

CHAPTER 1

INTRODUCTION

North-East India is renowned for having a variety of geography, soil, and climate, which is conducive to the growth of a wide range of food crops. Because of the agro-climatic diversity, North-East is native to several fruits that are rich in bioactive substances that in processed forms have the ability to reduce hunger, poverty, and food insecurity; but due to lack of awareness and proper knowledge, these fruits have not been fully exploited for maximizing the benefits of the bioactive compounds, and are categorized as underutilized fruits [13,14].

Passion fruit (*Passiflora edulis*) also known as Passiflora fruit is an attractive and nutrient-dense exotic fruit and it is appreciated for its characteristic aroma and flavor and is typically consumed fresh for its rich taste [10,32,35]. It is widely utilised in traditional medicine around the world, and the food, pharmaceutical, and cosmetics sectors all profit from its use [10,36,50]. Passion fruit is native to Brazil, where the fruit is consumed by the local population for its bioactive compounds that exhibit biological activity [42]. In 2021, the cultivation production of passion fruit in India was estimated to be approximately 5.02 ton/ha [33], whereas more than 70% of the total production was from Northeast India, specially Manipur and Nagaland [46].

In recent years, the fruit is being widely studied for its desirable chemical composition [2,10]. The vitamins and minerals that are necessary for life appear to be abundant in this fruit. It also contains a significant amount of nutraceuticals, like polyphenolic compounds, flavonoids, carotenoids, phytosterols etc. that exhibit several physiological health benefits, such as protection against degenerative and chronic diseases, neurological diseases, cardiovascular diseases, obesity, inflammations, infections, cancer, and allergy [2,10,20]. Although the fruit is rich in different bioactive compounds, due to lack of awareness about its health benefits, people have not been able to extract the full benefits of the fruit, and in India this fruit is still considered as an underutilized fruit [13,14].

There are more than 500 species of *Passifloraceae* family that are distributed in the world's tropical and subtropical areas, but the *Passiflora* species stands out for its economic importance [10]. The edible varieties are *Passiflora edulis Sims fo. flavicarpa*, *Passiflora edulis Sims fo. Edulis*, and *Passiflora caerulea* that are popularly known as sour or yellow, purple, and orange passion fruit, respectively. The purple and orange

fruits are best consumed as fresh products since they have a slight sweet taste [37,38]. The edible yellow fruit is a variety grown worldwide for commercial purposes and is used mainly by the juice processing industries for its yield and acidity [10]. **Table 1.1** gives the chemical composition of the different morphological parts of yellow passion fruit.

Table 1.1. Different parts of yellow passion fruit and its composition (average value) [38]

Parameters	Pulp	Peel	Seed
Yield (%)	27.71	64.05	8.24
Moisture (g/100 g d.w.)	90.06	87.14	57.09
Proteins (g/100 g d.w.)	8.57	3.40	13.07
Lipids (g/100 g d.w.)	1.11	4.20	12.31
Ash (g/100 g d.w.)	6.94	6.62	3.56
Carbohydrates (g/100 g d.w.)	83.37	85.78	71.07
Total fibre (g/100 g d.w.)	7.15	61.16	65.60
Total carotenoids ($\mu\text{g}/100\text{ g d.w.}$)	1785	918.41	-
β -carotene ($\mu\text{g}/100\text{ g d.w.}$)	1334	272.52	-
Phenolic compounds ($\mu\text{g}/100\text{ g d.w.}$)	1297.31	1061.87	346.69
DPPH (IC ₅₀ (g/100 mL))	0.20	1.69	1.18
ABTS (IC ₅₀ (g/100 mL))	0.82	2.22	3.84

d.w.: Dry weight basis; IC₅₀ : 50% effective radical inhibition concentration.

1.1. Passion fruit juice and its challenges

Passion fruit juice (PFJ) is a nourishing beverage that is full of vitamins, minerals, and bioactive substances that can improve human health [37]. PFJ is enriched with phenolic compounds, flavonoids, carotenoids with β -carotene being the major carotenoid, vitamins C and provitamin A.

These substances have the ability to act as antioxidants, which can reduce the risk of cardiovascular diseases like hypertension and arteriosclerosis, neurodegenerative problems, high cholesterol and blood glucose levels, control inflammatory, Alzheimer's, and infectious processes, and act as carcinogenesis and mutagenesis inhibitors, among other things [10,39,42]. Due to its unique taste and unique flavour, passion fruit juice is consumed as a fresh juice [37].

Although the pulp/juice of passion fruit is enriched with several health-benefiting compounds, its direct use is not preferred by customers because of its high acidity and astringent taste [4]. As the juice is rich in vitamin A and C, and can also be eaten in raw form, it is used to aid aroma in ice-cream, and make jams and refreshing drinks. Moreover, the excellent flavour of the juice is used to enhance the overall quality of blended products [6]. A blended beverage comprises of two or more different types of fruit juices. PFJ can be blended with any other juice to add its refreshing aroma and mask its high acidity. Mango and guava juice can be blended to obtain better sensory quality beverages. However, the juice spoils easily due to the growth of yeast and bacteria. Normally, at room temperature, raw PFJ undergoes alcoholic fermentation by yeast followed by the oxidation of alcohol by bacteria [4]. Some kind of treatment is therefore, needed to enhance the shelf life of the fruit juice.

Consumers prefer high quality fruit juice that is nutritious, has minimal chemical preservatives, and maintains the natural characteristics while conforming to legislative requirements. Passion fruit juice with 4% sugar as a preservative has been reported to spoil after three days [4]. The use of chemical preservatives is nowadays less accepted by customers. Therefore, other preservative techniques are used for the enhancement of the shelf-life. Generally, conventional thermal treatment is used but the delicate flavour of PFJ is extremely sensitive to heat treatment. Additionally, severe thermal treatment of passion fruit juice produces toxic compounds including 5-hydroxymethyl-2-furfural [16]. Further, clarification techniques are associated with a reduction of the nutritional value as most of the nutrients remain in the colloidal suspension [10].

1.2. Co-products of Passion fruit juice processing industries

In India, currently, the end use of passion fruit cultivation is juice processing only and passion fruit juice processing industries create a large amount of waste comprising of peels and seeds, which make up more than half (approximately 60%) of the whole fruit [37,50]. Dumping of this waste material into the ecosystem may cause environmental degradation, besides causing a loss of the valuable bioactive compounds present in the peel and seeds. In addition to the pulp, the peel and seeds of the passion fruit are of significance since they contain a significant number and amount of bioactive compounds such as carotenoids, polyphenolics, etc. [38,39].

As shown in **Table 1.1.**, peels represent the major portion of passion fruit. The extract of this fruit peel has been used to cure several diseases like cough, wheezing,

hypertension, asthma, anxiety, insomnia, etc. and has been utilized in folk medicine [49,50]. Passion fruit peel is enriched with phenolics and carotenoids and Watson et al. [49] reported the higher 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) antioxidant activity of peel as compared to pulp or seed portion.

The seed of passion fruit contains approximately 22-30 % oil [10,30,36] and high insoluble dietary fibre amounting to 64.1 g/100 g of fruit [8]. The edible nature of passion fruit seed oil and fibre is already well established [8,20]. The seed oil is enriched in several bioactive compounds such as phenolic acids, phytosterols, and essential fatty acids of which majority are unsaturated [31,36]. Passion fruit seed oil exhibits antimicrobial effects [21,22]. The seed oil contains approximately 570 mg/kg of total phenolic compounds and possesses strong antioxidant activity (34 mg/mL) [36,38,42]. The insoluble fibre-rich fraction (84.9-93.3 g/100 g) becomes the main component in the (defatted) seed after oil extraction and these components are primarily made of hemicellulose, cellulose, and pectic substances [8].

Due to the fact that dietary fibre is resistant to digestion and absorption in the human small intestine but can be partially or completely fermented in the large intestine, dietary fibre-rich products have become more and more popular as food ingredients for their health benefits. This has encouraged food scientists to look for new fibre sources and to develop fibre-rich functional products [1,5,12]. Numerous studies have shown that dietary fibre can lower the risk of gastrointestinal disease, support healthy intestine structure to operate as a mechanical barrier, and protect the gastrointestinal tract [1,34]. Some dietary fibre (obtained from various sources) can affect the metabolic processes, enzyme activity, and metabolite production of specific intestinal microbiota, which can have an impact on intestinal function, health, and structure [9]. Dietary fibres' physicochemical and compositional characteristics may both help to explain how they work in food. Passion fruit seed fibre also showed good antioxidant and antibacterial properties [28]. All of this knowledge might be used to better understand the physiological consequences of the dietary fibres in passion fruit, but to the best of our knowledge, there are only a few research works reported on passion fruit seed fibre. Whether passion fruit seed fiber can be used as a natural food ingredient needs to be explored.

1.3. Recovery of bioactive compounds

One of the most useful options for the management of fruit waste is the recovery

of its health-benefiting compounds. Traditionally, conventional extraction methods with the most frequent solvent (common solvent) have been used to extract nutritionally significant compounds from food matrices, however the procedure is expensive for operation and also requires an enormous amount of energy and the most common solvent (petrochemical) used in this extraction process has several disadvantages, such as prolonged exposure, thermal effect, generation of hazardous volatile organic compounds, low extraction yield, retention of small amount of residuals after solvent evaporation, etc [18,44,51], which show their negative impacts on health, safety and environment.

Therefore, in recent years, high-energy extraction technique such as ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE), etc. based on the principle of green chemistry/biorefinery techniques are gaining considerable interest among researchers for the extraction of bioactive compounds [15,18,24,41]. Modern extraction techniques with an appropriate solvent are being implemented in the food processing, cosmetic, and pharmaceutical related industries [18].

1.4. Emulsion based approach

An emulsion is a promising medium to maintain bioactivity and increase the bioavailability of lipophilic bioactive substances in a carrier system. Apart from solubility, an emulsion is also being used in order to regulate the distribution of bioactive substances and enhance the quality and shelf life of food products [11,29]. Different types of synthetic surfactants such as Tween 20, Tween 60, Tween 80, and combination of them are used to stabilize the emulsions [27]. However, surfactant usage in food has been restricted because of its toxicity, non-food grade and supersedance nature.

The generally recognised as safe (GRAS) status of food additives is a contentious area of research. As a result, natural label-friendly alternatives to synthetic emulsifiers are becoming more and more important in the food sector [3]. This has led the food industry to replace synthetic stabilizers with natural materials such as biosurfactants, phospholipids, polysaccharides, proteins (of the amphiphilic variety), and bioparticles, among others. So, fruit juice treated with different novel techniques followed by addition of Pickering emulsion with antimicrobial compounds may be applied to enhance the shelf life of fruit juice.

1.5. Research Gap

The North-Eastern region contributed more than 70% of India's passion fruit production in the year 2017 [46]. In India, currently, the end use of passion fruit cultivation is juice processing only and PFJ processing industries create a large amount of waste comprising of peels and seeds, which make up more than half (approximately 60%) of the whole fruit. In recent years, the fruit is being widely studied for its desirable chemical composition [10]. Despite being abundant in various health-promoting chemicals, passion fruit pulp and juice's high acidity and astringency make them unpopular for direct consumption [4]. For its blending quality, PFJ is often blended with juices of mango, pineapple, ginger etc [6,53] to enhance the flavour of the final product and also its shelf life, as it is very perishable in nature. Recently several novel treatments such as ultrasound [7,16,17,23,47], high-pressure homogenization [25,26,45,48,52], antimicrobial based emulsion [27,40] have been used to extend the shelf life. Very limited study has been reported about the shelf-life enhancement of PFJ.

The discarded portion of PFJ industries, mainly the peel and seeds are rich in several bioactive compounds such as alkaloids, flavonoids, carotenoids, tea polyphenols etc. [1,19,53]. Furthermore, in peel some compounds are higher in concentration compared to the other parts of the fruit [38]. So, extraction of these bioactive compounds and using them in food processing will lead to the development of novel food products. Combination of green solvent and novel extraction techniques such as UAE and MAE have been used for better extraction of bioactive compounds specially the hydrophobic compounds [15,18,43], but no study has been reported about the green extraction of bioactive compounds from passion fruits peel waste using green technology for industrial application.

One of the predominant by-products of the PFJ industry is seed, which contains significant amount of oil (22-30 %) [33,36] and dietary fibre (insoluble) (64.1 g/100 g) [8]. The passion fruit seed oil contains significant amount of polyphenols and strong antioxidant activity (34 mg/mL) [28,33] and exhibits antimicrobial effects [21,22]. Similarly, passion fruit seed fibre also showed good antioxidant and antibacterial properties [28]. So, passion fruit seed oil and fibre can be used as functional ingredient in juice. Direct incorporation of fibre and oil is a difficult task and emulsification is an emerging technique for the incorporation of fibre and oil in liquid food system. No study has found about the use of fibre and oil in passion fruit juice for shelf-life enhancement.

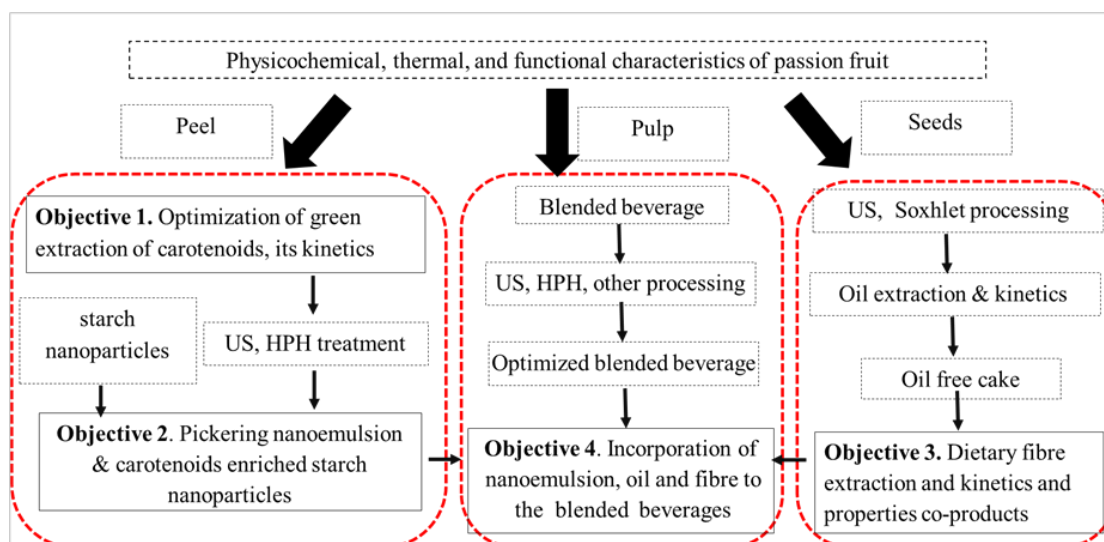
1.6. The objective of the present investigation

Since food waste management is a serious environmental issue, converting the peel and seeds generated as waste in the passion fruit processing industries into value-added components will mitigate environmental problems. So, the primary objective of this investigation was to valorise passion fruit and develop a beverage enriched with bioactive compounds extracted from its peel and seed using a green process that has scope for industrial application.

To achieve the goal, the following objectives were undertaken.

1. To optimize the extraction of carotenoids from passion fruit peel using novel and green techniques and study its kinetics for industrial application.
2. To develop and evaluate Pickering nanoemulsion containing passion fruit peel-derived bioactive compounds and stabilized with starch nanoparticles.
3. To optimize the extraction of oil and dietary fibre from seeds of passion fruit and study their extraction kinetics and properties.
4. To evaluate the quality of blended fruit juice of passion fruit and other fruits incorporated with the oil and dietary fibre extracted from the seeds of the fruit, and study the bio-accessibility of carotenoids.

The flowchart presents the overall plan of work covering all the objectives.



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