ABSTRACT

Introduction

North-East India is rich in several underutilized fruits that are rich in different bioactive compounds like carotenoids, polyphenols, phytosterols etc., which have protective action against cancer, neurological diseases, cardiovascular diseases, obesity, inflammations, infections, etc. [1,6,14]. Passion fruit *(Passiflora edulis)* also known as *Passiflora* fruit, is an attractive and nutritious exotic fruit consumed mostly in the fresh form because of its unique taste, and used in traditional medicine. It is also being exploited by food processing, pharmaceutical and cosmetics industries [15]. In 2017, the estimated cultivation area and production of passion fruits in India were around 19.01 thousand ha and 123.94 thousand tons, respectively, whereas more than 70 % of the total production is from Northeast India [18]. However, due to a lack of awareness about its health benefits, people have not been able to extract the full benefits of the fruit [5]. In recent years, the fruit is being widely studied for its desirable chemical composition [8,18]. This fruit, especially the pulp appears to be an excellent source of vitamins and minerals that are essential for life.

Although the pulp/juice of passion fruit is enriched with several health-benefiting compounds, its direct use has fewer acceptances by customers because of its high acidity and astringency [2]. Also, the juice spoils easily due to the growth of yeast and bacteria. Normally, at room temperature, raw passion fruit juice undergoes alcoholic fermentation by yeast followed by the oxidation of alcohol by bacteria [2]. The passion fruit is of interest not only because of the pulp but also for its peel and seeds, which contain a high amount and number of bioactive compounds such as carotenoids, polyphenolic groups, anthocyanins, etc. [6,16]. The extract of this fruit peel has been used to cure several diseases like wheezing, cough, and shortness of breath in adults with asthma, hypertension, anxiety, insomnia, etc. and also has been using in folk medicine [19]. Seeds of passion fruit also contain approximately 20-30% oil [8,15], which is rich in several bioactive compounds, such as polyphenols, phytosterols, and essential fatty acids with a higher proportion of unsaturated fatty acids [7]. Passion fruit seed oil exhibits antimicrobial effects [11]. The seed oil contains approximately 570 mg/kg of total phenolic compounds and possesses strong antioxidant activity [15]. The edible nature of passion fruit seed oil and fibre is already well established [3,10].

Passion fruit juice (PFJ) processing industries create a large amount of discarded portion comprising of peels and seeds, that make up more than half (approximately 60%)

of the whole fruit and is considered as a waste [13]. Dumping of this waste material into the ecosystem may cause environmental degradation, besides the loss of bioactive compounds present in the peel and seeds. One of the most useful options for the management of fruit waste is the recovery of its health-benefiting compounds using modern techniques like ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) with an appropriate solvent and using them in food processing, pharmaceutical, and cosmetic related industries [7,9].

An emulsion is a promising medium to incorporate lipophilic bioactive compounds in a carrier system for retaining their bioactivity. Apart from solubility, the emulsion is also being used to control the delivery of bioactive compounds and enhance the quality and shelf life of food products [4]. Pickering emulsions, which are stabilized by solid colloidal particles have many advantages over conventional emulsions in terms of stability and development of chemical-free products [4]. Different solid particles such as starch, protein, and fibre are being used in food products for the development of Pickering emulsion with oil that acts as a vehicle for delivery of antimicrobial bioactive compounds [17].

Seed, the waste portion of passion fruit, contains 20-30% oil and is loaded with bioactive compounds having antioxidant and antimicrobial activities [8,15]. Also, the seeds of passion fruit are high in insoluble dietary fibre (64.1 g/100 g) [3] and the basic health-benefiting effects of fibre are already well established. Apart from it, dietary fibre powder obtained from yellow passion fruit co-products showed antioxidant properties and antibacterial properties due to the presence of a significant amount of total phenolic and flavonoid content, which therefore, can be used as a natural food ingredient [12]. Since food waste management is a serious environmental issue, converting these wastes into value-added components must be given more attention.

Consumers prefer high-quality fruit juice that is nutritious, contains minimal chemical preservatives, and maintains natural characteristics while conforming to legislative requirements. The sensorial and shelf-life issues related PFJ are still big challenges with produced waste by-products, which limit the industrialization of passion fruit. So, the main aim of this study was to valorise passion fruit and develop a beverage enriched with bioactive compounds from its peel and seed using a process that has scope for industrial application.

Objectives

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 extracts from the seeds of the fruit, and study the bio-accessibility of carotenoids.

Methodology of the present work

The methodology followed in the present investigation to achieve the above-said objectives are given below.

Extraction of carotenoids from passion fruit peel using novel and green techniques and study its kinetics for industrial application

- Yellow colour passion fruit was collected from Bishnupur, Manipur and Kohima, Nagaland and its physicochemical properties were analysed.
- Effect of green processes Ultrasonic assisted extraction (UAE) and Microwave assisted extraction (MAE) using olive oil (OO) and sunflower oil (SO) as solvents on the extractability of carotenoids from passion fruit peel, and its optimization was done.
- Comparative study of the extraction techniques in terms of energy efficiency and quality evaluation of the optimized carotenoids enriched oil were performed.
- The mass transfer kinetics, effective diffusivity and thermodynamics properties of carotenoids extraction for the optimized process and solvent for an insight into the feasibility of industrial application of the green technologies were also studied.

Develop and evaluate Pickering nanoemulsion containing passion fruit peel-derived bioactive compounds and stabilized with starch nanoparticles

- Effect of ultrasound (US), and high-pressure homogenization (HPH) on starch particles were analysed.

- Physiochemical properties of developed US-treated, HPH treated and carotenoids enriched starch nanoparticles were evaluated.
- Fabrication of stabilized Pickering nanoemulsion with passion fruit peel derived carotenoids-enriched starch nanoparticles and its physicochemical and stability study was performed.

Optimize the extraction of oil and dietary fibre from seeds of passion fruit and study the extraction kinetics and properties

- Extraction of oil from the passion fruit seeds using novel technologies like UAE, conventional Soxhlet extraction (CE) method, and combination of both and its optimization and evaluation of oil properties were done.
- Kinetics study of extraction phenomena of oil from seed was analysed.
- Extraction of dietary fibre from the seed cake using different extraction techniques such as alkaline extraction, ultrasound-assisted extraction and combined alkaline and ultrasound-assisted extraction and its optimization and quality parameters were evaluated.

Evaluate the quality of blended fruit juice of passion fruit and other fruits incorporated with the oil and dietary fibre extracts from the seeds of the fruit, and study the bio-accessibility of carotenoids.

- Physicochemical properties and the shelf life of raw and sensory-optimized passion fruit-based blended beverages were studied.
- The effect of thermal pasteurization, US, and HPH on shelf stability and quality of juice was analysed.
- Physicochemical, functional and storage properties of oil and dietary fibre-based emulsion-incorporated beverages and also treated beverages, and the bio-accessibility of carotenoids were analysed.

According to the framework of the research, the thesis has been organized into seven chapters as discussed below:

Chapter 1: This chapter gives a brief introduction to the topic of research and states the research gap, the hypothesis and objectives.

Chapter 2: This segment of the thesis elucidates the reported works conducted by researchers that are relevant to the current work.

Chapter 3: This chapter discusses about objective 1. Briefly, the effect of conventional, UAE and MAE using OO and SO as solvents on the extractability of carotenoids from PFP and its kinetics and thermodynamics studies were investigated. Results suggested that both UAE and MAE processes showed the potential to enhance the extraction efficiency of carotenoids. Among UAE, MAE, and CE, UAE with OO as a solvent was found best for the extraction of carotenoids from PFP. The kinetics information mainly rate constant, effective diffusivity, and mass transfer coefficient obtained from the green extraction process using UAE and CE allowed efficient prediction of extraction operation conditions for industrial implementation.

Chapter 4: This chapter deals with objective 2. The effect of US and HPH on starch nanoparticles and the properties of nanoparticles were evaluated. The studies on the fabrication of stabilized Pickering nanoemulsion with PFP-derived carotenoids-enriched starch nanoparticles and its physicochemical properties and stability are reported. Results suggested that as the US treatment time increased, mean particles size decreased from micro size to 340 nm, and the subsequent HPH treatment of the suspension further decreased the particles size to less than 60 nm. The nanoemulsion that was developed was rich in naturally available carotenoids, total polyphenol groups, and possessed higher antioxidant activity. The developed carotenoids-enriched emulsion was found to be stable to heat and freeze-thaw cycles. Nanoemulsion developed under optimized conditions showed better stability of its antioxidant activity than normal emulsion. The developed nanoemulsion showed better oxidative stability as well as physical storage stability during storage at 6 and 25 ^{o}C.

Kinetics and thermodynamics studies on the thermal degradation of differently treated starch nanoparticles and estimation of the OH groups and H_2O content on particle surface were also studied. The results revealed that UST, HPH with the help of carotenoids-enriched oil were able to develop thermally stable starch nanoparticles (StNPs) with desirable kinetics properties. The OH group and physisorbed water on the starch surfaces increased after the treatment. The investigation made it possible to estimate the temperature ranges required to achieve the necessary attributes.

Chapter 5: Pertains to the studies performed to fulfil Objective 3. The extraction of oil from the seeds using a novel technology like UAE and conventional Soxhlet extraction method, optimization and evaluation of oil properties, and the extraction kinetics were performed. The results suggested that a combined ultrasonication followed by Soxhlet is a viable alternative method to only Soxhlet extraction with better extract quality. Finally, a phenomenological model with combined UAE and Soxhlet could be recommended for modelling the extraction kinetics with better quality oil from the seeds.

Further, the extraction of dietary fibre (DF) from the seed cake using different extraction techniques such as alkaline extraction, UAE, and combined alkaline and UAE, and its optimization and quality were evaluated. Results revealed that the alkaline extraction yielded 52.8 % DF, whereas UAE for only 90 min yielded 50.4 % and the ultrasonic-alkaline method yielded 61.35 % after 28.3 min of sonication/ extraction time with 284.8 W ultrasonic power and 8.33 g/100 mL of solid to liquid content. However, compared to ultrasound-alkali method, only ultrasonication enhanced the functional quality of DF by increasing the water-holding, oil-holding, swelling capacities, and emulsion capacity, while decreasing the cation exchange capacity, glucose adsorption capacity, and α-amylase activity. The cytotoxicity study revealed that ultrasonication treatment increased toxicity, but within the acceptable limit. These results indicated that dietary fibre from defatted passion fruit seeds could be used as a functional food ingredient.

Chapter 6: This chapter discusses (objective 4) the physicochemical properties of passion fruit juice and the sensory optimization of blended juice using fuzzy logic approach. The shelf life and quality parameters of pasteurization, US, and HPH were also studied. The physicochemical, functional and storage properties of oil and dietary fibre-incorporated juice and the bio-accessibility of carotenoids from the juice revealed that nonthermal treatment showed better retention properties. Results showed that passion fruit juice was enriched with several bioactive compounds but the shelf life of raw/untreated juice was very less (5 days in refrigerated and 2 days at 25 ^{o}C). After the treatment of US and HPH, the shelf life increased significantly but showed differences in biochemical properties. The shelf-life prediction of treated juices was also studied and the prediction was made efficiently using a predictive model. Finally, the oil and dietary fibre incorporated juice showed a shelf life of more than 8 weeks during storage at refrigerated temperature while maintaining the quality parameters.

Chapter 7: This chapter consists of the overall conclusion of the study. It includes the conclusion of each objective, salient findings and future scope of investigations.

Keywords: Passion fruit, Pickering nanoemulsion, bio-accessibility, Carotenoids, Functional foods

Bibliography

[1]. Adeyeye, E. Ii. and Aremu, M. O. Chemical Composition of the Raw Fruit Coat, Seed and Pulp of Passion Fruit *(Passiflora edulis*). *FUW Trends in Science & Technology Journal*, *2*(1):334–341, 2017.

[2]. Akpan, U. G. and Kovo, A. S. Preservation of passion fruit juice. *Leonardo J. Sci*, *7*, 17-22, 2005

[3]. Chau, C. F. and Huang, Y. L. Characterization of passion fruit seed fibres—a potential fibre source. *Food Chemistry*, *85*(2), 189-194, 2004.

[4]. Dammak, I., Sobral, P. J. D. A., Aquino, A., Neves, M. A. D., and Conte-Junior, C. A. Nanoemulsions: Using emulsifiers from natural sources replacing synthetic ones—A review. *Comprehensive reviews in food science and food safety*, *19*(5): 2721-2746. 2020.

[5] De, L. C. Valuable indigenous fruit crops of North-eastern Region of India. *International Journal of Research in Applied, Natural and Social Sciences*, *5*(3):21-42, 2017.

[6]. Reis, L. C., Facco, E. M. P., Salvador, M., Flores, S. hickmann, and Rios, A. de O. Antioxidant potential and physicochemical characterization of yellow , purple and orange passion fruit. *Journal of Food Science and Technology*, 55(7):2679–2691, 2018.

[7]. Elik, A., Yanik, D. K., and Göğüş, F. Microwave-assisted extraction of carotenoids from carrot juice processing waste using flaxseed oil as a solvent. *LWT - Food Science and Technology*, *123*: e109100, 2020.

[8]. Giuffre, A. M. Chemical composition of purple passion fruit (Passiflora edulis Sims var. edulis) seed oil. *Rivista Italiana Delle Sostanze Grasse* , 2007.

[9]. Goula, A. M., Ververi, M., Adamopoulou, A., and Kaderides, K. Green ultrasound-assisted extraction of carotenoids from pomegranate wastes using vegetable oils. *Ultrasonics - Sonochemistry*, 34:821–830, 2017.

[10]. He, X., Luan, F., Yang, Y., Wang, Z., Zhao, Z., Fang, J., ... and Li, Y. *Passiflora edulis:*An insight into current researches on phytochemistry and pharmacology. *Frontiers in pharmacology*, *11*, 617, 2020.

[11]. Jusuf, N. K, Putra, I. B., and Dewi, N. K. Antibacterial Activity of Passion Fruit Purple Variant *(Passiflora edulis Sims var. edulis)* Seeds Extract Against Propionibacterium acnes. *Clinical, Cosmetic and Investigational Dermatology*, 13:

99-104, 2020.

[12]. López-Vargas, J. H., Fernández-López, J., Pérez-Álvarez, J. A., and Viuda-Martos, M. Chemical, physico-chemical, technological, antibacterial and antioxidant properties of dietary fiber powder obtained from yellow passion fruit (*Passiflora edulis var. flavicarpa*) co-products. *Food Research International*, *51*(2), 756-763. 2013.

[13]. Oliveira, C. F., Gurak, P. D., Cladera-Olivera, F., and Marczak, L. D. F. Evaluation of physicochemical, technological and morphological characteristics of powdered yellow passion fruit peel. *International Food Research Journal*, *23*(4):1653–1662, 2016.

[14]. Pertuzatti, P. B., Sganzerla, M., Jacques, A. C., Barcia, M. T., and Zambiazi, R. C. Carotenoids, tocopherols and ascorbic acid content in yellow passion fruit (Passiflora edulis) grown under different cultivation systems. *LWT - Food Science and Technology*, *64*(1):259–263, 2015.

[15]. Ramaiya, S. D., Bujang, J. S., and Zakaria, M. H. Physicochemical, Fatty Acid and Antioxidant Properties of Passion Fruit (Passiflora Species) Seed Oil. *Pakistan Journal of Nutrition*, 18(5):421–429, 2019.

[16]. Rotta, E. M., Rodrigues, C. A., Jardim, I. C. S. F., Maldaner, L., and Visentainer, J. V. Determination of phenolic compounds and antioxidant activity in passion fruit pulp (*Passiflora spp*.) using a modified QuEChERS method and UHPLC-MS/MS. *LWT-Food Science and Technology*, 100:397–403, 2019.

[17]. Ruiz-Montañez, G., Ragazzo-Sanchez, J. A., Picart-Palmade, L., Calderón-Santoyo, M., and Chevalier-Lucia, D. Optimization of nanoemulsions processed by high-pressure homogenization to protect a bioactive extract of jackfruit (*Artocarpus heterophyllus* Lam). *Innovative Food Science and Emerging Technologies*, *40*:35–41. 2016.

[18]. Thokchom, R. and Mandal, G. Production Preference and Importance of Passion Fruit (Passiflora Edulis): A Review. *Journal of Agricultural Engineering and Food Technology* 4(1):27–30, 2017.

[19]. Waston, R. R., Zibadi, S., Rafatpanah, H., Jabbari, F., Ghasemi, R., Ghafari, J., Afrasiabi, H., Foo, L. Y., and Faridhosseini, R. Oral administration of the purple passion fruit peel extract reduces wheeze and cough and improves shortness of breath in adults with asthma. *Nutrition Research*, *28*:166–171, 2008.