacyanin extracted, the use of methanol as solvent is favorable as compared to pure water. Increasing the power and longer exposure to microwave does not significantly affect the percent yield of betacyanin but the data presented are not enough to draw a conclusion on the effect of the different parameters used in MAE of betacyanin. However, the use of this technology reduces the extraction time and energy expenditure. In addition, it is also considered safe due to its non-ionizing radiation and reduces the use of toxic organic solvents.

#### *4.4. Supercritical fluid extraction*

Supercritical fluid extraction (SFE) is a technique that uses supercritical fluids (systems formed by one or more compounds at conditions over their critical values of pressure and temperature) as an extraction solvent to separate one component (the extractant) from another (the matrix) (Rojas et al., 2016). The process involves a mobile phase subjected to pressure and temperature near or above the critical point to enhance its solvating power (Fathordoobady et al., 2019). Instead of using a traditional organic solvent, a supercritical fluid mostly CO<sub>2</sub> (T<sub>c</sub> = 31°C, P<sub>c</sub> = 74 bar) and water (T<sub>c</sub> = 374°C, P<sub>c</sub> = 221 bar) is widely used.

The delivery system of supercritical fluid is one of the major parts of the basic SFE system and it must be of high purity. The pumps are used to drive carbon dioxide at high pressure while maintaining constant flow at a controlled temperature in the furnace. While the restrictor depressurizes the fluid by passing existing supercritical conditions from the extraction cell to the atmospheric conditions (Azmir et al., 2013). The use of SFE can substantially reduce extraction time by using supercritical fluid like CO<sub>2</sub> which has higher diffusion coefficient, lower viscosity and surface tension than a liquid solvent leading to more penetration of the solvent into the sample matrix. The supercritical fluid is highly selective, so it can be used for targeted compound extraction and it also provides complete extraction with the repeated reflux of supercritical fluid. Moreover, SFE is operated at room temperature, making it an ideal method for extraction of thermolabile compounds. The only drawback of carbon dioxide is its low polarity which makes it ideal

for lipid, fat and non-polar substances, but unsuitable for most pharmaceuticals and drug samples (Azmir et al., 2013).

In the study of Fathordoobady et al. (2019) SFE was used to extract betacyanin from red pitaya fruit (*Hylocereus polyrhizus*) peel with CO<sub>2</sub> as solvent and EtOH/water (v/v) as co-solvent. It was optimized by applying 2<sup>3</sup> factorial experimental design and three independent variables were considered: pressure (20-30 MPa), temperature (40-60°C), and co-solvent concentration (10-20%). A flow rate of 2 mL/min of supercritical CO<sub>2</sub> solvent and 90 minutes for the dynamic time of extraction were found to give the optimal conditions of SFE with the maximum extraction yield of 4.09 ± 0.69%, and total betacyanin content of 25.49 ± 1.54 mg/100 mL.

In table 3, betacyanins can be efficiently extracted from pitaya by SFE-CO<sub>2</sub> with EtOH/water (10/90, v/v) as the co-solvent. The extracted total betacyanin was measured from both peel and pulp extract (24.58 and 91.27 mg/100 ml, respectively) at 25 MPa pressure and 10% EtOH/water (v/v) mixture as a co-solvent and could increase up to the value of 28.44 and 120.28 mg/100 ml for peel and pulp, respectively using the most desired parameters.

The major compound identified using HPLC-MS/MS analysis was betanin ([H]<sup>+</sup> at m/z 551) found at retention time of 7.9 min, followed by phyllocactin ([H]<sup>+</sup> at m/z 637, rt. 10.9 min), and their corresponding C-15 isomers. These betacyanins were previously identified in different species of *Hylocereus* (Wybraniec & Mizrahi, 2002; Roriz et al., 2022). The total betacyanin concentration from the extract was found to be 23.16 ± 1.08 mg/100 mL and expressed as betanin equivalent.

SFE has been widely explored especially for large scale extraction due to ease of availability of supercritical CO<sub>2</sub>, non-toxic, cheap and affordable, non-explosive, and better treatment of product during the process at temperature lower than 100°C as well as easy separation of solvent and extract. However, one of its main limitation is the use of relatively high pressure for supercritical CO<sub>2</sub> compared to hydrocarbon solvents which implies the use of larger machines and higher energy consumption.

The three emerging technologies discussed in this review were the most popular among other technologies and frequently developed and improved in various research studies. They have shown significant potential in increasing the extraction yield while, decreasing extraction time, energy, and solvent consumption. A comprehensive summary of the three-extraction methods on different pitaya species, quantification method, and concentration of the biomolecule extracted obtained is shown in Table 3. Freeze-drying is the commonly used sample preparation prior to extraction and alcohol-water mixture is the commonly used solvent. Data presented shows that SFE gives higher yield for extraction of phenolics and betacyanin in the peel while UAE has higher yield of the two biomolecules when used for extraction of the flesh of pitaya. Figure 3 summarizes the factors and parameters affecting the efficiency and percent yield using SFE, UAE, and MAE when extracting bioactive compounds. The time of exposure of the sample in the ultrasound wave greatly affects the yield and quality of extract as well as the US system, solvent, ambient condition, sample matrix, and solid/liquid ratio. Whereas for MAE, only the microwave parameter which includes power, frequency, power density and duration of exposure, and the material factors are the considerations. The SFE is dependent on the extraction temperature and pressure in order to reach the critical point of the CO<sub>2</sub> solvent which gives its high fluidity state allowing it to penetrate inside the material. The use of co-solvent, type of raw material, and solvent-to-feed ratio were also considered in increasing the percent yield of extraction.

## 5. Application of pitaya in various industries and emerging role in biomedical field

The growing interest in consumption of pitaya fruit due to its nutraceutical properties and extensive content of phytochemicals, especially phenolic compounds, betacyanin, vitamins and minerals, and terpenoids has drawn interest from researchers to further explore the application of this fruit and its byproducts. The fruit peel can be used as an anti-bacterial agent, natural dye, antioxidant and high source of pectin, whereas the fruit pulp was produced into jam, wine, juice and powdered starch or eaten fresh due to its nutritional benefits. The seeds are good source of oil which contains 50% essential fatty acids and added as ingredients in many food products such as syrups, ice creams, candies, and yogurts (Nishikito et al., 2023).

The natural food dye betacyanin found in pitaya peel and pulp is not only used in food products to improve appearance, flavor, nutrition and shelf-life, as well as for potential applications in food packaging and edible coatings, but it is also widely used in cosmetic industries. It was found that pitaya extract has a potential to be a good skin whitening product due to the presence of Vitamin C and good tyrosinase inhibition (Vijayakumar et al., 2018). In addition, it can also be added as a moisturizing agent to sunscreen giving high photoprotective value equivalent to sun protection factor (SPF) of commercial products (Vijayakumar et al., 2020).

Finally, recent advancement in biomedical and pharmaceutical field involves the use of organic synthesized carbon-dots with wide applications from drug formulation, imaging, photothermal and photodynamic therapy.

### 5.1. Food industry

#### 5.1.1. Active packaging incorporation in starch

The non-biodegradable synthetic polymer-based packaging has caused serious environmental problems. So, the development of natural and biopolymer-based packaging has become a global priority in recent years. Qin et al. (2020) has found a possible solution through development of active and smart-packaging by incorporating betalains from red pitaya (*Hylocereus polyrhizus*) peel into starch/polyvinyl alcohol films. Exploration of natural pigments like anthocyanin, betalains, and curcumins to be added into starch-based films for extended shelf life of food products and monitors the freshness of meat, milk and seafood have been extensively studied (Domínguez et al., 2018; Liu et al., 2017). In order to improve the physical properties of starch films, starch must be blended with other polymers. Polyvinyl alcohol (PVA) has been widely used in packaging materials due to its biodegradable, chemical resistant, film-forming, oxygen barrier and mechanical properties but one of its limitations is the lack of functional properties (e.g. antioxidant and antimicrobial activities) required for active packaging (Liu et al., 2017).

The study of Qin et al. (2020) aims to develop active and intelligent packaging films based on starch/polyvinyl alcohol incorporated with betalains-rich red pitaya (*Hylocereus polyrhizus*) peel extract. The incorporation of the extract enhanced the water vapor barrier and ultraviolet–visible light barrier ability, as well as the mechanical, antioxidant and antimicrobial potential of the films. They tested the thin film incorporating 1.00 wt.% of the betacyanin extract with ammonia and found that it was more sensitive than other films. It was also used in actual application to monitor the freshness of shrimps. The film containing

1.00 wt.% of the extract presented visible color changes due to the accumulated volatile nitrogen compounds during the spoiling process of shrimp.

### 5.1.2. *Fat replacer for ice cream*

The food industry aims to develop products which balances healthy diets while preserving their technological properties and flavor. One of the significant challenges for sweets and dairy related food products is the reduction of carbohydrates or lipids. Low-fat diets help prevent the risk of chronic diseases, like cardiovascular disease, diabetes, and cancer (Kalicka et al., 2019) but for ice cream making, fat has an essential function by increasing the richness of flavor, provides good structure and texture, and contributes to melting resistance, crystallization, and cold sensation.

A group of researchers, Utpott et al. (2020) from Brazil, characterized the pitaya peel flour and evaluated its potential application as a fat replacer in strawberry ice cream, through the determination of its chemical, physical, sensorial, rheological properties, and potential antioxidant. Technological properties of flour-derived from pitaya like solubility and oil/water holding capacity were studied. The study showed the product created had high sensorial acceptance and, despite differences in scores compared to reference products, it was in general well evaluated by the consumers, indicating that pitaya fiber is a potential fat replacer in ice creams. Upon using of pitaya fiber, a significant reduction of 73.5% on lipid content was observed.

In a similar study by Javidi et al. (2016), a 75% lipid content substitution by gum combinations were evaluated for rheological, physical, and sensory properties, and the results indicated that the ingredients were effective as fat replacers/stabilizers for low-fat ice creams. The strawberry ice cream added with pitaya peel powder also presented a higher protein value in relation to control, as well as higher mineral content, due to the high ash content of the flour. Similar value was obtained by McGhee et al. (2015) for protein and ash contents (0.9%–3.9%) when developing ice creams with reduced fat content. In terms of the physical parameters like color, melting rate, and overrun from ice creams, Utpott et al. (2020) noted that fat substitution did not cause color changes in the products. The flavoring of ice cream, besides improving its organoleptic characteristics, can be an excellent alternative to the standardization of color, in case of formulation modifications.

### 5.1.3. *Oxidative stabilizer for ground pork patty*

The novel technology of active packaging has been widely used for the improvement of the condition of packaged food and extension of its shelf-life by release of incorporated component in the food or to the environment. Meat products are most commonly used for preservation, shelf-life and active packaging studies due to its lipid oxidation and most likely oxidative processes that promote food deterioration, leading to changes in flavor, color, odor, and potentially causing consumer's rejection (Falowo et al., 2017). Popular techniques used to prolong quality and freshness of meat products are the use of high-pressure processing (HPP) (Cunha et al., 2018) and exposure to UV type C radiation (Cunha et al., 2021). Moreover, food industry uses synthetic antioxidants mainly butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) to improve the redox stability of food. Due to the toxic

effects associated with the consumption of synthetic antioxidants and the increasing demand for safe and healthy products, the meat industry seeks natural alternatives capable of maintaining the oxidative stability of meat during refrigerated storage. Therefore, studies with natural antioxidants into food products have increased.

Cunha et al. (2021), studied the effect of different levels of microencapsulated pitaya peel extracts (100 ppm, 1,000 ppm, and 2,000 ppm) on the oxidative stability of refrigerated ground pork patties subjected to UV-C ( $0.5 \text{ J/cm}^2$ ) for 10 days. Ground pitaya peel was microwave-assisted extracted utilizing different factors, and the extract with the highest polyphenol content (2.68 mg GAE/g sample) was microencapsulated using Maltodextrin MOR-REX 1910 and added to ground pork. It was observed that the highest concentration (2000 ppm) promoted higher protection (30%) of polyunsaturated fatty acids. The different concentration of extracts mitigated protein oxidation and the lipid oxidation induced by UV-C up to sixth day was hindered specifically with 2000 ppm concentration (about 44%).

Previous study of Cunha et al. (2018), focused on the effect of microencapsulated microwave assisted lower concentrations of pitaya extract and synthetic BHT on color, texture and oxidative stability of pork patties that underwent HPP at 500 MPa for 10 min and stored at 4 °C during 9 days. It was observed that HPP accelerated color and texture changes, as well as lipid and protein oxidation during refrigerated storage of meat patties. Although BHT demonstrated greater effectiveness against redness changes and lipid and protein oxidation, both synthetic BHT and pitaya extracts demonstrated a protective effect against the alterations promoted by HPP, minimizing hardness and chewiness changes in the pork matrix.

## *5.2. Pharmaceutical and biomedical benefits*

### *5.2.1. Fluorescent sensing of harmful substances in food*

Carbon quantum dots (CQD) are a type of fluorescent carbon materials first discovered in 2004 and having important applications in many fields such as biological imaging, sensing, drug carrying, photocatalysis, optoelectronics, photothermal therapy (Ali et al., 2020; Wu et al., 2020; Naik et al., 2022). The CQDs can be functionalized and combined with other kinds of materials to form the nanostructured composites with unique properties, having a very broad application prospect for related research of many fields. The low toxicity and cost of CQD production makes it an ideal raw material for developing effective sensing devices. Recent remarkable researches on trace amount monitoring of harmful food substance utilizes green-synthesized CQDs or those derived from plant extracts. In the field of food analysis, CQDs acts as a sensing probe to detect and analyze functional components of food such as protein, vitamins, phenolic compounds.

In a study of sulfoquinoxaline detection in food, a fluorescent probe based on white pitaya peel-derived carbon dots were used as a sensor. The synthesized pitaya based CQDs exhibit excellent fluorescent response towards the sulfoquinoxaline due to the inner filter effect and aggregation-caused quenching. A detection limit of 24.5  $\mu\text{g/L}$  sulfoquinoxaline was detected and the prepared fluorescent probe showed a good linear relationship indicating high selectivity, specificity, sensitivity, reliability and good reproducibility for the detection of the compound.

The practicability of this fluorescent probe was further validated in milk, honey, eggs, carp, pork, chicken, beef and pork liver, and the recoveries of the target compound range from 93.38% to 103.86% (Zhang et al., 2023).

#### 5.2.2. *Organic dye synthesis of fluorescent green carbon dots*

Though CDs are a promising technology some of its limitations are on the synthesis sources and approaches including the use of toxic chemical materials, requiring high temperature, time-consuming process, and the exorbitant cost of synthesis (Radnia et al., 2020).

The most recent and innovative approach to improve CDs and to overcome these restrictions is based on the use of green precursors which caused growing attention to benign preparation of CDs, called “green-CDs” (Radnia et al., 2020). CDs have been widely researched for their *in vivo* and *in vitro* biomedical applications, such as drug delivery, bioimaging, antioxidant and antibacterial activity, and photothermal and photodynamic therapy (Innocenzi & Stagi, 2020; Radnia et al., 2020). The work of Guo et al. (2020) explored the used of red pitaya peels in acetic acid for synthesis of carbon dots (ACD) using solvothermal method in fluorometric and colometric dual mode system cellular imaging of gold ( $\text{Au}^{3+}$ ) ion in actual samples. The high number of organic compounds found in pitaya peels makes it an appropriate raw material for fabrication of carbonaceous materials. The ACD produced was also successfully applied for intracellular  $\text{Au}^{3+}$  sensing and imaging due to its good photostability, low cytotoxicity, and excellent biocompatibility. In addition, they measured an extraordinarily high antioxidant activity with ACD having  $\text{IC}_{50}$  value for DPPH radical scavenging ( $0.70 \mu\text{g/ml}$ ) much lower than that of ascorbic acid ( $4.34 \mu\text{g/ml}$ ), which further expanded its biological application.

#### 5.2.3. *Biogenic eco-friendly material for synthesis of silver nanoparticles*

The emergence of nanoparticles for pharmaceutical applications has been long studied due to its numerous advantages like *in vitro* diagnostic but specifically in drug delivery (Wagner et al., 2006). Drugs bound to nanoparticles (NPs) have been claimed to have advantages over the conventional forms of the drugs in terms of extended half-life *in vivo*, longer circulation time, and effective drug delivery system for targeted area. Nanoparticle drug size is easily modified to achieve the desired delivery characteristics thus, increasing drug loading capacity and they are easily suspended in bodily fluids causing them to penetrate deep in organs and tissues. Silver nanoparticles can be created using several physico-chemical methods with the drawback of being economically expensive and involving toxic chemicals (Phongtongpasuk et al., 2016). Biogenic synthesis of nanoparticles from salts of the corresponding metals as well as the use of microorganisms, whole plants, plant tissue and fruits, plant extracts and marine algae (Mittal et al., 2013) is favored because of its reduced environmental impact and it can be used to produce large quantities of nanoparticles free of contamination and with a well-defined size and morphology (Hutchison, 2008). However, there are only few studies on the synthesis of silver nanoparticles using agricultural wastes.

In the study conducted by Phongtongpasuk et al. (2016), they introduced a facile, green, and eco-friendly approach for synthesis of silver nanoparticles using dragon fruit peel extract as reducing and capping agent. It is said that the organic compounds in the dragon fruit peel could have the ability for the reduction of metal salt ions in the synthesis of metal nanoparticles. The high antioxidant capacity of

the extract allows it to neutralize free radicals due to the availability of electrons. The characterization of synthesized silver nanoparticle was fully elucidated by microscopic and spectroscopic techniques and Ag<sub>2</sub>O in the final product was shown present in XRD and EDX analysis. The results showed that pitaya peel extract can be used for biogenic silver nanoparticle synthesis with a promising antibacterial activity against pathogenic bacteria.

## **6. Growing interest of European market, limitations, and market size of pitaya**

Pitaya is widely cultivated and consumed in Asia, Vietnam being one of the main countries to export this fruit in the world. Pitaya was introduced to Vietnam by the French over a hundred years ago (Mizrahi et al., 1997). It is one of the key export fruits with export sales worth US\$ 895.7 million in 2016 equivalent to 32% of the total export value of Vietnamese vegetables and fruits and 50.3% of the country's total fresh fruit exports (The Asia Foundation, 2019). This is one of the leading world's exotic tropical fruit exports with 80-85% of the output production in the country being exported to over 40 countries with main key markets like China, Thailand, Indonesia, Malaysia, Singapore, Australia, Canada, United States, The Netherlands, Spain, Germany, and United Kingdom (Luu et al., 2021). It was in 2005 when pitaya was accepted to the EU market following the GlobalGAP (Good Agricultural Practices) and packing house code and three years after the US market accepted the exotic fruit following a more stringent requirement (The Asia Foundation, 2019). In a survey conducted by the Centre for the Promotion of Imports (CBI, 2019) from developing countries, Europe was found as a potential big market to export pitaya. However, international and national standard for good agricultural practices must be secured in order to secure high quality standard, clean and safe products for health and environment prior to expanding to the European market.

In France, tropical fruits are produced in the overseas departments, in particular in the Reunion Island concerning the pitaya fruits. However, this land is difficult to exploit (e.g. land with steep slopes, areas suffering from a lack or excess of water, (Le Bellec, 2003) and in this context, an approach was adopted in 2000s to select, develop, and support a new fruit species. Considering the island's agricultural economy, the objective was not to seek a substitute crop for the main crop of farms, but to enable farmers to diversify their agricultural income through other opportunities. The pitaya (*Hylocereus sp.*) was selected among the twenty other candidate species due to the new shape of this fruit, its attractive colors, its rapidity in fruiting and the fruit's good keeping qualities were some of the considerations (Le Bellec, 2006). With the growing awareness of people all over EU of this exotic tropical fruits and a promising cultivation of the fruit in Reunion Island, this fruit given its nutraceutical and potential health benefits as stated in various literature has grown interest not only to researchers but also to consumers.

## **7. Conclusion and future perspective**

Different species of pitaya varies in color of pulp and peel due the presence of two main organic food dye betacyanin and betaxanthin responsible for the red and yellow coloration of the outer layer of the peel, respectively. The cultivation, maturity, and harvesting period greatly affects this pigment formation in the cell. Fruit harvest must be within the 22-30 days of anthesis to ensure maturity and development of physico-chemical and sensorial properties of the fruit. Pitaya contains nutritional qualities and bioactive molecules such as protein, fat,

fiber, sugar, polysaccharide, vitamins and minerals, and bioactive molecules like the natural pigment betacyanin, polyphenols, flavonoids, and triterpenoids. Green extraction technologies like UAE, MAE, and SFE due to its reported high- and better-quality yield of extracts. They are proven to effectively extract bioactive compounds but further study is needed for an efficient and optimized parameter in order to eliminate recurring limitations with economic implications such as energy consumption and industry scale-up. Extracts of the fruit byproducts are used in food industry as food-grade dye, fat replacer, oxidative stabilizer, and smart food packaging and edible coatings. In addition, the emerging role of this organic dye as substance for green-synthesized CQD has continuously attracts researchers to explore its possible application in pharmaceutical drug delivery system, biomedical fluorescent imaging and harmful food substance sensing probe detector. Making pitaya an interesting and promising superfruit that is full of potential for future innovations not only for the application in the food industry but also to cosmetic and pharmaceutical industries.

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### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **CRedit authorship contribution statement**

**Feby Carmen:** Conceptualization, Writing – original draft, Writing – review & editing.

**Christine Frances:** Supervision, Conceptualization, Validation, Writing – review & editing.

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## Tables and Figures

Table 1. Major pitaya-producing countries with average yield per hectare for the year 2017-2018. Source: ICAR-National Institute of Abiotic Stress Management Technical Bulletin No.47, (Nangare et al., 2020) and Overall Dragon Fruit Production and Global Marketing presented by Paull and Chen (2019)

	Production area (ha)	Percentage (%) per production area	Production Volume (MT)
<i>Vietnam</i>	55 419	48.8	1074242
<i>China</i>	40 000	35.2	700000
<i>Indonesia</i>	8 491	7.5	2221832
<i>Thailand</i>	3 482	3.1	26000
<i>Taiwan</i>	2490.6	2.2	49108
<i>*Malaysia</i>	1641	0.8	11000
<i>Philippines</i>	485	0.4	6062.5
<i>Cambodia</i>	440	0.4	4840
<i>India</i>	400	0.4	4200
<i>USA</i>	324	0.3	5832
<i>*California</i>	160		
<i>*Florida</i>	150		
<i>*Hawaii</i>	80		
<i>Australia</i>	40	0	740
<i>South Africa</i>	12	0	100

ha: hectare, MT: metric ton, ICAR: Indian Council of Agricultural Research, \*data collected in 2019

Table 2. Botanical classification of various pitaya fruits according to literature.








Species name	Botanical characteristics	Image	Reference
<i>H. polyrhizus</i> (F.A.C. Web.) Britton & Rose (syn. <i>H. monacanthus</i> ) (red pitaya or red pitaya with red flesh)	Flowers are 25–30 cm long. Perianth is red, especially at the tips. Stigma is short, lobed, and yellow in color. Fruit is 10–12 cm long and 130–350 g in weight. It is oblong and covered with scales with different size. It has a red flesh with many small black seeds, pleasant flesh texture and sweeter taste. <i>H. polyrhizus</i> has several varieties.	 Image available via <a href="https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit">https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit</a>	Ariffin et al. (2009) ; Le Bellec et al. (2006) ; Wybraniec et al. (2001)
<i>H. undatus</i> (Haw.) Britton & Rose (white pitaya or red pitaya with white flesh)	Stems are long and green. Flowers are very long (up to 29 cm). Perianth has outer green (or yellow-green) and inner white segments. Fruit is rosy-red with 15–22 cm length and 300–800 g weight. It is oblong and covered with large and long scales, which are red and green at the tips. It has a white flesh with many small black seeds, pleasant flesh texture, and a good taste. Its fruit is slightly to significantly less sweet than the red-fleshed pitaya fruit.	 Image available via <a href="https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit">https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit</a>	Ariffin et al. (2009) ; Le Bellec et al. (2006)
<i>H. megalanthus</i> (K. Schumann ex Vaupel) Ralf Bauer (syn. <i>Selenicereus megalanthus</i> ) (yellow pitaya)	Stems are green, robust, three-ribbed, 1.5 cm thick, with slightly undulating margins, white areoles bearing 1–3 yellowish spines, 2–3 mm long. Flowers are nocturnal, large, white, and funnel-shaped, 32–38 cm long. Perianth has outer green and inner white segments. Stigma is lobed and green in color. Fruit is ovoid, tuberculate, spiny, yellow with numerous black seeds embedded in a sweet, juicy white pulp, and much smaller than the red pitaya. It is the sweetest varieties, with relatively smaller sized fruits.	 Image available via <a href="https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit">https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit</a>	Ariffin et al. (2009) ; Wybraniec et al. (2001)
<i>H. purpusii</i> (Weing.) Britton & Rose	Flower is 25 cm long with margins. Perianth has more or less reddish outer, golden middle, and white inner segments. Fruit is oblong covered with large scales. It is 10–15 cm in length and 150–400 g in weight. It has red flesh with many small black seeds, and pleasant flesh texture, but not very pronounced.	 Image available via <a href="https://www.edpsciences.org/fruits">https://www.edpsciences.org/fruits</a>	Le Bellec et al. (2006)
<i>H. ocamponis</i> (S.D.) Britton & Rose	It is closely related to <i>H. purpusii</i> . They can be distinguished only by the acicular and slender spines of <i>H. ocamponis</i> .	 Image available via <a href="https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit">https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit</a>	Le Bellec et al. (2006)
<i>H. costaricensis</i> (Web.) Britton & Rose	Stems are waxy white. Flower is nearly the same as <i>H. polyrhizus</i> . Fruit is 10–15 cm in diameter and 250–600 g in weight. It is ovoid and covered with scales with different size. It has a red purple flesh with many small black seeds, pleasant flesh texture and good taste.	 Image available via <a href="https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit">https://www.tropicalfruitnursery.com/variety-viewer-variety-dragon-fruit</a>	Le Bellec et al. (2006)
<i>H. trigonus</i> (Haw.) Saff	Stem is slender, green with margins, not horny. Fruit is red, ovoid or oblong, becoming nearly smooth. It is 7–9 cm in diameter and 120–250 g in weight. It has white flesh with many small black seeds and pleasant flesh texture, but not a very pronounced flavor.	 Image available via <a href="https://www.edpsciences.org/fruits">https://www.edpsciences.org/fruits</a>	Le Bellec et al. (2006)

Table 3. Bioactive compounds found from the different species of pitaya fruit using various methods.

Pitaya species and plant part	Extraction method	Target compounds	Quantification method	Concentrations (mg/kg) in dry weight	reference
Red pitaya peel, pulp and seed  ( <i>Hylocereus polyrhizus</i> )	Freeze-dried red pitaya peel, pulp and seed were mixed in a hydroalcoholic solution at pH 2 to obtain the extracts. The extracts were filtrated, and the solvent was evaporated by vacuum drying under reduced pressure.	Phenolic	Folin-Ciocalteu agent	294.8 ± 12.9 (peel), 277.6 ± 14.2 (pulp) and 375.1 ± 12.6 (seed) mg GAE/g	Saenjum et al. (2021)
		Flavonoids	Aluminium chloride method	193.8 ± 11.7 (peel), 177.4 ± 12.5 (pulp), and 264.4 ± 10.8 (seed) mg QE/g	
		Anthocyanin	Modified pH differential method (cyanidin chloride as positive control)	135.4 ± 9.3 (peel) and 159.7 ± 8.9 (pulp) mg CE/g	
Red pitaya peel  ( <i>Hylocereus polyrhizus</i> )	Freeze-dried peels were homogenized with deionized water at pH 5.5, 40 °C for 20 min. The extract was stored in the dark in a refrigerator until analysis.	Phenolic	Folin-Ciocalteu reagent	35.81 ± 3.11 mg GAE/g	Thaiudom et al. (2021)
		Betacyanin	Liquid Chromatography Mass Selective Detector (LCMS)	15.21 ± 0.04 mg/g	
Red pitaya peel  ( <i>Hylocereus polyrhizus</i> )	Oven-dried pitaya peel or flesh (5 g) were extracted with 50 mL ethanol solvent (100%, 90%, 70%, 50%, 30%, 10% and 0%) for 20 min at 25 °C.	Phenolic	Folin-Ciocalteu agent	139 ± 0.41 (peel, 50% ethanol), 105 ± 0.53 (flesh, 50% ethanol), 135.75 (peel, supercritical fluid) and 99.42 (flesh, supercritical fluid) GAE/100 g	Fathordoobady et al. (2019)
	For supercritical fluid extraction 5 g of sample was treated with 99.9% purity of CO <sub>2</sub> at 2 mL/min flow rate and 10% ethanol solvent (50 mL). The extraction was conducted under 25 MPa pressure and 50 °C.	Betacyanin	McIlvaine buffer method (at 538 nm)	28.44 ± 1.98 (peel, 50% ethanol), 84.25 ± 0.89 (flesh, 50% ethanol), 24.58 ± 2.16 (peel, supercritical fluid), and 91.20 ± 2.23 (flesh, supercritical fluid) mg/100 mL	
Red Pitaya peel  ( <i>Hylocereus undatus</i> )	Freeze-dried peels were mixed with different proportions of ethanol (from 30% to 60%) at varied solvent/solid ratios (10:1–30:1) in an ultrasonic bath in the dark for 5–25 min at different temperatures (30–70 °C).	Phenolic	Folin-Ciocalteu reagent	4.51–8.54 mg GAE/g	Bhagya Raj & Dash (2020)
		Betacyanin	McIlvaine buffer method (at 537 nm absorption)	1.02–1.60 mg BCE/g	
Red pitaya peel  ( <i>Hylocereus costaricensis</i> )	The red pitaya peel extract was obtained by UAE at 500 W for 22 min, using a mixture of H <sub>2</sub> O:EtOH (90:10, v/v) as extraction solvent and a solid/liquid ratio of 5 g/L.	Betacyanin	HPLC-DAD-ESI/MS	48.3 ± 0.8 mg/g extract	Roriz et al. (2022)
		Tocopherols	HPLC-DAD-ESI/MS	15.93 ± 0.07 g/100 g dw	
		Organic acid	HPLC-DAD-ESI/MS	2.65 ± 0.03 g/100 g dw	

Red pitaya peel <i>(Hylocereus polyrhizus)</i>	The ultrasound-assisted extraction (UAE) was conducted using a horn-type ultrasonic probe for 20 g dried dragon fruit with a frequency of 40 kHz, power and sonication time levels were varied between 0.5, 1.5, 2.5, 3.5, and 4.5 W/g dry weight material; and 2.5, 5.0, 7.5, 10.0, and 12.5 min.	Betacyanin (UAE)	UV-/NIR ( $\lambda=538$ nm)	0.3402 mg/g	Le Van et al. (2002)
Red pitaya peel and pulp <i>(Hylocereus undatus)</i>	A microwave-assisted extraction with different ratios of solid/water, from 1/50–1/150 g/mL, at 600 W for 5–65 min) was used to extract phenolic compounds and betacyanins in freeze-dried pulps followed by centrifugation twice at 4000× g for 10 min each time.	Phenolics Flavonoids Betacyanin	HPLC-DAD-ESI/MS HPLC-DAD HPLC-DAD	2.39–10.14 mg/g 3.22–5.04 mg/g 0.43–1.66 mg/g	Ferreres et al. (2017)
Red pitaya peel <i>(Hylocereus polyrhizus)</i>	Supercritical fluid extraction (SFE) with CO <sub>2</sub> as solvent and EtOH/water (v/v) as co-solvent of 5.00 ± 0.01 g ground sample (particle size > 0.60 mm, mesh 30) was optimised by applying 23 factorial experimental design. Co-solvent (EtOH/ water 10/90 v/v) was spiked to the sample prior to each run of extraction performed at pressures 20-30 MPa and temperatures 40-60°C	Betacyanin Inhibition concentration assay	HPLC/LC-ESI-MS/MS standard curve (dose–response plot) of DPPH absorbance versus sample concentration	25.49 ± 1.54 mg/100 mL, 1.34 ± 0.12 mg/mL	Fathordoobady et al. (2019)
Red pitaya peel and pulp <i>(Hylocereus polyrhizus)</i>	One g of the flesh was homogenized with 25 mL of distilled water for a few minutes at 1/25 (w/v). The mixture was then centrifuged at 6000 rpm for 15 minutes at room temperature.  For the peel, 55 mL of distilled water was added to 1 g of the sample at 1/55 (w/v). The mixture was then placed in ultrasonic bath and sonicated at 50 kHz for 30 minutes at ambient temperature (25° C).	Betacyanin  Total Phenolic content  Total Flavonoid	Spectrophotometric method  Folin Ciocalteu reagent (absorbance measured at 750 nm)  aluminium trichloride colorimetric (absorbance measured at 430 nm)	Peel CV 18.67 ± 0.50 mg/100 g dry extracts UAE 17.64 ± 0.03 mg/100 g dry extracts Pulp CV 82.79 ± 0.55 mg/100 g dry extracts UAE 71.34 ± 1.00 mg/100 g dry extracts  Peel CV 73.84 ± 15.94 mg% dry basis UAE 65.16 ± 1.52 mg% dry basis Pulp CV 121.86±4.89 mg% dry basis UAE 128.30 ± 8.17 mg% dry basis  Peel CV 145.88 ± 2.33 mg% dry basis UE 195.82 ± 55.0 mg% dry basis Pulp CV 510.69 ± 7.54 mg% dry basis UAE 245.31 ± 6.04 mg% dry basis	Ramli et al. (2014)

HPLC-DAD: high performance liquid chromatography-diode array detector, HPLC-DAD-ESI/MS: high performance liquid chromatography-diode array detector electrospray ionization and mass spectrometry, HPLC-ESI/MS/MS: high performance liquid chromatography-electrospray ionization and tandem mass spectrometry, DPPH: 2,2-diphenyl-1-picrylhydrazyl, GAE: gallic acid equivalent, BCE: betacyanin equivalent, CE: catechin equivalent, QE: quercetin equivalent, UAE: ultrasound assisted extraction, CV: conventional extraction, SFE: supercritical fluid extraction

Table 4. Phytochemical composition of pitaya extract based on reported results.

Polyphenols	mg/ 100 g extract	References
caffeic acid	20.10	Arivalagan et al. (2021)
ferulic acid	14.37	Arivalagan et al. (2021)
protocatechuic acid	3.30-10.32	Arivalagan et al. (2021)
p-coumaric acid	-	Saenjum et al. (2021)
sinapic acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
vanillic acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
gallic acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
o-coumaric acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
t-cinnamic acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
salicylic acid	-	Saenjum et al. (2021) ; Morais et al. (2021)
<b>Flavonoids</b>		
catechin		Arivalagan et al. (2021)
Quercetin		Arivalagan et al. (2021)
Epicatechin		Arivalagan et al. (2021)
epigallocatechin gallate		Saenjum et al. (2021)
procyanidin B1		Saenjum et al. (2021)
procyanidin B2		Saenjum et al. (2021)
kaempferol 3-glucoside		Saenjum et al. (2021)
quercetin 3-glucoside		Saenjum et al. (2021)
rutin		Saenjum et al. (2021)
<b>Organic acids</b>		
	$\mu\text{g}/ 100 \text{ g extract}$	
malonic acid	46.7–64.6	Arivalagan et al. (2021) ; Saenjum et al. (2021)
citric acid	25.8–77.2	Arivalagan et al. (2021) ; Saenjum et al. (2021)
shikimic acid	0.156–0.394	Arivalagan et al. (2021) ; Saenjum et al. (2021)
malic acid	0.094–0.295	Arivalagan et al. (2021)
fumaric acid	0.099–0.329	Arivalagan et al. (2021)
syringic acid	0.095–0.790	Arivalagan et al. (2021)



Figure 1. Maturation and pigment formation in the cells of both pulp and peel of pitaya fruit (a) four stages of 'Jinghonglong' pitaya white-pulp fruit (top row) and 'Zihonglong' (bottom row) fruit samples from 22 days post-anthesis to 30 DPA (b) distribution of pigment in single cells of Zihonglong peel at coloring stage (c) single pigment cell at mature stage of peel (d) distribution of pigment in single cells of Zihonglong pulp at coloring stage (e) single pigment cell at mature stage of pulp (adapted from Wu et al., 2020a).

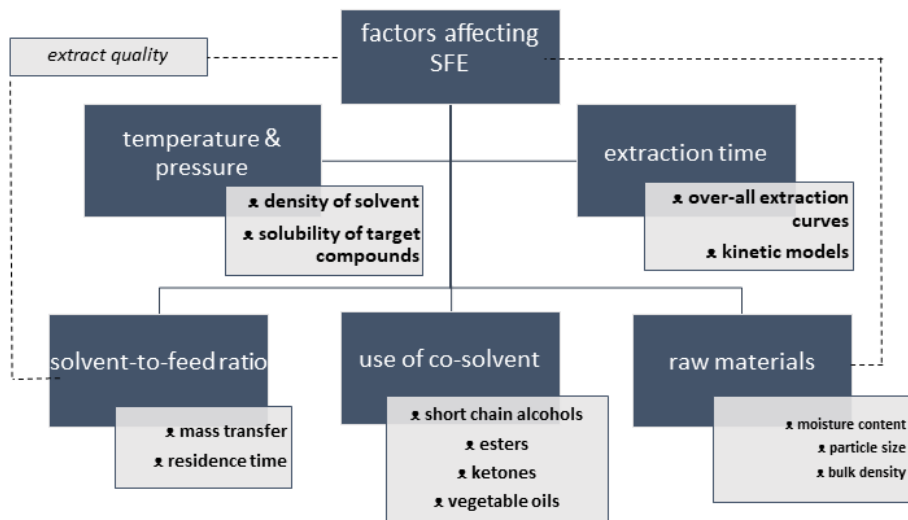
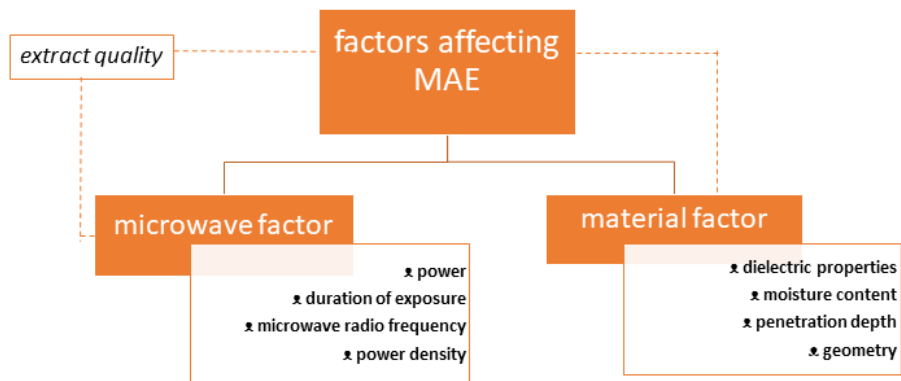
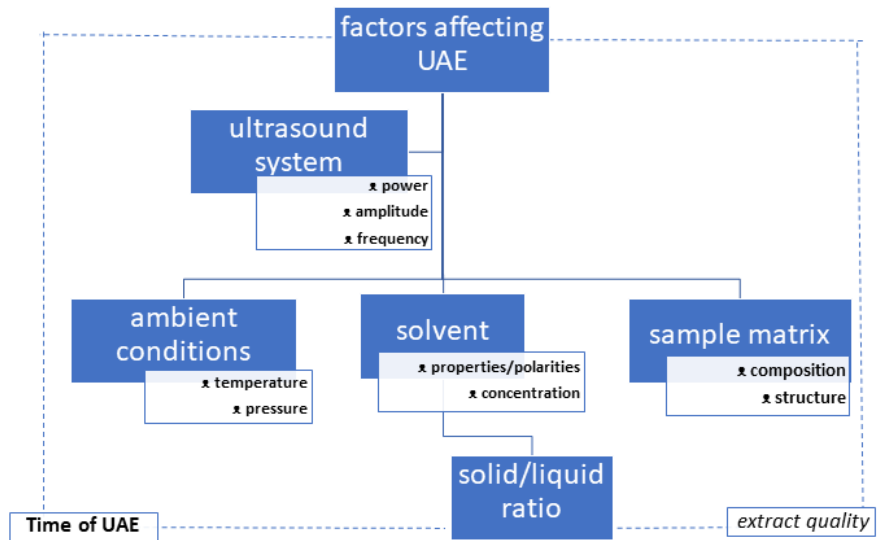


Figure 2. Factors and parameters affecting the UAE, MAE, and SFE processes.

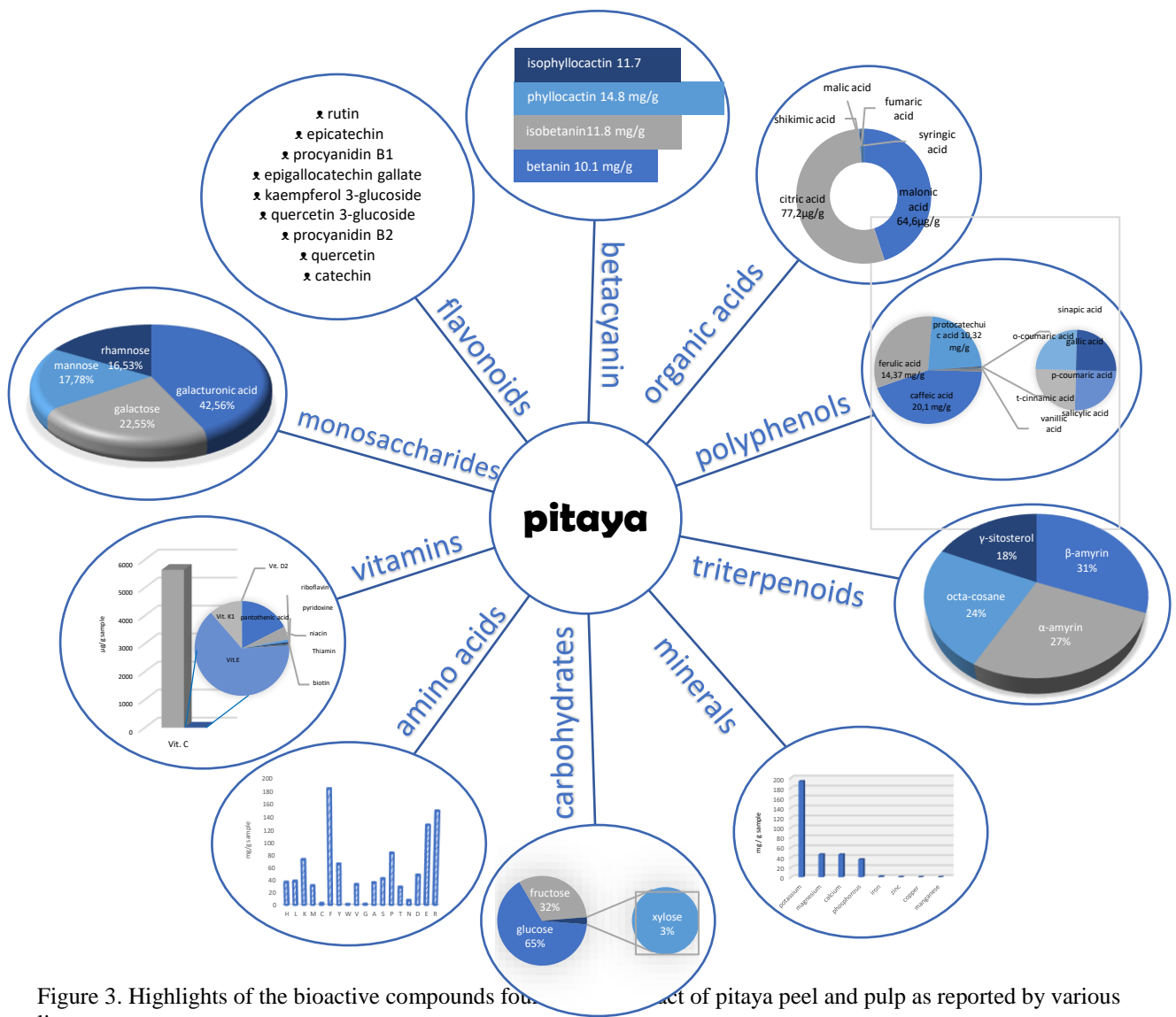


Figure 3. Highlights of the bioactive compounds found in the peel and pulp of pitaya as reported by various literatures.