Chapter-2

Review of Literature

Chapter 2

Review of Literature

2.1 Papaya production and utilization

Papaya is a popular tropical and subtropical fruit with both medicinal and nutritional properties. The papaya is currently included in the Caricaceae botanical family, comprising 35 species that produce latex and are categorized into four genera: Carica, Jarilla, Jacaratia, and Cyclicomorpha **[78]**. It was previously classified under the Passifloraceae, Bixaceae, Cucurbitaceae, and Papayaceae families. Papaya is native to southern Mexico, the Philippines, and Central America **[56,62]**. FAOSTAT **[49]** lists India, Mexico, Brazil, Indonesia, and Nigeria among the top producers.

The papaya plant is a tall, single-stemmed, perennial herbaceous tree that grows 20–30 feet in height (Figure 2.1a). The plant possesses hollow stems that measure 8 inches in diameter, displaying a range of colors from light green to tawny brown, with evident marks or blemishes **[29]**. The fruits are large and oval shaped, and have a central seed chamber just like melon, thus frequently referred to as pepo-like berries (Figure 2.1c). Different papaya types may be identified by their differences in leaf structure, stomatal shape, numbers of central leaf veins, lobes at the leaf margins, waxed coating on the surface of the leaf, and petiole coloring.

Papaya has a huge export market in the global trade of tropical fruits due to its excellent nutritional content **[107]**. After mango and pineapple, papaya is presently placed third with 15.36% of total tropical fruit output. Papaya has a global production of 13.4 million metric tons. India's production capacity is 6.39 million metric tons. On the basis of state-by-state value of papaya production, Andhra Pradesh is the first major producer and Gujarat is the second largest **[49].**

Many bioactive components found in papaya, including carotenoids, phenolic compounds, vitamins A, C, and E, pantothenic acid, minerals (potassium and magnesium), folate, and fiber, have several positive health benefits on the body that are attributed to their ant oxidation characteristics **[154]**. Based on nutritional evaluations, papaya is ranked among the top five fruits, with watermelon, kiwi, guava and apple **[4,125]**. Furthermore, papaya is a natural source of papain, a digestive enzyme that finds application in the food processing, pharmaceuticals, brewing, and cosmetics industries. The majority of products made from papaya include papaya powder, chips, tutu fruity, skin care products, papain, candies, jam, jelly, pickles, sauce, squash, etc. Therefore, it should come as no surprise that using these fruits for the numerous uses stated above creates a lot of waste and by-products, especially during the harvesting and post-harvesting processes **[113]**.

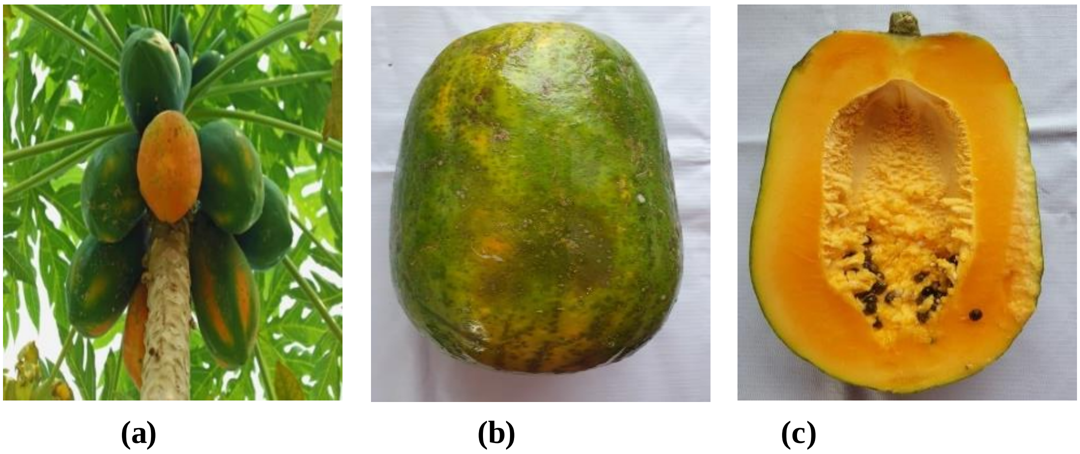

Figure 2.1: (a) Papaya tree (b) Papaya fruit (c) Fresh cut papaya fruit

Processing of papaya results in the production of 6.51% of seeds, 8.47% of skin, 32% of pulp, which is unusable, and 52.96% of finished products **[11]**. The production of such significant volumes of waste material shows that the value addition of all these by-products would greatly benefit to papaya growers. Characterizing the various ripe and unripe papaya parts, along with their peels and seeds, is a useful approach to show that their complete usage may provide vital elements including vitamins and bioactive substances **[67]**. Carotenoids, phenolic acids, and flavonoids are the principal bioactive compounds found in papaya pulp, skin, and seeds, respectively **[12]**. Papaya by-products might be employed as antimicrobials, antioxidants, colourants, flavourings, thickening agents, and other biologically useful substances because of the biological characteristics of these bioactive compounds **[12]**. Edible papaya and its by-products are attractive food components because of the bioactive substances they contain, especially when it comes to the development of functional food products or nutraceutical ingredients.

2.1.1 Nutritional and phytochemical profile

According to the papaya's chemical analysis, it contains significant quantities of sodium, calcium, phosphorus, zinc, iron, copper, magnesium, and manganese (as shown in Table 2.1). Additionally, it has detectable quantities of potassium, with a measurement of 223 mg per 100 grams of fresh fruit. In terms of vitamin A, C, B1, B2, thiamine, folate, niacin, riboflavin, calcium, iron, potassium, and fiber content, papaya has a prominent position among fruits. It is high in vitamins and minerals yet low in calories. For 100 g of fruit, around 60% of the mature fruit is edible. Papaya has an energy content of 200 kJ/100 g. The three primary sugars are sucrose (48.3 g/100 g), fructose (21.9), and glucose (29.8 g/100 g). Ascorbic acid content in fresh fruit is around 108 mg/100 g, which is greater than oranges with content of 67 mg/100 g **[83]**. Volatile compounds, such as hydrocarbons, alcohols, aldehydes, ketones, terpenes, esters, benzyl isothiocyanate, and organic acids, are responsible for sensory properties, such as taste and aroma **[7,57]**. Nevertheless, fruit aroma is attributed to ethyl hexanoate, ethyl 2-methyl butanoate, and ethyl acetate **[17,116]**. The classes of hydrocarbons are found to be more abundant and to contribute significantly to aroma are aliphatic and aromatic hydrocarbons **[54]**. According to Flath & Forrey **[54]**, the volatile compound linalool is quite prevalent among papaya species, accounting for 94% of them. The pulp color of papayas is thought to be a key indicator of their nutritional value **[153]**. Ripe fruit has a soft texture and amber to orange colour development. It has a cantaloupe-like flavour that is sweet and juicy with a touch of musk **[102]**, and the pulp has total soluble solids (TSS) of 10-11.5%. The substances identified in the fruit includes: caffeic acid (175.51 - 112.89 mg/100 g), p-coumaric acid (229.59 - 135.64 mg/100 g), and ferulic acid (277.49 -186.63 mg/100 g of fresh fruit).

However, the levels of the following carotenoids, rise with ripening stage: lycopene (0.36 - 3.40 mg/100 g), β-criptoxanthin (0.28 - 1.06 mg/100 g), β-carotene (0.23 - 0.50 mg/100 g), and vitamin C (25.07 - 58.59 mg/100 g). Numerous scientific investigations have been conducted to assess the biological functions of the fruits, peels, leaves, latex, seeds, and roots of the papaya. The plant's various tissues contain a wide range of bioactive compounds with distinct functions. For instance, the fruit pulp contains linalool **[148]**, while the leaves contain carpaine, pseudocarpaine, dehydrocarpaine I and II, alkaloids **[73]**, and the latex contains glutaminyl cyclase **[13]**.

The shoots contain cysteine endopeptidases, quercetin, and kaempferol **[100]**, while the roots contain cyanogenic compounds. Additionally, benzyl glucosinolate and its breakdown product, benzyl isothiocyanate, are present in all tissues collectively. These components can be identified with the help of chemical characterization of isolated metabolite **[109]**.

Table 2.1: Nutritional value in papaya fruits, seeds, and leaf (mg/100 g)

Parameter	Fruit	Seed	Leaf
Moisture (%)	84.32-90.76	2.30-9.73	58.5-64.10
Ash (%)	0.27-0.38	6.84-10.7	1.87-11.20
Carbohydrate (%)	7.66-13.54	8.3-26.6	10.47-58.40
Protein (%)	0.37-0.45	24.3-31.80	16.08-29.50
Lipid (%)	0.20-0.29	20.97-30.1	2.70-6.31
Dietary fiber (%)	0.37-0.60	17.0-22.6	1.27-2.13
β-carotene (μg/g)	208.67-4534.26	888.00	-
Ascorbic acid (mg/g)	35.32-43.80	0.12-0.15	126.2
Na	6.79-9.53	39.80	0.03 g/kg
K	18.36-24.78	743.30-1635.50	2.75
Fe	0.61-0.85	5.23-5.80	0.46-1.84
Ca	27.88-32.48	725.00-8435.10	4.80-32.73
Zn	-	5.00-6.17	0.04-0.05
P	11.54-16.81	566.9	-
Cu	-	0.50-1.09	0.01-0.06
Mn	-	2.50-3.10	0.12-0.66
Mg	9.45-13.63	218.80-332.50	16.18

Source: Dotto & Abihuda **[44]** and Boshra &Tajul **[24]**

2.2 Papaya peel

Papaya leaves, fruits, seeds, and peels all have nutritional benefits that make them valuable for human consumption. Different papaya portions contain varied amounts of macro- and micronutrients. Moreover, studies have shown that the papaya seeds and peels are a good source of minerals (Table 2.1). As stated by Santos et al. **[127]** and Morais et al. **[101]**, minerals play a crucial role in maintaining the effective operation of physiological and metabolic systems within the body. Dietary fibers may be found in significant amounts in papaya peels. Dietary fibers provide numerous health advantages, including their ability to reduce cholesterol levels and eliminate toxins from the digestive system **[19,151]**. Papaya peels contains 67–86% moisture, 8–20% protein, 0.19–2% fat, and 3–12% ash **[14,89,127]**. The peel is a source of both macronutrients and trace minerals. The literature demonstrates that papaya leaf, seed, and peel extracts with their antioxidant properties can fight against cancer by reducing tumor development, inducing apoptosis, and can prevents metastasis in some cancer cells that were the subject of the studies **[113,127]**. The extracts obtained from by-products such as the peel, leaf, and seed contain bioactive compounds such as benzyl isothiocyanate, phenolics, carotenoids, glucosinolates, flavonoids, α-tocopherol, and lycopene, which contribute to their anticancer properties **[95]**. Numerous studies on the seeds, leaves, and peels of papaya fruit have been conducted throughout the world in an effort to examine the activities of their bioactive compounds, which have been found to have anticancer, antioxidant, antimicrobial, antifungal, antiparasitic, gastroprotective, , hypoglycemic, , contraceptive, and hepatoprotective properties **[93,116]**.

According to the information presented in Table 2.2, it was observed that papaya peels have the ability to serve as a promising natural source of antioxidants. According to the findings of Martial-Didier et al. **[95]**, the phytochemical analysis showed elevated concentrations of total phenolics (65.58 mg GAE/100 g DW), flavonoids (5.68 mg QE/100 g DW), and tannins (10.41 mg TAE/100 g DW).

Table 2.2 Bioactive compounds of papaya peel and their activities

Sl. No.	Bioactive compounds	Activities	References
1.	Soluble polyphenols	Antioxidant, anti-inflammatory	Faller & Fialho **[47]**
2.	Hydrolysable polyphenols	Antioxidant, antibacterial, antiviral, cytoprotective activity	Faller & Fialho **[47]**
3.	Total phenolics	Antioxidant, antiviral, anticancer	Matsusaka & Kawabata **[96]**
4.	Caffeic Acid	Anti-inflammatory, anticancer, and antiviral	Rivera-Pastrana et al., **[122]**
5.	Rutin	Antioxidant, anti-allergies, anti-viruses	Rivera-Pastrana et al., **[122]**
6.	Ferulic Acid	Antioxidants rich in vitamin A, vitamin C, and vitamin E, anti-aging	Rivera-Pastrana et al., **[122]**
7.	Catechin	Anti-inflammatory, anti-mutagenic, and anti-carcinogenic effects	Marina & Noriham **[93]**
8.	Epicatechin	Anti-diabetes and cardiovascular diseases	Marina & Noriham **[93]**
9.	Tannins	Reduces blood pressure	Martial-Didier et al., **[95]**

Papaya peel has the potential to serve as a valuable source of essential mineral nutrients, making it a viable option as an alternative food source. However, it is important to note that the bioavailability of these minerals can be influenced by various factors, including the concentration and oxidation state of the minerals, solubility, and the presence of inhibitors or compounds that may have synergistic effects within the food **[44]**. Therefore, it is not advisable to consume papaya peel on a daily basis. However, by employing appropriate processing methods, extracts derived from papaya peel could be utilized as a mineral source. It should also be noted that the mineral content in the peel and leaves of the papaya plant tends to be higher compared to the pulp **[113]**.

According to Pathak et al. **[113]**, the levels of calcium and magnesium, essential elements in chlorophyll, decrease as the papaya ripens **[13]**. Papaya peel also contains various trace minerals, including Br, Cr, Cs, Fe, La, Na, Rb, Sc, and Zn, although their specific functions in the human body are not well-known. It is worth noting that the concentration of these minerals, such as Br, can be influenced by pesticide usage, but even with chemical treatments, all values remain within the typical range (> 3 mg/kg) found in plants and plant-derived foods **[14]**. As a result, regular consumption of papaya peel may not be advisable; however, extracts obtained from the peel, after appropriate processing, could serve as a viable source of minerals.

2.2.1 Valorization of papaya peel

Papaya peel contains a variety of beneficial constituents, such as polyphenols, fibers, carbohydrates, proteins, minerals, tannins, and fatty acids. These components make papaya peel a valuable raw material with the potential to produce various high-value products. The presence of polyphenols makes it suitable for remedial uses, including cosmetics and therapeutic applications **[113]**. Furthermore, its carbon and nitrogen content creates favorable conditions for microbial activity, leading to the synthesis of enzymes, biogas, methane, and other valuable fermented products. The diverse range of compounds found in papaya peel allows for multiple applications, such as adsorption, meat tenderization, animal feed and growth, corrosion inhibition, utilization as a capping agent, green synthesis of nanomaterial’s, and extraction of value-added chemicals.

2.3 Papain

The plant proteolytic enzyme, papain (EC 3.4.22.2) belongs to the cysteine proteinase family. Papain is extracted by cutting the skin of immature papaya, where fluid (latex) is released and then assembled before being dried. Unwanted compounds must be removed by purification, which includes solubilization and active papain extraction. Papain activity is influenced by the fruit's ripeness and level of greenness. Papain belongs to the superfamily and is crucial to several vital functions in all living organisms **[142]**. A wide range of peptides with short chains, proteins, esterified amino acids, and amide linkages may be broken down by papain, which has vast applications in both food and medical products **[91]**. Lysine, arginine, and phenylalanine residues are the primary positively charged amino acids peptide linkages that it selectively breaks **[98]**.

The globular protein papain comprises 212 protein building units with 4 disulfide bridges and a molecular weight of 23.4 KDa. In the 3D structure, there are two structural domains and a cleft that include the active site at various places of histidine, cysteine, and aspartate **[130,142]**. Papain, cysteine hydrolase is active under a various circumstances and remains stable at high temperatures **[35]**. The optimal pH range for papain activity is 3–9 **[46,60]**. The hydrophobicity of papain affects the protein folding and conformation, with the inner hydrophobic core maintaining the tertiary structure and the outside hydrophilic core interacting with water. The catalytic activity of papain promotes the breakdown of proteins **[87]**. Papain activates by attacking the carbonyl in peptide backbones with a free part of the amino-terminal cysteine_25 and His_159 in the triad active site **[119]**.

Papain is used to separate cells in preparations for cell culture. It helps in cleaning to eliminate contaminated cells or tissues in various enzymatic preparations. It is a whitening agent included in dental pastes, but because saliva easily dilutes it, prolonged usage is necessary for effective effects. Papain is a component of the gel papacaries, which is used to treat tooth decay. It acts as a detox and inhibits the results of urine diagnostic testing. Papain breaks down antibodies into three compounds that don't undergo precipitation, agglutination, etc **[27]**.

It may grow all year round in tropical climates since there are no seasonal restrictions on its production. Papain is composed of enzymes such as 1). Peptidase breaks down protein to create polypeptides and dipeptides. 2). Rennin acts on milk casein as a coagulating enzyme. 3). An enzyme called amylolytic converts carbs into monosaccharide’s. Papain is widely utilized for a variety of reasons across industries including food, pharmaceuticals, breweries, leather, detergent, fish, and meat. There is significant demand for its export **[16,70]**.

The enzyme has the ability to break down organic polypeptide molecules amino acids, and as a result, it is essential for many biological processes in both physiological and pathological conditions, drug development, and industrial applications like cheese production, meat tenderization s, and pharmaceutical preparations. Papain's distinctive structure is the reason for its functionality that proofs its importance in numerous applications **[72]**. Papain is a well-known traditional treatment for relieving pain, swelling, and inflammation. Moreover, it has been used to treat allergies, infections, diarrhea, and poor digestion **[91]**. According to Fernández-Lucas et al. **[54]**, papain has further uses in the pharmaceutical sector, the tanning procedure used in the cosmetic sector, the manufacturing of chewing gum, and the clarification of beer.

2.4 Extraction of bioactive compound

Extraction is a crucial step to be followed in the production of herbal products since it affects the active components in the samples qualitatively and quantitatively **[66]**. Currently, "modern" extraction techniques perform better than traditional ones since doing so is necessary to provide herbal products that are of outstanding quality for consumers. Because there are so many different plant species and chemical compounds that are physiologically active, screening methods must be both conventional and thorough **[51]**. Only with the appropriate extraction methods can physiologically active compounds be separated, identified, and characterized from other compounds. The extraction of physiologically active substances is affected by a number of variables, such as the extraction method, the solvent and the raw materials **[141]**. To understand the selectivity of extraction from various natural sources, different extraction methods must be applied in different contexts. The extraction of phenolic compounds from natural sources has recently attracted a lot of attention; consideration must be given to variables such as separation, identification, and uses **[115]**. A few innovative and creative techniques used in food processing include supercritical fluid extraction, ultrasound, high-pressure processing, pulsed electric field, cold plasma, and UV irradiation. Similarly, for routine analysis of antioxidant metabolites, alternative methods like solid-phase microextraction and matrix solid-phase dispersion can be utilized **[108]**.

2.5 Pre-treatment for extraction

For the extraction of bioactive chemicals, pre-treatment of plant material is essential. The type of the plant material and separated compounds should be considered while choosing an extraction procedure for preventing the compound degradation and the formation of undesirable bacteria. The composition of plant material particles will be impacted differently by each chosen process, which will then influence the amount of released phytochemical compounds. Pre-treatment is often carried out to improve cell wall disruption and bioactive compound extraction from plant sources. Pre-treatments including microwave, ultrasound, and enzymatic treatments have recently made it easier to extract the phytochemicals from plant tissues. Recently, several researchers have discussed how various treatments, either individually or in combination, significantly enhanced the amount of bioactive compounds that could be isolated **[92,99,108]**.

According to recent findings from multiple studies, the synthesis and activity of different bioactives have both increased **[92]**. As per pervious study, microwave pretreatment has simultaneously increased extraction yield and polyphenol richness. Microwaves' tendency to speed up the process of extraction kinetics for polyphenols was more significant than it was for other compounds like sugar and fibers. Microwave pretreatments were recommended as a means of extracting bio capability which also enhancing the quality and quantity of the final product in this case **[8]**. Shah **[128]** reported that extraction with enzyme pre-treatment and ultrasonication was a proficient method for oil extraction from Jatropha seed kernels. When ultrasonication is used before enzyme pre-treatment, the oil yield percentage rises to 97% as opposed to the 92% yield achieved with enzyme pre-treatment alone. Since enzymes treatment leads to weakening integrity of the cell walls, the recovery increases with their use, making the extraction process more effective **[84]**. Enzymatic maceration has been selected as one of the pretreatment methods for this investigation based on these factors.

2.6 Extraction of papain

Papain has an 80–90% activity level when extracted from unripe papaya. Papain is widely utilized in the food and biotechnology sectors, such as a meat tenderizer, to cure edoemas, defibrinate wounds, and shrink-proof wool, among many other things application **[106]**. However in order to produce enzymes with high purity and activity, which requires the downstream processing for extraction and purification of this enzyme. Methods like chromatography, precipitation, and electrophoresis are often utilized in the majority of organizations. These processes are batch-based, time-consuming, costly, and challenging to scale up **[75]**. Papain is extracted from papaya latex using conventional techniques including precipitation. Unfortunately, the purified protein is compromised by the presence of extra proteolytic enzymes. As a result, other purification methods are being explored that can continuously separate, concentrate, and purify the protein while also being conveniently scaled up **[106]**. Enormous efforts have been undertaken over the past few decades for scaling up u downstream approaches for the separation of required biomolecules in an inexpensive, continuous, and highly selective manner. To achieve separation of proteins and enzymes, liquid-liquid extraction-based techniques like aqueous two-phase extraction (ATPE) and reverse micellar extraction (RME) are among the several cost-effective downstream procedures that are being utilized **[42,69]**.

2.7 Extraction techniques

The efficacy and efficiency of the selected extraction techniques are crucial for the extraction of diverse phytochemical compounds derived from agri-food **[15,147]**. Bioactive compounds have been extracted from various natural sources using traditional or conventional extraction techniques **[129]**. The extraction parameter is chosen in a way that facilitates quick, accurate, and selective separation of the bioactive components. Traditional plant extraction techniques still play a significant role in providing consumers with high-quality plant products, but more advanced extraction techniques have been created that offer advantages over conventional ones. Another common method of extraction, maceration, required a significant period of time (3–15 days), and high-temperature decoction affected the active components of the plants. The infusion was regarded as a method for extraction from delicate plant components (restricted use); regular reflux requires high temperatures and takes time as well. Novel extraction techniques, such as ultrasonic-assisted extraction (UAE), microwave-assisted extraction

(MAE), and enzyme-assisted extraction (EAE), according to Handa et al. **[64]**, are among the most advanced and offer numerous benefits, including ease of extraction with high efficiency and limited destruction of the active constituents as it is not exposed to high temperatures. According to Vinatoru **[144]**, the conventional approach for extracting bioactive compounds efficiently needs greater quantities of solvents, significant energy consumption, and longer extraction times. For increasing efficiency of extraction while overcoming the drawbacks of conventional techniques, more advanced strategies for the extraction of bioactive compounds are being studied.

Nowadays, extracting biomolecules (such proteins and enzymes) from the crude mixture in any biotechnological process has grown difficult since the majority of the techniques used are either costly or risk affecting the characteristics of the biomolecules. It is also crucial to consider the final product's purity and yield. In order to recover biomolecules created by biotechnological methods, downstream processing operations must be effective, affordable, quick, easy, and environmentally acceptable. One such strategy for extraction is aqueous two-phase systems (ATPS) **[136]**. While ATPS separation potentially solve these issues, and its use as a high-value biomolecule separation approach is constrained by a lack of awareness and expertise about ATPS among researchers and in the industry.

2.7.1 Ultrasound-assisted extraction

The Ultrasound technique can be proved as a vital technique to help achieve the aim of sustainable “green” chemistry. Ultrasound system has an important role in extraction, which help in determining the key design parameters of the extraction process like acoustic energy density, ultrasonic intensity, ultrasonic power and the operation mode (e.g. pulsed or non-pulsed) **[141]**. The ultrasound waves can propagate through any medium as a mechanical wave within a frequency range of 20 kHz to 100 MHz, by generating phases of expansions and compressions. The ultrasonic bath and ultrasound probe-type method are the two different types of UAE instruments which are widely used for ultrasound applications. The system can ease down the process of extraction with high reproducibility, less solvent consumption, simpler manipulation and work up, highly pure final product, elimination of wastewater after treatment and less consumption of fossil energy than normally required for conventional extraction techniques like Soxhlet extraction and Clevenger distillation **[32]**. This technique in the field of chemical and food industry is well known for significantly influencing the processing rate of different processes used **[39,97]**. The system can effectively extract various compounds such as aromas, antioxidants, pigments and other organic compounds from food components both processed and formulated from a range of matrices (mostly animal tissue, yeasts, microalgae, food and plant materials). Ultrasound can be used in a wide range of application including processes like extraction, fermentation, emulsification, crystallization, reactions kinetics, and extraction technique **[134]**.

2.7.2 Microwave-assisted extraction

The method of microwave-assisted extraction (MAE) involves transferring solutes from a solid matrix into a solvent. The procedure is complicated by phenomena including electromagnetic transfer, mass transfer, heat transfer, and momentum transfer. The features of heat and mass transmission are crucial for the development of process engineering. Since microwave radiation may enter and integrate with a substrate, accurate and controlled heating is feasible **[82]**. The procedure of MAE involves the application of heat to solvents in direct contact with a sample, allowing the separation of analytes from the sample matrix and transferring them into the solvent. Hence, the microwave process may be designed to provide electromagnetic radiation with a precise power to the site of the relevant compounds in the material **[124]**. When compared to other extraction procedures, the energy-saving features and quick processing times result in lower manufacturing costs, improved product uniformity and yields, and high-quality results. The sample solvent mixture may be heated quickly due to MAE's intrinsic properties **[81]**.

2.7.3 Enzyme-assisted extraction

Enzymes are incredibly adaptable substances that can catalyse a variety of chemical reactions. Due to their properties, which function in aqueous solutions at room temperature and atmospheric pressure, they are the perfect catalyst for use in analytical applications. In addition, the usage of enzymes has been expanded to include the extraction of intracellular components from plant cells. The utilization of enzyme for extraction process is vast, and it goes beyond the mechanical action used to break down any plant cell, such as grinding, milling, and ultrasonication. This is because enzyme extraction is more cost-effective and environmentally friendly **[65]**. Enzyme based extraction has over-served application in commercial processes to extract oil from seeds or fruits. In comparison to the conventional approach, introducing enzyme treatment to the process has produced extraction oils of higher quality, contain more protein, and take less time to finish **[41]**. While the total phenol release was often larger when enzymatic treatment was used, Pinelo **[115]** suggested that the utilizing cell wall disintegrating enzymes can enhance the phenol extraction. The main benefit of enzyme-assisted extraction (EAE) is an increased yield of bioactive components recovered from diverse sources **[61]**.

2.7.4 Aqueous Two-Phase Extraction (ATPS)

Conventional purification techniques utilized for recovery and purification of enzymes consists of several steps. These procedures have been proven tedious, expensive, costly, time consuming with a lower recovery rate. Thus, alternative recovery techniques such as aqueous two-phase extraction (ATPE), thermo-separation (TMP) and three-phase partitioning (TPP) are developed to overcome the mentioned disadvantages **[45]**.

ATPS are well recognized for their application in the isolation and purification of biological materials, such as proteins, enzymes, nucleic acids, cell organelles, viruses, and others (see Figure 2.2). Several books and review papers have been published on these applications **[5,40,76,123]**. ATPE offers high capacity, low interfacial tension, good yield, high selectivity, shorter processing time and energy, and ease of scaling. It also allows for continuous processes, and most importantly, the equipment and approaches used in conventional organic-aqueous phase extraction might be easily adapted. ATPE, on the other hand, is not selective enough to achieve the high levels of purity that are normally needed. It is acknowledged the first purification stage in the overall purification process of protein **[1,131]**, with final purification performed with chromatography or crystallization. This method can further be used to remove contaminating elements like nucleic acids and polysaccharides **[77,136]**.

ATPS can be generating on adding two water-soluble polymers or by adding a polymer with salt to aqueous media above their critical concentrations results in the generation of an ATPS. Two aqueous phases are generated without using an organic solvent in this method. Polyethylene glycol (PEG)/potassium phosphate is the most widely used polymer/salt system, and polyethylene glycol/dextran (PEG/DX) is the most extensively investigated polymer/polymer system as they are easier to design and take less time to segregate, having greater protein extraction selectivity **[135]**. Furthermore, simple dialysis can be applied to easily recover the proteins from the salt phase.

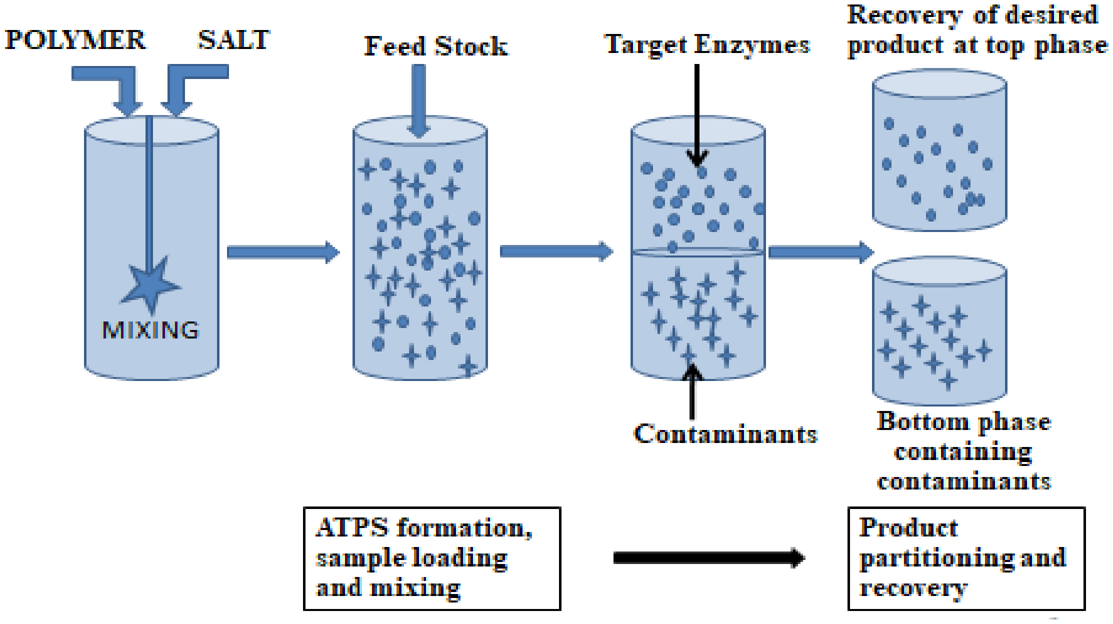

Figure 2.2: Schematic diagram of ATPS

2.8 Integration of different extraction techniques

According to the previous assertion, it is now of utmost significance to seek for high-quality products that have significant bioactivity and little negative influence on human health and the environment. Although while each of the extraction methods described in this section works well on its own, and combining several of them at once can produce even better results. Also, a thorough understanding of the process underlying the severe mixing effect caused by the ultrasound wave propagation inside liquid medium allows for the augmentation of mass transfer while also speeding up the migration of solute. Acoustic streaming on a macroscopic scales and acoustic micro-streaming on a local level are responsible for the mixing effect **[143]**. Ultrasonic extraction efficiency may be improved by combining the effects of mixing and physical ultrasound impact on raw material. Also, this method enables the purification of valuable components from a diversity of source materials and the gradual extraction of various compounds from a sample **[25]**. Because to recent quick advancements, extraction techniques have evolved significantly. This approach primarily promotes resource and energy conservation by combining sample preparation and analysis procedures **[103]**.

2.8.1 Ultrasound combined with microwave technology

Combining UAE with microwave assisted extraction (MAE) is one of the most effective hybridized methods (UMAE) which provide quick and efficient extraction **[132]**. Several researchers have studied combination UMAE irradiation in natural extraction Cravotto & Binello **[36]**, but the considerate capability of the hybrid procedure is still not been appropriately utilized. We assume UMAE possess immense potential in academic as well as industrial research activities due to its highly efficiency and significantly shorter extraction period. This is a cost-efficient method for rapid sample preparation with newer process intensification strategies. Also, double simultaneous irradiation triggers additional along with synergistic effects towards vegetal matrix extraction phenomenon while non-metallic horns are only employed at moderate power. As defined in Cravotto & Cintas **[37]**, horns made of Pyrex ®, quartz or Peek ® could be safely utilized till 90 W, beyond which material internal structure is destroyed irreversibly. But, it develops slight drawback as UMAE needs low marks of power by just two singly energy sources. Ultrasound significantly enhances the target extraction component via the cavitation phenomena. The effect of mechanical ultrasonication facilitates the withdrawal by plant body of soluble compounds by cell walls disruption promoting mass movement enabling access towards cell material by solvents. During the meantime, the entire sample is heated by microwave very fast triggering the movement of dissolved molecules. Simultaneously, irradiation improves the solvent penetration inside matrices promoting the solvation of analytes with typically increasing the solubility of target components. Garcia-Vaquero et al. **[59]** observed that UMAE extracted higher yield of phenolic compound and antioxidant from brown macroalgae as compared to UAE and MAE separately. Also, UMAE of flavonoid compounds extraction from Eucommia ulmoides leaves was studied by Wang et al. **[146]** and observed that UMAE extracted 2.454% high yield than ultrasound and microwave extraction. Cravotto et al., **[38]**, successfully implemented UMAE as a complementing method for extracting oils through vegetable source i.e., soybean germ and docosahexaenoic acid (DHA)-abundant cultivated seaweed.

2.8.2 Ultrasound combined with enzymatic extraction

Since current time, combined ultrasound with enzyme-assisted separation process in bioactive components, referred as Ultrasound assisted enzymatic extraction (UAEE) has been explored in certain researches **[43,86,104,137]**. The UAEE is known to be combined complementary strategies of extraction, providing a few extra points of interest. Enzymes encourage recuperation through the degradation and rupture of cell wall and films within enzyme-assisted extraction (EAE). Enzymes, in any case, such as cell walls, don't hydrolyze the matrix totally **[28,149]**. UAE enhances the process of enzymatic extraction, as the cavity process is stimulated by ultrasonic power which easily breaks down the matrices, to permit enzyme aided reactions along with consequent discharge of targeted components. Contrary to this, the mass migration of solvents, target particles, and enzymes itself inside or in the exterior of the media can't be improved by enzymes alone. Hence, in EAE, other physical iterations such as shaking can be utilized to make strides in mass exchange, among which UAE is a perfect choice for its mass transfer enhancement, as it were not for exterior but moreover interior of the matrices **[31,68]**. Apart from this, ultrasonic power within the UAE raised matrix surfaces and area of contact among stages, will uncover the enzymes to more substrates within the EAE treatment and contribute to the exposing of more target compounds. Several researches have emphasized about increase in enzymatic reaction rates in EAE due to ultrasonic treatment which can upgrade enzyme-substratum collisions and allow a better rate of discharge **[26,28]**. A few other types of research have shown that through certain conditions, low-frequency ultrasound was competent in progressing the operation of cellulose **[6,105]**. In a few cases, on the other side, the enzymatic reaction within the EAE might increase the effect of ultrasound within the UAEE in return. As said over, the impact of ultrasound would be significantly affected by the viscosity of the extrication system. While separating constituents, the consistency of the extraction process might be enhanced, especially when water is utilized as a dissolving agent due to the discharge of a few untargeted compounds, such as certain hydrocolloids. A few particular enzymes may corrupt numerous of these untargeted compounds, bringing down the viscosity and resulting in about the negative viscous impact upon improvement of ultrasonic treatment. UAEE is utilized for extracting polysaccharides from numerous plant resources, like epimedium leaves **[33]**, wheat brans **[145]**, pumpkin **[149]**, blackcurrant **[152]** and Ginkgo biloba clears out **[155]**, in which UAEE has illustrated its capacity to boost production. Wu et al. **[149]** found out that with EAE and UAEE on the recovery of pumpkin polysaccharides. The investigation demonstrated that when enzymes and ultrasound were utilized at the same time (ultrasonic illumination with dynamic proteins) or in the arrangement (ultrasonic light with inert enzymes) for sample, superior recovery of polysaccharides was accomplished in comparison to only EAE and traditional water extraction method or only using UAE. Collectively with the best polysaccharide production around 4.33 ± 0.15 percent were obtained through synchronous UAEE handle. Moreover, to remove arabinoxylan from wheat bran, Wang et al. **[145]**, utilized EAE with UAE in the sequential arrangement. Building on ultrasound enhanced viability in enzyme treated with UAEE delivered high extracted yields (14.26 ± 0.17%) of arabinoxylan by wheat bran correlating to either EAE or UAE only, they found out. Amiri-Rigi et al. **[10]** utilized UAEE to extract lycopene from residues of tomatoes within the tomato industry. Tchabo et al. **[140]**, inspected impact of UAE and EAE for extracting phytochemical component (flavonoid, phenolic, anthocyanin, etc.). In this study, extraction is performed using UAE in combination with EAE and after that application of micro-emulsification extraction in tomatoes residue brought about improved partition in lycopenes when UAEE method is employed. A better extraction and productivity was observed using final products of phyto-bioactive mulberry components in UAEE extracted samples in comparison for UAE, EAE or conventional extracted procedures. They observed an increment using final products of phyto-bioactived mulberry components with better productivity in UAEE extracted in comparison for UAE, EAE or conventional extracted procedures. Amigh & Dinani **[9]** performed an enzymatic treatment to recover oil from date seeds. To enhance the extraction performance of oil from date seeds, these same authors applied the simultaneous combination of ultrasound and enzymes. The results showed that strategy allows increasing the extraction yield of oil by using the sample to solvent ratio of 1:3 in comparison to the treatment using only enzymes. Bora et al., **[23]** combined enzymatic treatment with ultrasound treatment for extraction of juice from banana pulp and observed that juice yield increased while viscosity of the juice decreased. The total soluble solids and clarity of the juice obtained by the combined treatment was also higher.

2.9 Freeze drying of extracted bioactive compounds

Freeze drying is a dehydration technique that takes place at low pressures and temperatures for preserving the targeted biological components and heat-sensitive food items **[120]**. The method immediately sublimes water present in the substance directly from the solid to the gaseous phase **[22]**. The process of freeze-drying has recently become more significant in the bio product and pharmaceutical sectors. The bioactive compounds must be preserved in three processes using a freeze drying procedure. According to Tang et al. **[139]**, these processes include (i) freezing, (ii) sublimation (primary drying), and (iii) evaporation (secondary drying). At the first phase of the process, freezing, the liquid solution is cooled to a low temperature where it will solidify. For instance, water has to be below 0°C and under 0.006 atm of pressure in order to change to solid from a liquid form and later to the gaseous form **[90]**. At this stage, the water molecules transform into ice while the targeted bioactive substances stay in an amorphous or glass state. As the process requires low temperature and pressure, it has a significant operational cost **[121]**. As the pressure is decreased to levels below the vapor pressure of ice, sublimation begins in the second step, which removes the majority of the water present in the substance. This process continues until the temperature of the product has reached the shelf temperature. The third process, which is evaporation, continues to evaporate the remaining water after this. In comparison to the sublimation step, time taken for evaporation is less while using less energy **[48]**. The shelf temperature is increased throughout this procedure while the pressure is kept constant **[57]**.

2.10 Spray drying of fruit juices

Spray drying is a method that often used to make powdered juices from different fruits. In this process, the juice is dispersed into small drops, which are then quickly dried with hot air, making a dry powder. Spray drying has benefits like a longer shelf life, being easy to handle, as well as easy to store. It keeps the healthy and tasty parts of the fruit juices while making them easier to use in the food and drink business. For example, spray-dried blackberry juice keeps its original colour, taste, and bioactive substances **[55]**. This means that it can be used to make quick beverage mixes. Spray drying has also been used to turn juices from other fruits, like pineapple, strawberry, and mango, into solid powders that are easy to rehydrate and have a longer shelf life **[30,138]**.

While spray drying is a commonly used method for drying fruit juices and preserving their bioactive compounds, it does have some disadvantages that can affect the retention and functionality of these compounds. One big problem is that heat-sensitive bioactive chemicals may be lost or broken down during the drying process. Spray drying is done at high temperatures, which can break down enzymes, vitamins, antioxidants, and other beneficial substances in plant juices that are sensitive to heat. Also, when the juice is atomized and dried, it is exposed to air, which can cause sensitive chemicals to oxidise and lose their bioactivity. Also, the choice of drying factors, such as air flow rate and inlet temperature, can affect how well bioactive chemicals are kept. If these factors aren't controlled well enough, the biological components may be exposed to too much heat or dry for too long, which can damage them even more. Quek **[118]** found that beneficial chemical substances can be destroyed or broken down when watermelon juices are sprayed dried for a long time at high temperatures. Fang & Bhandari **[48]** say that heat-sensitive compounds and volatile components, like phenolic compounds and antioxidants, can be degraded or oxidised by heat, which makes them less concentrated in the dry product. It is important to carefully optimize the spray-drying process to reduce these problems and keep as much of the fruit juice's bioactivity as possible.

2.11 Application of bioactive compounds in development of food product

Food bioactive substances are unprocessed, naturally occurring elements with biological activity and, in certain cases, nutritional value. Because they have been found to have beneficial functions in human development and growth, in addition to lowering illness risks, they offers o key role in the health and safety of the community. The utilization of bioactive components necessitates the use of acceptable and approved extraction techniques. Consumer attraction towards product with energy-boosting, anti-aging, general well-being, and calming properties is high in the food application industry, which includes food and drinks, nutraceuticals, and animal feed category. To produce an item, the extracted components are simply mixed with the food. Oftenly the correct delivery strategy is required to be adopted to ensure compound’s efficiency, prevent oxidation and destruction caused by certain environmental variables (light, for example), and improve the overall sensory characteristics of the food item **[126]**. In this regard, the food sector may employ microencapsulation as a promising technology for the manufacture of functional bioactive compounds through the use of spray drying, freeze drying, emulsion, extrusion, coacervation, liposome entrapment, co-crystallization, etc. **[111]**. With its potential to enhance consumer quality of life, provide value to industrial waste, and promote additional patent applications in these fields, the rising interest in these items might be advantageous for both consumers and the industry **[79]**.

2.11.1 Functional Foods

Functional foods are food products that offer additional health benefits beyond basic nutrition, as they contain bioactive compounds that can positively impact physiological functions in the body. These compounds can include antioxidants, probiotics, prebiotics, dietary fibers, and plant-based phytochemicals **[18]**. The consumption of functional foods has been allied with a reduced risk of chronic diseases such as cardiac disease, cancer, and diabetes, as well as improved overall health and well-being.

For instance, antioxidants like polyphenols, carotenoids, and flavonoids can protect against oxidative stress and chronic diseases, leading to the incorporation of berries, green tea, and dark chocolate into functional food products. Omega-3 fatty acids derived from fatty fish and nuts have been linked to improved cardiovascular health and brain function, leading to the development of fortified foods like fish oil supplements and omega-3 enriched eggs **[117]**. Probiotics and prebiotics, such as those found in yogurt and certain cereals, support gut health and boost the immune system. Fiber-rich foods, including whole grains and legumes, aid in digestion and weight management. Additionally, functional foods enriched with plant sterols and stanols effectively reduce cholesterol levels and the risk of cardiovascular diseases. By harnessing the power of these bioactive compounds, functional foods are designed to provide targeted health benefits and contribute to overall well-being. However, it is important to remember that functional foods should complement a balanced diet and individual nutritional needs **[112]**.

Functional foods play a significant role in preventive medicine and public health, as they offer a proactive approach to improving health and decreasing the risk of diseases. Incorporating functional foods into the daily diet can be an effective strategy to optimize nutrition and support overall well-being. However, it is important to note that functional foods are not intended to replace a balanced diet or medical treatments but rather complement them in promoting health and disease prevention.

2.12 Application of papain

Papain, finds diverse applications in various industries. It is used as a meat tenderizer, in the production of digestive aids, as a clarifying agent in beer brewing, and in the textile and pharmaceutical industries for its proteolytic properties. Additionally, it finds use in cosmetics for exfoliation and skin brightening.

2.12.1 Food processing

Papain's functional qualities are becoming more and more desired for a various industrial applications, namely in the food, brewing, meat tenderization, as well as in textile industries **[2]**. Also, papain is an excellent illustration of an industrial enzyme that is effective and covers every phase of the bio-catalytic cycle required for the industrial application of any bioprocess **[3]**. The production and extraction of the relevant enzyme (from natural or recombinant sources), structural and functional characterization, genetic improvement, immobilization, and, eventually, industrial use are all included in this cycle. As a result, papain has a variety of applications and is marketable **[53]**.

2.12.1.1 Meat tenderization

Recently, there is a surge in the usage of exogenous proteases to enhance meat softness. The ability of the meat company to fulfill the rising demand for extremely tender meat and do value addition to lower-grade meat cuts is a primary concern. Increasing post-mortem softness has been the focus of several approaches, including mechanical tenderization, water content improvement, and other enzymatic treatments **[114]**. Meat is traditionally kept at 4°C for 7–10 days while allowing autoproteolysis (mostly mediated by cathepsins and capains) to set in for inducing softness. Papain is an effective meat tenderizer because it can hydrolyze any protein type found in muscle tissue, tendons, and ligaments when acting in the ideal circumstances (pH range between 7-8 and temperature around 60 to 65°C) **[21]**. When animal gets infused with the papain before slaughtering, it allows a homogeneous distribution of the enzyme in the animal's meat, and the meat-softening impact of papain has been witnessed throughout a number of experiments. Due to the fact that feeding active papain to animals might result in their suffering and discomfort, this method is no longer employed nowadays due to ethical considerations. A possibility is injection of inactive papain. Papain is often given to the animal as an injection after being treatment with hydrogen peroxide that oxidizes the catalytic cysteine. Thus, when animal is killed, the catalytic cysteine is reduced under anoxic circumstances cause, which reactivates papain **[21]**. Even so, this ante-mortem approach has certain limitations, primarily because it is challenging to estimate the degree of tenderization because it differs with various physiological aspects of the animal. Other issues that might arise include textural discrepancies when compared to slices of high-quality meat, over-tenderization, undesirable tastes or odors, or deterioration of organs that could be of economic importance. For inferior meat slices, post-mortem application is typically acceptable. Commercially available forms of papain include powder, liquid, and combining forms with other proteases like bromelain. Several commercial solutions are readily accessible for use by processors and individuals and may contain additional components (salt, phosphates, or flavor enhancers like sodium glutamate).

Kang & Rice **[71]** demonstrated increased papain activity for the myofibrillar fraction and better connective tissue solubilizing activity. According to Maiti **[88]**, the infusion of papain combined with forking technique demonstrated superior effectiveness in tenderizing cuts of spent hen meat compared to the injection method. A synergistic effect of papain and sodium tripolyphosphate was shown by Grover et al. **[63]** to increase the tenderness of chicken gizzard

2.12.1.2 Dairy processing

Papain enzyme is also used in the cheese processing business in a number of ways. One of the main ways that papain is used is to make cheese. It is used as a coagulant and a digestive enzyme to help cheese get better flavours and soften faster **[130]**. Papain breaks down certain milk proteins, like alpha-s1 casein, which makes peptides and amino acids that give cheeses their desirable structure, smell, and taste. It is often used with traditional cheese cultures to speed up the proteolysis process and give the cheese certain qualities.

Papain is also a key part of making milk protein hydrolysates, which are used in a lot of baby feeds and other special nutrition goods **[74]**. Papain breaks down the milk proteins into smaller peptides and amino acids by using enzymes to break them down. This makes them easier to digest and more bioavailable. People who can't handle lactose or have trouble eating whole proteins can use milk protein hydrolysates made with papain because they are easier for the body to absorb **[80]**.

Papain is not only used to make cheese and milk protein hydrolysate, but it is also used to make dairy-based desserts and drinks. It helps improve smoothness by breaking down proteins and making dairy products less thick. This action of enzymes makes things like custards, puddings, ice creams, and creamy drinks creamier, smoother, and more pleasant to eat or drink **[50]**.

2.12.2 Pharmaceutical industry

Due to its adaptable properties, papain is utilized extensively in the pharmaceutical industry. It has been widely studied and utilized for its enzymatic activity in various pharmaceutical formulations **[20]**. Papain is known for its ability to break down proteins, making it valuable in the production of protein-based drugs, such as antibodies and enzymes, by cleaving them into smaller, more manageable fragments. This enzymatic activity also enables papain to assist in the formulation of pharmaceutical tablets by improving their disintegration and dissolution properties **[94]**. Moreover, papain's anti-inflammatory and wound-healing properties make it a potential ingredient in topical formulations for treating skin conditions and promoting tissue repair. Research is ongoing to explore its potential applications in drug delivery systems and as a tool for targeted drug release. The use of papain in the pharmaceutical industry offers promising opportunities for developing novel formulations and improving drug efficacy **[85]**.

2.12.3 Textile industry

In the textile industry, papain has been discovered to have certain benefits, particularly in the process of fabric preparation and finishing. Its proteolytic properties make it effective in removing protein-based stains and improving fabric softness **[34]**. Papain acts as a natural fabric softener and stain remover, breaking down protein molecules that cause stains and odors on fabrics. It is particularly useful in treating stains such as blood, grass, or food stains, which are often protein-based. By breaking down the proteins responsible for the stains, papain helps to lift and remove them from the fabric fibers, restoring the fabric's appearance and cleanliness **[52]**. Moreover, papain is gentle on fabrics and does not cause damage or discoloration, making it suitable for use on various types of textiles. Its eco-friendly nature as a natural enzyme-based solution also aligns with the growing demand for sustainable and environmentally friendly textile treatments **[150]**. Overall, papain offers a natural and effective solution for stain removal and fabric softening in the textile industry.

2.12.4 Cosmetic industry

Papain, has gained significant attention and application in the cosmetic industry. Its ability to break down proteins makes it a valuable ingredient in skincare and hair care products. In skincare, papain is utilized as an exfoliating agent due to its gentle yet effective enzymatic action on the skin's surface. It helps remove dead skin cells, unclog pores, and promote a smoother and brighter complexion **[133]**. Papain is also known for its skin lightening properties, as it can assist in reducing the appearance of hyperpigmentation and uneven skin tone. Additionally, papain's anti-inflammatory properties contribute to its soothing and calming effects on the skin, making it beneficial for individuals with sensitive or acne-prone skin. In haircare, papain is used in shampoos and conditioners to help remove build-up and clarify the scalp, resulting in healthier hair growth. The inclusion of papain in cosmetic formulations offers natural and effective solutions for exfoliation, brightening, and scalp care **[110]**.

2.13 References

1. Abbot, N. L., Blankschtein, D., & Hatton, T. A. On protein partitioning in two-phase systems. *Bioseparation*, 1, 195, 1990.
2. Abe, N., Wu, C. Y., Kim, Y. K., Fujii, T., and Abe, K. Development of an efficient soymilk cream production method by papain digestion, heat treatment, and low-speed centrifugation. *Bioscience, Biotechnology, and Biochemistry*, 79:1890–1892, 2015.
3. Agyei, D., and Danquah, M. K. Industrial-scale manufacturing of pharmaceutical grade bioactive peptides. *Biotechnology Advances*, 29:272–277, 2011.
4. Alara, O. R., Abdurahman, N. H., & Alara, J. A. Carica papaya: comprehensive overview of the nutritional values, phytochemicals and pharmacological activities. *Advances in Traditional Medicine*, 1–31, 2020.
5. Albertsson, P. Å. Partition of cell particles and macromolecules in polymer two-phase systems. *Advances in protein chemistry*, 24, 309–341, 1970.
6. Aliyu, M., & Hepher, M. J. Effects of ultrasound energy on degradation of cellulose material. *Ultrasonics Sonochemistry*, 7(4), 265–268, 2000.
7. Almora, K., Pino, J. A., Hernández, M., Duarte, C., González, J., & Roncal, E. Evaluation of volatiles from ripening papaya (Carica papaya L., var. Maradol roja). *Food Chemistry*, *86*(1), 127–130, 2004.
8. Alvarez, A., Poejo, J., Matias, A. A., Duarte, C. M., Cocero, M. J., and Mato, R. B. Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity. *Food and Bioproducts Processing*, 106, 162–170, 2017.
9. Amigh, S., & Taghian Dinani, S. Combination of ultrasound-assisted aqueous enzymatic extraction and cooking pretreatment for date seed oil recovery. *Heat and Mass Transfer*, 56(8), 2345–2354, 2020.
10. Amiri-Rigi, A., Abbasi, S., & Scanlon, M. G. Enhanced lycopene extraction from tomato industrial waste using microemulsion technique: Optimization of enzymatic and ultrasound pre-treatments. *Innovative Food Science & Emerging Technologies*, 35, 160–167, 2016.
11. Ayala-Zavala, J. F., Rosas-Domínguez, C., Vega-Vega, V., and González-Aguilar, G. A. Antioxidant enrichment and antimicrobial protection of fresh-cut fruits using their

own byproducts: Looking for integral exploitation. *Journal of food science*, 75(8):175–181, 2010.

12. Ayala-Zavala, J., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J. A., Siddiqui, M. W., and González-Aguilar, G. A. Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, 44(7):1866–1874, 2011.
13. Azarkan, M., Clantin, B., Bompard, C., Belrhali, H., Baeyens-Volant, D., Looze, Y., Villeret, V. & Wintjens, R. Crystallization and preliminary X-ray diffraction studies of the glutaminyl cyclase from Carica papaya latex. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications*, 61(1), 59–61, 2005.
14. Azevedo, L. A., & Campagnol, P. C. B. Papaya seed flour (Carica papaya) affects the technological and sensory quality of hamburgers. *International Food Research Journal*, 21(6), 2141, 2014.
15. Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., and Omar, A. K. M. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4):426–436, 2013
16. Babalola, B. A. Extraction, Purification and Characterization of Papain Enzyme (EC 3.4. 22.2) From the Leaves of Carica Papaya, 2019.
17. Balbontín, C., Gaete-Eastman, C., Vergara, M., Herrera, R., & Moya-León, M. A. Treatment with 1-MCP and the role of ethylene in aroma development of mountain papaya fruit. *Postharvest Biology and Technology*, 43(1), 67–77, 2007.
18. Banwo, K., Olojede, A. O., Adesulu-Dahunsi, A. T., Verma, D. K., Thakur, M., Tripathy, S., ... & Utama, G. L. Functional importance of bioactive compounds of foods with Potential Health Benefits: A review on recent trends. *Food Bioscience*, 43, 101320, 2021.
19. Basu, A., Devaraj, S., & Jialal, I. Dietary factors that promote or retard inflammation. *Arteriosclerosis, thrombosis, and vascular biology*, 26(5), 995–1001, 2006.
20. Beeley, J. A., Yip, H. K., & Stevenson, A. G. Chemochemical caries removal: a review of the techniques and latest developments. *British dental journal*, 188(8), 427–430, 2000.
21. Bekhit, A. A., Hopkins, D. L., Geesink, G., Bekhit, A. A., & Franks, P. Exogenous proteases for meat tenderization. *Critical reviews in food science and nutrition*, 54, 1012–1031, 2014.

22. Bhatta, S., Stevanovic Janezic, T., & Ratti, C. Freeze-drying of plant-based foods. *Foods*, 9(1), 87, 2020.
23. Bora, S. J., Handique, J., & Sit, N. Effect of ultrasound and enzymatic pre-treatment on yield and properties of banana juice. *Ultrasonics Sonochemistry*, 37, 445–451, 2017.
24. Boshra, V., & Tajul, A. Y. Papaya-an innovative raw material for food and pharmaceutical processing industry. *Health and the Environment Journal*, 4(1), 68–75, 2013.
25. Boyadzhiev, L., Dimitrov, K., and Metcheva, D. Integration of solvent extraction and liquid membrane separation: an efficient tool for recovery of bio-active substances from botanicals. *Chemical Engineering Science*, 61(12), 4126–4128, 2006.
26. Bracey, E., Stenning, R. A., & Brooker, B. E. Relating the microstructure of enzyme dispersions in organic solvents to their kinetic behavior. *Enzyme and microbial technology*, 22(3), 147–151, 1998.
27. Burrows, D. L., Nicolaides, A., Rice, P. J., Dufforc, M., Johnson, D. A., & Ferslew, K. E. Papain: a novel urine adulterant. *Journal of analytical toxicology*, 29(5), 275–295, 2005.
28. Capelo, J. L., & Mota, A. M. Ultrasonication for analytical chemistry. *Current Analytical Chemistry*, 1(2), 193–201, 2005.
29. Chattopadhyay, P., Mandal, G., Sar, K., & Das, S. S. Effect of integrated nutrient management practices on productivity of papaya (Carica papaya L.) in Terai Dooars region, 2022.
30. Chegini, G. R., & Ghobadian, B. Spray dryer parameters for fruit juice drying. *World Journal of Agricultural Sciences*, 3(2), 230–236, 2007.
31. Chemat, F., & Khan, M. K. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics sonochemistry*, 18(4), 813–835, 2011.
32. Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, 34, 540–560, 2017.
33. Chen, R., Li, S., Liu, C., Yang, S., & Li, X. Ultrasound complex enzymes assisted extraction and biochemical activities of polysaccharides from Epimedium leaves. *Process Biochemistry*, 47(12), 2040–2050, 2012.

34. Cheng, Y., Zhu, T., Li, S., Huang, J., Mao, J., Yang, H., ... & Lai, Y. A novel strategy for fabricating robust superhydrophobic fabrics by environmentally-friendly enzyme etching. *Chemical Engineering Journal*, 355, 290–298, 2019.
35. Cohen, L. W., Coghlan, V. M., & Dihel, L. C. Cloning and sequencing of papain-encoding cDNA. *Gene*, 48(2-3), 219–227, 1986.
36. CRAVOTTO, G., & Binello, A. R. I. A. N. N. A. Innovative techniques and equipments for flavours extraction-Application and effectiveness of ultrasound and microwaves. *HOUSEHOLD AND PERSONAL CARE TODAY*, 30–32, 2010.
37. Cravotto, G., & Cintas, P. The combined use of microwaves and ultrasound: improved tools in process chemistry and organic synthesis. *Chemistry–A European Journal*, 13(7), 1902–1909, 2007.
38. Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M., & Cintas, P. Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. *Ultrasonics sonochemistry*, 15(5), 898–902, 2008.
39. Dastkhoon, M., Ghaedi, M., Asfaram, A., Jannesar, R., & Sadeghfar, F. Magnetic based nanocomposite sorbent combination with ultrasound assisted for solid-phase microextraction of Azure II in water samples prior to its determination spectrophotometric. *Journal of colloid and interface science*, 513, 240–250, 2018.
40. Diamond, A. D., & Hsu, J. T. Aqueous two-phase systems for biomolecule separation. *Bioseparation*, 89–135, 2006.
41. Dominguez, H., Nunez, M. J., and Lema, J. M. Enzyme-assisted hexane extraction of soya bean oil. *Food Chemistry*, 54(2):223–231, 1995.
42. Dong, J., Cai, J., Guo, X., and Xiao, J. Effect of the spacer of gemini surfactants on reverse micellar extraction of bovine serum albumin. *Soft Matter*, 9(47):11383–11391, 2013.
43. Dong, X., Wang, J., & Raghavan, V. Critical reviews and recent advances of novel non-thermal processing techniques on the modification of food allergens. *Critical reviews in food science and nutrition*, 61(2), 196–210, 2021.
44. Dotto, J. M., & Abihudi, S. A. Nutraceutical value of Carica papaya: A review. *Scientific African*, 13, e00933, 2021.
45. Dreyer, S. E. *Aqueous two-phase extraction of proteins and enzymes using tetraalkylammonium based ionic liquids*, 2008.

46. Edwin, F., & Jagannadham, M. V. Single disulfide bond reduced papain exists in a compact intermediate state. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, 1479(1-2), 69–82, 2000.
47. Faller, A. L. K., and Fialho, E. F. N. U. Polyphenol content and antioxidant capacity in organic and conventional plant foods. *Journal of Food Composition and Analysis*, 23(6):561–568, 2010.
48. Fang, Z., & Bhandari, B. Effect of spray drying and storage on the stability of bayberry polyphenols. *Food chemistry*, 129(3), 1139–1147, 2011.
49. Food and Agriculture Organization (FAO) of the United Nations. *FAOSTAT Database*. Retrieved from http://www.fao.org/faostat/en/#home, 2021.
50. Farahat, A. M., & El-Batawy, O. I. Proteolytic activity and some properties of stirred fruit yoghurt made using some fruits containing proteolytic enzymes. *World Journal of Dairy & Food Sciences*, 8(1), 38–44, 2013.
51. Farnsworth, N. R., Akerele, O., Bingel, A. S., Soejarto, D. D., and Guo, Z. Medicinal plants in therapy. *Bulletin of the World Health Organization*, 63(6), 965, 1985.
52. Feng, Y., Lin, J., Niu, L., Wang, Y., Cheng, Z., Sun, X., & Li, M. High molecular weight silk fibroin prepared by papain degumming. *Polymers*, 12(9), 2105, 2020.
53. Fernández-Lucas, J., Castañeda, D., and Hormigo, D. New trends for a classical enzyme: Papain, a biotechnological success story in the food industry. *Trends in Food Science & Technology*, 68:91–101, 2017.
54. Flath, R. A., & Forrey, R. R. Volatile components of papaya (Carica papaya L., Solo variety). *Journal of Agricultural and Food Chemistry*, 25(1), 103–109, 1977.
55. Franceschinis, L., Salvatori, D. M., Sosa, N., & Schebor, C. Physical and functional properties of blackberry freeze-and spray-dried powders. *Drying Technology*, 32(2), 197–207, 2014.
56. Fuentes, G., & Santamaría, J. M. Papaya (Carica papaya L.): origin, domestication, and production. *Genetics and genomics of papaya*, 3–15, 2014.
57. Fuggate, P., Wongs-Aree, C., Noichinda, S., & Kanlayanarat, S. Quality and volatile attributes of attached and detached 'Pluk Mai Lie'papaya during fruit ripening. *Scientia Horticulturae*, 126(2), 120–129, 2010.
58. Gaidhani, K. A., Harwalkar, M., Bhambere, D., & Nirgude, P. S. Lyophilization/freeze drying–a review. *World journal of pharmaceutical research*, 4(8), 516–543, 2015.

59. Garcia-Vaquero, M., Ummat, V., Tiwari, B., & Rajauria, G. Exploring ultrasound, microwave and ultrasound–microwave assisted extraction technologies to increase the extraction of bioactive compounds and antioxidants from brown macroalgae. *Marine drugs,* 18(3), 172, 2020.
60. Ghosh, S. Physicochemical and conformational studies of papain/sodium dodecyl sulfate system in aqueous medium. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 264(1-3), 6–16, 2005.
61. Gligor, O., Mocan, A., Moldovan, C., Locatelli, M., Crișan, G., and Ferreira, I. C. Enzyme-assisted extractions of polyphenols–A comprehensive review. *Trends in Food Science & Technology,* 88:302–315, 2019.
62. Gonsalves, C., Lee, D. R., & Gonsalves, D. The adoption of genetically modified papaya in Hawaii and its implications for developing countries. *The Journal of Development Studies*, 43(1), 177–191, 2007.
63. Grover, R. K., Sharma, D. P., & Ahlawat, S. S. Standardization of chicken gizzard pickle using sodium tripolyphosphate and papain as tenderizer. *Indian Journal of Poultry Science*, 40(2), 202–205, 2005.
64. Handa, S. S. An overview of extraction techniques for medicinal and aromatic plants. *Extraction technologies for medicinal and aromatic plants*, 1(1), 21–40, 2008.
65. Hanmoungjai, P., Pyle, D. L., & Niranjan, K. Enzyme-assisted water-extraction of oil and protein from rice bran. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 77(7), 771–776, 2002.
66. Huie, C. W. A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants. *Analytical and bioanalytical chemistry*, 373, 23–30, 2002.
67. Ikram, E. H. K., Stanley, R., Netzel, M., and Fanning, K. Phytochemicals of papaya and its traditional health and culinary uses–A review. *Journal of Food Composition and Analysis*, 41:201–211, 2015.
68. J Mason, T., Chemat, F., & Vinatoru, M. The extraction of natural products using ultrasound or microwaves. *Current Organic Chemistry*, 15(2), 237–247, 2011.
69. Kadam, K. L. Reverse micelles as a bioseparation tool. *Enzyme and microbial technology*, 8(5), 266–273, 1986.
70. Kamalkumar, R., Amutha, R., Muthulaksmi, S., Mareeswari, P., & Rani, W. B. Screening of dioecious papaya hybrids for papain yield and enzyme

activity. *Research Journal of Agriculture and Biological Sciences*, 3(5), 447–449, 2007.

71. Kang, C. K., & RICE, E. E. Degradation of various meat fractions by tenderizing enzymes. *Journal of Food Science*, 35(5), 563–565, 1970.
72. Khanna, N., & Panda, P. C. Effect of papain on tenderization and functional properties of spent hen meat cuts. *Indian Journal of Animal Research*, 41(1), 55–58, 2007.
73. Khuzhaev, V. U., & Aripova, S. F. Pseudocarpaine from Carica papaya. *Chemistry of Natural Compounds*, 36(4), 2000.
74. Kocabaş, D. S., Lyne, J., & Ustunol, Z. Hydrolytic enzymes in the dairy industry: Applications, market and future perspectives. *Trends in Food Science & Technology*, 119, 467–475, 2022.
75. Krishna, S. H., Srinivas, N. D., Raghavarao, K. S. M. S., & Karanth, N. G. Reverse micellar extraction for downstream processing of proteins/enzymes. *History and trends in bioprocessing and biotransformation*, 119–183, 2002.
76. Kula, M. R., Kroner, K. H., & Hustedt, H. Purification of enzymes by liquid-liquid extraction. In *reaction engineering*, pages 73-118. Berlin, Heidelberg: Springer Berlin Heidelberg, 2005.
77. Kula, M. R., Kroner, K. H., Hustedt, H., & Schutte, H. Scale-up of protein purification by liquid-liquid extraction. In *Enzyme Engineering:* volume 6, pages 69-74. Springer US, 1982.
78. Kumar, L. S. S., & Srinivasan, V. K. Chromosome number of carica dodecaphylla vell fl. Flum. *Current Science*, 13(1), 15–15, 1994.
79. Lagos, J. B., Vargas, F. C., de Oliveira, T. G., da Aparecida Makishi, G. L., & do Amaral Sobral, P. J. Recent patents on the application of bioactive compounds in food: a short review. *Current Opinion in Food Science*, 5, 1–7, 2015.
80. Law, B. A. Enzymes in dairy product manufacture. *Enzymes in food technology*, 92–93, 2010.
81. Letellier, M., & Budzinski, H. Microwave assisted extraction of organic compounds. *Analusis*, 27(3), 259–270, 1999.
82. Liazid, A., Palma, M., Brigui, J., & Barroso, C. G. Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A*, 1140(1-2), 29–34, 2007.

83. Lim, Y. Y., Lim, T. T., & Tee, J. J. Antioxidant properties of several tropical fruits: A comparative study. *Food chemistry*, 103(3), 1003–1008, 2007.
84. Liu, J. J., Gasmalla, M. A. A., Li, P., & Yang, R. Enzyme-assisted extraction processing from oilseeds: Principle, processing and application. *Innovative Food Science & Emerging Technologies*, 35, 184–193, 2016.
85. Lopes, M. C., Mascarini, R. C., da Silva, B. M. C. G., Flório, F. M., & Basting, R. T. Effect of a papain-based gel for chemomechanical caries removal on dentin shear bond strength. *Journal of Dentistry for Children*, 74(2), 93–97, 2007.
86. Luo, X., Bai, R., Zhen, D., Yang, Z., Huang, D., Mao, H., ... & Fu, C. Response surface optimization of the enzyme-based ultrasound-assisted extraction of acorn tannins and their corrosion inhibition properties. *Industrial Crops and Products*, *129,* 405–413, 2019.
87. Madej, T., Addess, K. J., Fong, J. H., Geer, L. Y., Geer, R. C., Lanczycki, C. J., ... & Bryant, S. H. MMDB: 3D structures and macromolecular interactions. *Nucleic acids research*, 40(D1), D461–D464, 2012.
88. Maiti, A. K., Ahlawat, S. S., Sharma, D. P., & Khanna, N. Application of natural tenderizers in meat–A review. *Agricultural Reviews*, 29(3), 226–230, 2008.
89. Malacrida, C. R., Kimura, M., & Jorge, N. Characterization of a high oleic oil extracted from papaya (Carica papaya L.) seeds. *Food Science and Technology*, 31, 929–934, 2011.
90. Malik, N., Muttakin, S., Lopez-Quiroga, E., Watson, N. J., Fryer, P. J., Bakalis, S., & Gouseti, O. Microstructure and reconstitution of freeze-dried gum Arabic at a range of concentrations and primary drying temperatures. *Food Hydrocolloids*, 104, 105712, 2020.
91. Mamboya, E. A. F., & Amri, E. Papain, a plant enzyme of biological importance: A review. *Am. J. Biochem. Biotechnol*, 8(2), 99–104, 2012.
92. Marathe, S. J., Jadhav, S. B., Bankar, S. B., Dubey, K. K., & Singhal, R. S. Improvements in the extraction of bioactive compounds by enzymes. *Current Opinion in Food Science*, 25, 62–72, 2019.
93. Marina, Z., & Noriham, A. Quantification of total phenolic compound and in vitro antioxidant potential of fruit peel extracts. *International Food Research Journal*, 21(5), 2014.
94. Marques, M. R. Enzymes in the dissolution testing of gelatin capsules. *AAPS PharmSciTech*, 15(6), 1410–1416, 2014.

95. Martial-Didier, A. K., Hubert, K. K., Parfait, K. E. J., & Kablan, T. Phytochemical properties and proximate composition of papaya (Carica papaya L. var solo 8) peels. *Turkish Journal of Agriculture-Food Science and Technology*, 5(6), 676–680, 2017.
96. Matsusaka, Y., & Kawabata, J. Evaluation of antioxidant capacity of non-edible parts of some selected tropical fruits. *Food Science and Technology Research*, 16(5), 467–472, 2010.
97. McClements, D. J. Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science & Technology*, 6(9), 293–299, 1995.
98. Menard, R., Khouri, H. E., Plouffe, C., Dupras, R., Ripoll, D., Vernet, T., ... & Storer, A. C. A protein engineering study of the role of aspartate 158 in the catalytic mechanism of papain. *Biochemistry*, 29(28), 6706–6713, 1990.
99. Micard, V., Renard, C. M., & Thibault, J. F. Studies on enzymic release of ferulic acid from sugar-beet pulp. *LWT-Food Science and Technology*, 27(1), 59–66, 1994.
100. Miean, K. H., & Mohamed, S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. *Journal of agricultural and food chemistry*, 49(6), 3106–3112, 2001.
101. Morais, D. R., Rotta, E. M., Sargi, S. C., Bonafe, E. G., Suzuki, R. M., Souza, N. E., ... & Visentainer, J. V. Proximate composition, mineral contents and fatty acid composition of the different parts and dried peels of tropical fruits cultivated in Brazil. *Journal of the Brazilian Chemical Society*, 28, 308–318, 2017.
102. Morton, J. Papaya. In: *Fruits of Warm Climates*, 1st Ed.; Morton, J.F.; Ed.; Florida Flair Books: Miami, FL, 336–346, 1987.
103. Mustafa, A., & Turner, C. Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. *Analytica chimica acta*, 703(1), 8–18, 2011.
104. Nag, S., & Sit, N. Optimization of ultrasound assisted enzymatic extraction of polyphenols from pomegranate peels based on phytochemical content and antioxidant property. *Journal of Food Measurement and Characterization*, 12, 1734–1743, 2018.
105. Nguyen, T. T. T., & Le, V. V. M. Effects of ultrasound on cellulolytic activity of cellulase complex. *International Food Research Journal*, 20(2), 557, 2013.
106. Nitsawang, S., Hatti-Kaul, R., & Kanasawud, P. Purification of papain from Carica papaya latex: Aqueous two-phase extraction versus two-step salt precipitation. *Enzyme and Microbial technology*, 39(5), 1103–1107, 2006.

107. Nunes, M. C. N., Emond, J. P., and Brecht, J. K. Brief deviations from set point temperatures during normal airport handling operations negatively affect the quality of papaya (Carica papaya) fruit. *Postharvest Biology and Technology,* 41(3):328–340, 2006.
108. Oancea, S., Daniela, G., and Otto, K. The effect of ultrasonic pretreatment and sample preparation on the extraction yield of antioxidant compounds and activity of black currant fruits. *Acta Chimica Slovenica*, 62(1), 242–248, 2014.
109. Olafsdottir, E. S., Jørgensen, L. B., & Jaroszewski, J. W. Cyanogenesis in glucosinolate-producing plants: Carica papaya and Carica quercifolia. *Phytochemistry*, 60(3), 269–273, 2002.
110. Packianathan, N., & Kandasamy, R. Skin care with herbal exfoliants. *Functional Plant Science and Biotechnology*, 5(1), 94–97, 2011.
111. Paini, M., Aliakbarian, B., Casazza, A. A., Lagazzo, A., Botter, R., & Perego, P. Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. *LWT-Food Science and Technology,* 62(1), 177–186, 2015.
112. Papierska, K., & Ignatowicz, E. Functional food in prevention of cardiovascular diseases and obesity. *Acta Poloniae Pharmaceutica-Drug Research*, 76(6), 945–958, 2019.
113. Pathak, P. D., Mandavgane, S. A., and Kulkarni, B. D. Waste to wealth: a case study of papaya peel. *Waste and Biomass Valorization*, 10(6):1755–1766, 2019.
114. Pietrasik, Z., & Shand, P. J. Effects of moisture enhancement, enzyme treatment, and blade tenderization on the processing characteristics and tenderness of beef semimembranosus steaks. *Meat Science*, 88, 8–13, 2011.
115. Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., and Núñez, M. J. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry*, 53(6), 2111–2117, 2005.
116. Pino, J. A., Almora, K., & Marbot, R. Volatile components of papaya (Carica papaya L., Maradol variety) fruit. *Flavour and fragrance journal*, 18(6), 492–496, 2003.
117. Punia, S., Sandhu, K. S., Siroha, A. K., & Dhull, S. B. Omega 3-metabolism, absorption, bioavailability and health benefits–A review. *PharmaNutrition*, 10, 100162, 2019.

118. Quek, S. Y., Chok, N. K., & Swedlund, P. The physicochemical properties of spray-dried watermelon powders. Chemical Engineering and Processing: *Process Intensification*, 46(5), 386–392, 2007.
119. Rajasekaran, E., & Vijayasarathy, M. CARBANA: Carbon analysis program forprotein sequences. *Bioinformation*, 5(10), 455, 2011.
120. Ramírez, M. J., Giraldo, G. I., & Orrego, C. E. Modeling and stability of polyphenol in spray-dried and freeze-dried fruit encapsulates. *Powder Technology*, 277, 89–96, 2015.
121. Ratti, C. Hot air and freeze-drying of high-value foods: a review. *Journal of food engineering*, 49(4), 311–319, 2001.
122. Rivera-Pastrana, D. M., Yahia, E. M., and González-Aguilar, G. A. Phenolic and carotenoid profiles of papaya fruit (Carica papaya L.) and their contents under low temperature storage. *Journal of the Science of Food and Agriculture*, 90(14):2358–2365, 2010.
123. Rnghavarno, K. S. M. S., Guinn, M. R., & Todd, P. Recent Developments in Aqueous two-Pease Extraction in Bioprocessing. *Separation and Purification methods*, 27(1), 1–49, 1998.
124. Routray, W., and Orsat, V. Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology*, 5(2):409–424, 2012.
125. Saeed, F., Arshad, M. U., Pasha, I., Naz, R., Batool, R., Khan, A. A., ... & Shafique, B. Nutritional and phyto-therapeutic potential of papaya (Carica papaya Linn.): an overview. *International Journal of Food Properties*, 17(7), 1637–1653, 2014.
126. Saikia, S., Mahnot, N. K., & Mahanta, C. L. Optimisation of phenolic extraction from Averrhoa carambola pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food chemistry*, 171, 144–152, 2015.
127. Santos, C. M. D., Abreu, C. M. P. D., Freire, J. M., Queiroz, E. D. R., & Mendonça, M. M. Chemical characterization of the flour of peel and seed from two papaya cultivars. *Food Science and Technology*, 34, 353–357, 2014.
128. Shah, S., Sharma, A., & Gupta, M. N. Extraction of oil from Jatropha curcas L. seed kernels by combination of ultrasonication and aqueous enzymatic oil extraction. *Bioresource technology*, 96(1), 121–123, 2005.

129. Sharayei, P., Azarpazhooh, E., Zomorodi, S., & Ramaswamy, H. S. Ultrasound assisted extraction of bioactive compounds from pomegranate (Punica granatum L.) peel. *Lwt*, 101, 342–350, 2019.

130. Shouket, H. A., Ameen, I., Tursunov, O., Kholikova, K., Pirimov, O., Kurbonov, N., ... & Mukimov, B. Study on industrial applications of papain: A succinct review. In *IOP Conference Series: Earth and Environmental Science,* volume 614, page 0.12171 IOP Publishing, 2020.

131. Sikdar, S. K., Cole, K. D., Stewart, R. M., Szlag, D. C., & Todd, P. C. H. Jr. *Aqueous two-phase extraction in bioseparations: An assessment. Bio/Technology*, *9*, 253–256, 1991.

132. Sillero, L., Prado, R., & Labidi, J. Simultaneous microwave-ultrasound assisted extraction of bioactive compounds from bark. *Chemical Engineering and Processing-Process Intensification*, 156, 108100, 2020.

133. Sim, Y. C., Lee, S. G., Lee, D. C., Kang, B. Y., Park, K. M., Lee, J. Y., ... & Rhee, J. S. Stabilization of papain and lysozyme for application to cosmetic products. *Biotechnology letters*, 22, 137–140, 2000.

134. Singla, M., & Sit, N. Application of ultrasound in combination with other technologies in food processing: A review. *Ultrasonics Sonochemistry*, 73, 105506, 2021.

135. Singla, M., & Sit, N. Isolation of papain from ripe papaya peel using aqueous two-phase extraction. *Journal of Food Measurement and Characterization*, 17(2), 1685–1692, 2023.

136. Singla, M., & Sit, N. Theoretical Aspects and Applications of Aqueous Two-Phase Systems. *ChemBioEng Reviews*, 10(1), 65–80, 2023.

137. Sit, N., Deka, S. C., & Misra, S. Combined effect of ultrasound and enzymatic pretreatment on yield and functional properties of taro (Colocasia esculenta) starch. *Starch-Stärke*, 66(11-12), 959–967, 2014.

138. Sonone, E. V. S., Unde, P. A., & Kad, V. P. Effect of spray dryer parameters on different properties of fruit juice powder. *International Journal of Advanced Engineering, Management and Science*, 2(8), 239601, 2016.

139. Tang, X., & Pikal, M. J. Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharmaceutical research*, *21*, 191-200, 2004.

140. Tchabo, W., Ma, Y., Engmann, F. N., & Zhang, H. Ultrasound-assisted enzymatic extraction (UAEE) of phytochemical compounds from mulberry (Morus nigra)

must and optimization study using response surface methodology. *Industrial Crops and Products*, 63, 214–225, 2015.

141. Tiwari, B. K. Ultrasound: A clean, green extraction technology. *TrAC Trends in Analytical Chemistry*, 71, 100–109, 2015.
142. Tsuge, H., Nishimura, T., Tada, Y., Asao, T., Turk, D., Turk, V., & Katunuma, N. Inhibition mechanism of cathepsin L-specific inhibitors based on the crystal structure of papain–CLIK148 complex. *Biochemical and Biophysical Research Communications*, 266(2), 411–416, 1999.
143. Vilkhu, K., Manasseh, R., Mawson, R., & Ashokkumar, M. Ultrasonic recovery and modification of food ingredients. In *Ultrasound technologies for food and bioprocessing*, pages 345–368. New York, NY: Springer New York, 2010.
144. Vinatoru, M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, 8(3):303–313, 2001.
145. Wang, J., Sun, B., Liu, Y., & Zhang, H. Optimisation of ultrasound-assisted enzymatic extraction of arabinoxylan from wheat bran. *Food chemistry*, 150, 482–488, 2014.
146. Wang, X., Peng, M. J., Wang, Z. H., Yang, Q. L., & Peng, S. Ultrasound-microwave assisted extraction of flavonoid compounds from Eucommia ulmoides leaves and an evaluation of their antioxidant and antibacterial activities. *Archives of Biological Sciences*, 72(2), 211–221, 2020.
147. Wen, L., Zhang, Z., Sun, D. W., Sivagnanam, S. P., and Tiwari, B. K. Combination of emerging technologies for the extraction of bioactive compounds. *Critical reviews in food science and nutrition*, 60(11):1826–1841, 2020.
148. Winterhalter, P., Katzenberger, D., & Schreier, P. 6, 7-Epoxy-linalool and related oxygenated terpenoids from Carica papaya fruit. *Phytochemistry*, 25(6), 1347–1350, 1986.
149. Wu, H., Zhu, J., Diao, W., & Wang, C. Ultrasound-assisted enzymatic extraction and antioxidant activity of polysaccharides from pumpkin (Cucurbita moschata). *Carbohydrate Polymers*, 113, 314–324, 2014.
150. Wu, J., Cai, G., Liu, J., Ge, H., & Wang, J. Eco-friendly surface modification on polyester fabrics by esterase treatment. *Applied Surface Science*, 295, 150–157, 2014.

151. Wulansari, D. D., Wulandari, D. D., Risthanti, R. R., & Kirtishanti, A. Ameliorative effect of Carica papaya seed extract on diabetic rat model with muscle atrophy. *Media Pharmaceutica Indonesiana*, 2(4), 208–215, 2019.
152. Xu, Y., Zhang, L., Yang, Y., Song, X., & Yu, Z. Optimization of ultrasound-assisted compound enzymatic extraction and characterization of polysaccharides from blackcurrant. *Carbohydrate Polymers*, 117, 895-902, 2015.
153. Yan, P., Gao, X. Z., Shen, W. T., & Zhou, P. Cloning and expression analysis of phytoene desaturase and ζ-carotene desaturase genes in Carica papaya. *Molecular Biology Reports*, 38, 785–791, 2011.
154. Yogiraj, V., Goyal, P. K., Chauhan, C. S., Goyal, A., and Vyas, B. Carica papaya Linn: an overview. *International Journal of Herbal Medicine,* 2(5):01–08, 2014.
155. Zhang, L., Guo, S., Wang, M., & He, L. PEG-based ultrasound-assisted enzymatic extraction of polysaccharides from Ginkgo biloba leaves. *International Journal of Biological Macromolecules*, 80, 644–650, 2015