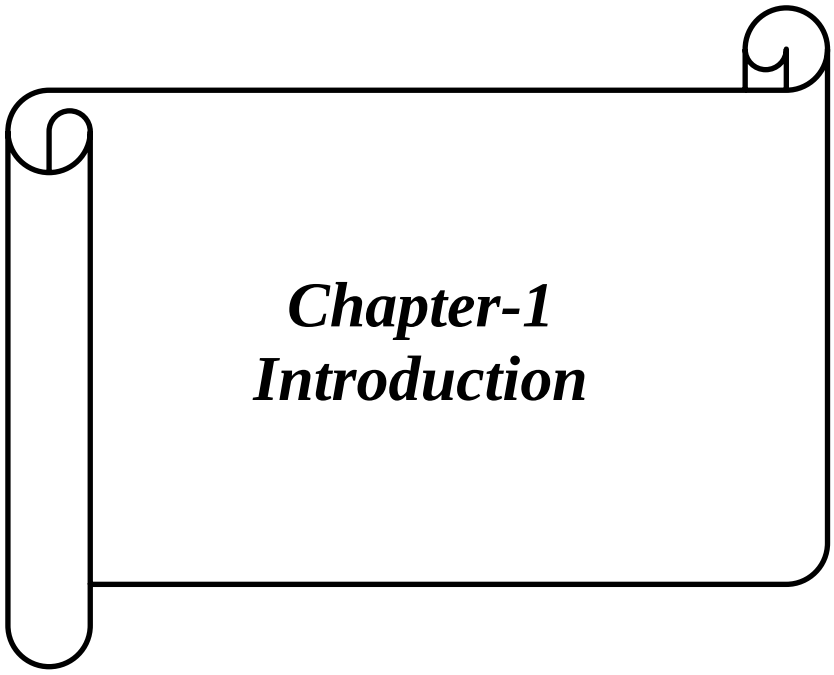

Chapter-1
Introduction

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Tropical fruits are consumed frequently in both India and other parts of the world due to their medicinal and nutritive benefits. Tropical fruits are usually processed to remove important desirable items from plant parts, which thus produce by-products such as skin, seeds, and inedible pulp **[4]**. According to Sun Waterhouse et al. **[27]**, the disposal of these byproducts poses major challenges for waste management, environmental concerns, legal limitations, and financial considerations. The application of such by-products for producing functional products or nutraceutical supplements is considered a possible solution to overcome the aforementioned challenges. These by-products prove to be a vital source of the crucial bioactive substances, including vitamin C, phenolic compounds, flavonoids, and carotenoids that have antioxidant characteristics and are beneficial to health **[30]**.

The papaya (*Carica papaya* L.), a tropical fruit in the *Caricaceae* family, has been used as a therapeutic medicine for centuries. It is also named to as 'Papaya' in English, 'Papita' in Hindi, and 'Erandakarkati' in Sanskrit. The plant originates from North America and was brought to India in the 16th century **[33]**. Papaya is a year-round crop, highly valued in the Caricaceae family and ranks third in total tropical fruit production after mango and pineapple, contributing to 15.36% of the total. Globally, papaya production amounts to 13.4 million metric tons, with India's capacity at 6.39 million metric tons **[9]**.It has wide consumption owing to its superior taste and great nutrition value. It is a great source of the papain enzyme, antioxidants, including carotenoids, and vitamins B and C. Vitamin C content is found in notable quantities in papaya **[15]**. The mineral composition of papaya includes calcium, iron, manganese, phosphorus, zinc, and K and Mg **[14]**.

Additionally, it contains the digestive enzyme papain, which decreases the possibility of allergies, sports injuries, and trauma. It also eases down the digestion of proteins found in food at alkaline, neutral, and acidic pH levels. As a whole, papaya improves cardiovascular health and protects against heart attacks, colon cancer, and strokes. This fruit is a very high source of carotene, thereby preventing free radical damage, which can lead to cancer **[20]**. It lowers high cholesterol levels since it is abundant in fiber. Moreover, it has been used as a stabilizer, chewing gum, meat

tenderizer, and to clarify beer in commercial preparations. Although few studies have been documented in the literature on using papaya to make value items like beverages, jam, jelly, etc.,.

Moreover, the papaya peel has soluble solids, fiber, vitamin C, phenolic compounds, papain enzyme, and minerals including calcium, copper, salt, and potassium. Its peel is frequently viewed as waste that poses a risk to the environment if produced in excess. It is discarded and does not find usage in terms of usage or recycling **[25]**. Interestingly, papaya fruit byproducts that is the peel and seed are found to contain numerous useful components including carotenoids, flavonoids, and polyphenols, similar to the fruit pulp itself, might exhibit great antioxidant potential **[1,16,28]**. Papaya peel is utilized as a food ingredient in some of the less developed tropical nations. The increased use of papaya pulp for processing makes it necessary to assess the possibilities for using papaya peel in human and/or animal diets **[21]**. Papaya peel has traditionally been used to tenderize the meat. Numerous fruit products have been studied, including pomegranate peel, which in comparison to the pulp found to have higher antioxidant activity **[19]**; and tomato pomace, seeds, and skin containing higher levels of the antioxidant lycopene **[8]**; and grape seed which have higher levels of the antioxidant pro-anthocyanidin than the pulp and can scavenge various species of reactive oxygen free-radical species **[12]**. While papaya pulp contains the highest concentration of carotenoids found in nature with great antioxidant activity **[22]**, its peel is also a good source of antioxidants and phenolics. Papaya peel offers anti-dandruff, soothing, and moisturizing properties that are significant for the cosmetics industry **[3]**.

The extraction of bioactive components depends on numerous variables, including the extraction technique, the solvent and the raw materials **[29]**. Numerous novel extraction approaches have been put forward in the past few years to address a number of drawbacks that the traditional extraction technique confronts. Several studies have noted that combination of several novel extraction techniques could result in effective and quick extraction methods. The most often described and widely utilized novel extraction techniques for bioactive compounds are ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE) **[11]**. Moreover, the implementation of these novel approaches results in a considerable reduction in the amount of solvent utilized thus reduces extraction costs and energy consumption and obtaining excellent extraction efficiency for bioactive components

[10]. Thermal pre-treatments and other treatments that continually increase the temperature must be optimized in order to prevent component degradation. Unfortunately, these procedures might result in an excessive loss of important substances, particularly volatile components. Soaking the material in ethanol and water over time, may degrade the material's cellular structure and facilitate solvent penetration **[31]**. Energy is directly transmitted when microwave radiation is used on plant material, which causes electromagnetic field interactions with molecules. It is possible to quickly create constant heating in this way. This improves the material's porosity, thus allowing easy solvent penetration and the chemicals to be accessible **[23]**. When plants are exposed to microwave radiation, the energy released primarily targets the moisture present in them. If moisture and microwave exposure are combined, it can lead to a significant enhancement in the permeability of the material within a brief period. Enzymatic pre-treatment is a technique used to break down the cell wall of plants using mild conditions, aiding in biodegradation. The plant may release more oil when exposed to this treatment **[18]**. Another advantage of the enzymatic pre-treatment is that it doesn't need expensive equipment **[17]**.

Industries are in search of rapid and cost-effective methods for purifying protein products, which can achieve both high yield and high purity **[13]**. The aqueous two-phase system (ATPS) is a technique that fulfills these requirements. The main advantage of this system is that it provides a mild operating environment, making scaling up straightforward, while also enabling a rapid process with minimal material requirements. Furthermore, protein denaturation is limited in this system **[2,7]**. However, only few papers on the total phenolic contents of papaya peel are published. In practical terms, there is no study available on the kinetics of novel technique based total phenolic component extraction from papaya peel. Moreover, papaya peels are a great source of the papain enzyme, which is known to be a key component of papaya peels and is known for its proteolytic activities **[6]**. The properties and protein content of proteases extracted from papaya peels have not yet been examined. There is a demand for a systematic research and engineering strategy for extraction, especially for the peel, because the extraction efficiency and quality of the extract depend on numerous processing factors **[26]**.

However, it was recognized that naturally occurring bioactive compounds were chemically unstable and prone to oxidation, therefore their direct usage was relatively

constrained in formulations and required protection from the surrounding **[24,32]**. As per, Baghero et al. **[5]**, there are various method of microencapsulation are employed in the food sector to preserve these extracted bioactive compounds, including freeze-drying, spray-drying, coacervation, inclusion complexation, emulsification, and liposome entrapment.

The research work was conducted to make use of the by-product of papaya process fruit by utilizing the natural sources such as phytochemical compounds and papain enzyme. The present study aimed to evaluate the effects of various parameters and different extraction methods related to bioactive compounds, from papaya peel and to enhance them through different novel pre-treatment approaches and extract using ultrasound-assisted extraction (UAE). The extracted bioactive compounds are freeze dried and a new product is developed by incorporating the extracted bioactive compounds. Additionally, extraction of papain enzyme using aqueous two-phase extraction has been done that would be further used in food processing.

The whole work is divided into the following objectives to achieve the aim of the present research/thesis:

1. To extract bioactive compounds from papaya peels using ultrasound, microwave and enzymes.
2. To extract papain from papaya peel using Aqueous Two-Phase Extraction (ATPE) and compare with conventional extraction.
3. To study the application of extracted compounds in food processing.

   A. To develop a food product by incorporating extracted bioactive compounds.

   B. Application of papain enzyme in food processing.

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