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Review article

RECENT PROGRESS IN THE VALORIZATION OF PINEAPPLE WASTE INTO VALUE-ADDED BIOACTIVE COMPONENTS: A SYSTEMATIC APPROACH

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ABSTRACT

Pineapples are widely consumed across the world, particularly in tropical regions and during processing generates massive waste, such as peel, core, pomace, crown, leaves, stems etc. Unfortunately, the disposal of these wastes results in environmental hazards. However, these wastes contain various value added constituents and can be effectively utilized in different industries, such as cosmetics, food, pharmaceuticals etc. Extraction of these compounds can be achieved through conventional e.g Soxhlet extraction, maceration, and hydro-distillation etc and non-conventional techniques e.g. ultrasound assisted (UAE), microwave assisted (MAE), supercritical fluid (SCFE), pressurized liquid (PLE), pulse electric field (PEF), enzyme assisted extraction (EAE), and liquid-liquid extraction (LLE) techniques are reviewed in this paper. The characterization of these compounds is also critical aspect to determine the quality and purity of the extracted compounds. HPLC, NMR spectroscopy are some of the technique which can be used to separate, quantified and characterized the bioactive components. In the present investigation, valorization of pineapple waste as a sustainable waste management strategy by extraction of valuable bioactive compounds using conventional and non-conventional extraction techniques, their isolation, quantification and characterization techniques are thoroughly reviewed.

1.Introduction

The pineapple (Ananas comosus) which is from the Bromeliaceae family is a fruit which is sweet and has slightly acidic taste is consumed across the world. This fruit juice is the third most preferred worldwide after orange and apple juices. The plant has approximately height of 75-150 cm and width 90-120 cm. It is short, having a stout stump with narrow, fibrous and spiny leaves (Upadhyay et al. 2013). In north-east India pineapple is mostly grown compared to other horticulture crops. India ranks 5th in the production of pineapple with about 1.2 million tons annually. The ministry of commerce and industry has recently sanctioned the Agri-export zone scheme for the entire NER at Tripura (Saloni et al. 2017). The total annual world production of pineapple was 25439366 tonnes during the year 2014 (FAO stat). India is the fifth largest producer of pineapple with annual output of about 1.2 millions. During the year 2014-15 the area and production was 116000Ha and 1984000MT while for the year 2015-16 the estimated production is 1964000MT in 110000Ha area (NHB, India)(Saloni et al., 2017). Commercially, it is mainly produced as canned fruits and consumed worldwide (Upadhyay et al. 2013). The term "pineapple waste" refers to the byproducts and remains produced during the preparation and eating of pineapples. As customers enjoy the tropical treat, a substantial amount of plant debris is left behind, posing both obstacles and possibilities. Because of its potential for creative and sustainable solutions, this often-overlooked component of the pineapple business has gained growing attention. This investigation will dive into the numerous facets of pineapple trash, from its environmental effect to the imaginative ways it may be recycled, reflecting an increasing worldwide emphasis on waste reduction and resource efficiency. A large number of wastes generated everyday from human consumption to different agribusinesess and also from process industries. These waste has a lot of organic matter (Vieira et al. 2022). It is a terrestrial, monocot, herbaceous, and perennial plant (Sun et al. 2016; Vieira et al. 2022) The valorization

of pineapple waste marks an extraordinary attempt to turn what was previously a wasted byproduct into valuable resources, so addressing environmental issues and supporting sustainable practices. Pineapple waste includes a variety of products, including as peels, cores, and leaves, that were previously disregarded but are now garnering attention for their possible application.

2.Pineapple Waste

Whenever pineapple is harvested from the field a significant amount of waste generates and different portions of the pineapple is thrown during processing. Processing residue or waste generally includes peel, core and residual pomace. The development of new technologies, supplements, food, medicines, and also the manufacture of biofuels can all benefit from the presence of diverse compounds in pineapple waste (Rabiu et al. 2018). Peels constitutes a large percentage of residue. Theoretically pineapple waste can be composted but only a 4% of compostable materials are actually composted. Rest always ends up in landfills. Throwing organic waste into landfills causes a huge problem. It generates methane gas which is very harmful green-house gas. Also it impacts the environment. Landfills generates bad odor and other aesthetic issues. Soil and ground water contamination can also be seen. Various waste management techniques can be employed to treat the waste and also can be converted into valuable products. Peel (30-42%), cores (9-10%), stems (2-5%), and crowns (2-4%) are the trash produced by the processing of pineapples (Lobo and Paull. 2017). Because of this, the weight of the pineapple is comprised of half pineapple waste(Ketnawa et al., 2012).Pineapple peel contains significant amount of sugar which is the main nutrition for microorganisms. The peel has the potential to be used as a substrate for the production of CH_4, C_2H_5OH, and H_2 (Diaz-Vela et al. 2013). In the western countries 95% of the pineapple harvested are processed whereas in India only 10% of the pineapple is processed. Example of those processed product includes mostly canned pineapple, pineapple juice, pineapple juice concentrate, jam and jelly and pineapple preserve etc. But due to the health benefits of pineapple juice, people in India also considering processed products from pineapple due to that reason pineapple processing industries are also growing in India. As the industries are growing, waste is also growing proportionally. Therefore, it is necessary to investigate alternate methods of turning trash into value-added goods to lessen the environmental impact and socioeconomic issues.

3.Valorization techniques for the synthesis of bioactive constituents from pineapple waste as a potential agro-waste residue

Extraction of bioactive compounds is the method of carefully separating valuable and physiologically active molecules from natural sources including plants, fruits, and marine life. Liquid-liquid, supercritical fluid, and enzyme-assisted extraction are a few techniques used to accomplish this. Based on the characteristics of the desired chemicals, an extraction technique and solvent are chosen. Extracted bioactive substances are used in industries, including comprehensive screening method to weed out compounds that are important to human health (Azmir et al. 2013). Some of the techniques described here used for hundred of years. All of these methods share the following objectives: (a) to remove targeted bioactive compounds from complex plant samples; (b) to enhance analytical methods' selectivity; (c) boosting the sensitivity of bioassays by increasing the concentration of targeted compounds; (d) changing the bioactive compounds into suitable form that for better detection and separation.

3.1.Conventional Extraction Techniques:

To extract bioactive compounds from plants, conventional extraction methods are: (1) Soxhlet extraction, (2) Maceration, and (3) Hydro-distillation(Azmir et al., 2013). Most of these methods are based on the extracting power of different solvents in use and the application of heat and/or mixing (Azmir et al. 2013).

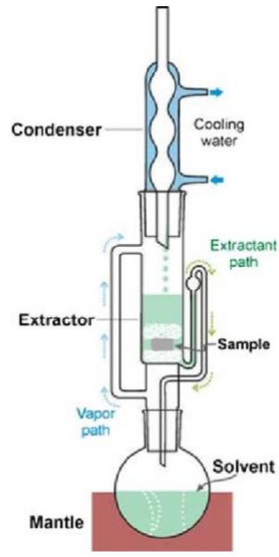

Figure 1. Schematic diagram of a Soxhlet extractor (Arsad et al. 2014)

3.1.1.Soxhlet Extraction:

Soxhlet extractor proposed by German chemist Franz Ritter Von Soxhlet in the year 1879 (Lone et al. 2020). At first this new extractor was only used for lipid extraction but it had a lot of potential in extracting other components also. It can be used to extract various components from natural resources. A lot of new techniques uses this old technique to compare the extraction efficiency. This technique follows the following protocol. A)

Firstly dry pineapple peel powder is placed inside the thimble, B) The thimble is then placed inside the distillation flask where the solvent is poured. Generally, petroleum ether or n-hexane. Once the overflow level is reached, a siphon is used to aspirate the solution from the thimble holder. When the solvent is heated the solvent evaporates and the vapor flows through the extractor and goes to the condenser and liquifies the solvent. The solvent falls into the distillation flask afterwards. Whenever it reaches the level the siphon transfers the solvent to the flask, which contains the extracted components. That completes a cycle, and this process continues for several cycles depending upon the requirements and the plant material used.

3.1.2.Maceration:

Maceration is one of the oldest techniques known to extract valuable components from natural resources. Back in the days this method was used to prepare tonics for various health issues. This method is still used by the people as this method is very convenient and protocol is quite easy to follow. Essential oils can be extract using this method also though the yield is low compared to other techniques available. Modifying some of the parameters in maceration process also increases the yield. Normally dried and grounded plant materials like pineapple peel powder is used mixing with a suitable solvent. The solvent used in the maceration process is called the menstruum. The menstruum is added to the plant material and after closing the vessel, it is kept for one to two days upto several days for the better absoption of the components. Occasional shaking increases absorption of the components. After that the solution is filtered whatever left is called marc. Sometimes new solvent is added to the older menstruum for more yield.

3.1.3.Hydro-distillation:

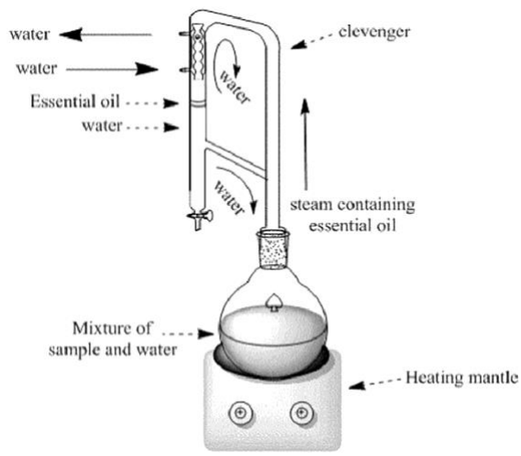

Figure 2. Schematic diagram of Cleavenger Apparatus(Hydrodistillation Unit) (Samadi et al. 2016)

Bioactive substances, essential oils are traditionally extracted from plant matrix via hydro-distillation. In this method raw plant material can be used and since organic solvents are not needed. There are three types of hydro-distillation: 1)direct steam distillation, 2) water distillation, and 3) water and steam distillation (Yang and Jin, 2024). In the process of hydro-distillation, plant
materials are kept in a container and water is added to the container and the solution is heated till boiling. In direct steam technique uses hot steam injected directly into the dried plant sample. The main influencing factors that extract the bioactive chemicals from plant are steam and hot water. Hydro-distillation, however, enhances the extraction yield when combined with other extraction techniques, such as microwave pretreatment (Diana and Santosa, 2024). The vapor containing oil and water is cooled using indirect water-cooling method. In the condenser section oil and other components are separated due to their different specific gravity.

3.2.Non-conventional extraction techniques:

3.2.1.Ultrasound assisted extraction:

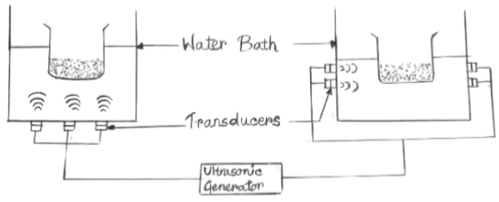

Figure 3. Schematic diagram of an ultrasonicator (Hussain et al., 2020)

The application of ultrasonic technology in food processing includes extraction, filtration, freezing, packing, cutting, and nano-formulations. Additionally, its use in the extraction of bioactive chemicals from plant matrices has been thoroughly investigated on a lab scale (Belwal et al. 2020).

Usually, ultrasound ranges from 20 kHz to 100 MHz. Like other waves, it passes through a medium by creating compression and rarefaction. Compression and rarefaction produces a phenomenon called cavitation, implosion of bubbles which means production, growth and collapse of bubbles. The primary advantage of UAE(Ultrasound Assisted Extraction) is seen in plant samples because ultrasonic energy promotes the leaching of organic and inorganic components from plant matrix(Purkait et al. 2023; Kumar et al. 2021). Two types of ultrasonic equipments are there, ultrasonic bath type and ultrasonic probe type. Generally solvents used in the extraction process are Ethanol and methanol at various concentrations(Qadariyah et al. 2018). (Dhar and Deka, 2023) reported a significant yield of dietary fibre using UAE from pineapple waste compared to convention extraction techniques.

3.2.2.Microwave assisted extraction:

Microwave energy is developed recently for the extraction of organic compounds from plant

matrices after its use for trace metal analysis in inorganic chemistry. The requirement for a quick, secure, and affordable solution led to the invention of microwave technology. Modern extraction methods like microwave-assisted extraction (MAE) successfully extract bioactive chemicals from natural sources. In MAE, dried pineapple waste fraction is mixed with solvent and subjected to microwave radiation, quickly heating the mixture and hastening the extraction process. Different solvents extracts different bioactive compounds(Table 1). This technique is beneficial for a variety of sectors, from pharmaceuticals to food manufacturing and cosmetics, since it reduces extraction time, increases yields, and preserves heat-sensitive chemicals. By lowering energy and solvent usage, MAE also supports sustainability initiatives. To maximise its advantages and avoid any problems caused by overheating or compound degradation, adequate parameter management and specialised equipment are necessary. The frequency of microwave ranges from 300 MHz-300 GHz. Microwave oven's are of two types, monomode(fig. 4a) and multimode cavity(fig.4b) (prado et al.,2015). A frequency that only excites one mode of resonance can be produced via the monomode cavity. MAE is highly potential for extraction of high yield of pineapple peel pectin and provide better quality pectin (Zakaria et al. 2021). Due to the size of the multimode cavity, several modes of resonance can be affected by the incident wave. Since the modes are superimposed, the field may be homogenized. Rotating plates are included as homogenization systems (Prado et al. 2015).

3.2.3.Pulsed-electric field extraction:

Pulsed-electric field is a novel extraction method that has attracted research interest in recent years because of it's efficacy in the food, pharmaceutical, and nutraceutical industries. The PEF approach was initially used as a non-thermal method to preserve and enhance the quality of food and medical materials by inactivating the majority of microorganisms and certain enzymes at room temperature (Yan et al. 2017). The principle behind electroporation, when biological materials are subjected to quick, high-voltage electric pulses. The target source, whether it be plant tissue, fruits, or other biological materials, has its cell membranes temporarily perforated or opened by these electric pulses. Rapid and accelerated intracellular bioactive molecule release is made possible by this brief disturbance of the cell membrane's structure. PEF extraction thus offers a considerable improvement in mass transfer rates and extraction effectiveness over traditional extraction techniques. PEF extraction offers a productive and environmentally friendly way to acquire important chemicals, particularly heat-sensitive and labile ones, while minimizing damage and decreasing the need for chemical solvents. This is accomplished by optimizing variables including intensity of electric field, electric pulse length, and the number of electric pulses. Plant tissue is observed to be destroyed by PEF treatment at a modest electric field (500 and 1000 V/cm; for 104-102 s) with no rise in temperature. Several factors affect the PEF treatment process. Such as a) Electric-field intensity b) Pulse wave shape c) Solvent selected, concentration, pH d) Ratio to solvent to raw material e) Pulse duration and f) Treatment temperature.

3.2.4.Enzyme-assisted extraction:

Chemicals present in the plant matrix are distributed in the cell cytoplasm, and some substances are kept in the polysaccharide-lignin network by hydrogen or hydrophobic bonds, are inaccessible to a solvent in a typical technique. Enzymatic pre-treatment is viewed as a cutting-edge and successful method to liberate bound chemicals and boost total yield (Rosenthal et al. 1996). To proceed with enzyme assisted extraction, generally two processes are there: 1) Enzyme-assisted aqueous extraction(EAAE) and 2) Enzyme-assisted cold pressing(EACP) (Azmir et al. 2013). The extraction of bioactive molecules from natural resources, primarily plant materials, is done using an environmentally friendly and sustainable technique called enzyme-assisted aqueous extraction. Cellulases, hemicellulases, or proteases are only a few of the particular enzymes that are used in this extraction procedure, which is carried out in an aqueous solution. The structural elements of cells(plant), for example cellulose, hemicellulose, and proteins, are the main targets of these enzymes, which break them down and disturb the plant cells in order to liberate important chemicals. The extraction of oils from oilseeds, such as nuts and seeds, using enzyme-assisted cold pressing is a cutting-edge and environmentally beneficial technique, especially for industrial and culinary uses. The principles of cold pressing, which extracts oil from seeds mechanically at low temperatures, are combined with the use of certain enzymes, often lipases or proteases, in this procedure. These enzymes aid in the disintegration of cellular walls and the hydrolysis of proteins in oilseeds, making oil extraction simpler while preserving lower processing temperatures. The benefit of cold pressing in conjunction with enzyme aid is that it maintains the quality of the extracted oil by avoiding the use of heat or chemical solvents that can destroy heat-sensitive components (Azmir et al. 2013).

3.2.5.Pressurized liquid extraction:

In 1995, Dionex Corporation originally presented PLE at the Pittcon Conference under the name Accelerated Solvent Extraction Technology (ASE®). This process is sometimes referred to as improved solvent extraction, accelerated solvent extraction, and pressurized liquid extraction (Ganjeh et al. 2023).

Pressurised-Hot Water Extraction (PHWE), sub-critical water, or superheated water extraction are all terms used to describe the method where water is utilised as the extraction solvent. Both of these methods will be referred to as PLE and PHWE in this essay. This technique makes use of high pressure and temperature to improve target chemical extraction from solid or semi-solid materials. In Pressurized Liquid Extraction, the sample is put into an extraction cell, and after that a heated liquid solvent is pumped into the cell under high pressure. When pressure and temperature are combined, the extraction process is sped up, leading to noticeably shorter extraction periods than with conventional techniques. PLE is frequently used to extract a broad variety of analytes from difficult matrices in analytical chemistry, environmental studies, food science, and pharmaceutical research. It is a useful tool for sample preparation and analysis because to its benefits including increased extraction efficiency, automation possibilities, and reduced solvent usage. PLE was observed to considerably reduce time consumption and solvent use when compared to the conventional Soxhlet extraction(Richter et al., 1996). Because just a little quantity of organic solvent is used, PLE is widely reorganized as a green extraction method (Azmir et al. 2013).

3.2.6.Supercritical-fluid extraction:

Supercritical-fluid extraction has several operational benefits over traditional extraction procedures since it uses supercritical-solvents, which differ from them in terms of density, diffusivity, viscosity, and dielectric constant because their low viscosity and, relatively high diffusivity, supercritical fluids may extract substances from solid materials more quickly than liquids since they can diffuse through them more quickly. One of the main characteristics of a supercritical fluid is its capacity to alter the density by changing its temperature and pressure. Due to the relation of density and solubility, altering the extraction pressure will affect the fluid's solvent strength (Madhumeena et al. 2021). There are a number of substances that may be utilized as supercritical fluids, as shown in Table 2, however CO_2 is the most widely employed solvent for three main reasons: First, it is safe for use around people and the environment, adhering to the sustainability standards that increasingly determine whether chemical processes are appropriate, Second, its moderate critical temperature of 31.2°C is crucial for the preservation of bioactive compounds in extracts, Third, the extract is protected from coming into contact with air, where light oxidation reactions may occur(da Silva et al. 2016). Since carbon dioxide is a gas at ambient temperature, it is eliminated when the extraction is finished and the system is decompressed, producing an extract without any solvent. When carbon dioxide consumption is large on an industrial scale, the process can be managed to recycle it. Nevertheless, because to its low polarity, CO_2 is less successful in removing highly polar chemicals from organic matrices (da Silva et al. 2016). However, compared to Soxhlet extraction yield of pineapple peel powder with SCFE technique is lower. 79% mg of yield was observed compared to 90% mg after Soxhlet extraction. When the yield was measured for antioxidant and TPC it was found to be 21% and 1.165mgGAE/g extract(Madhumeena et al. 2021) The solubilization of the chemical components existing on the solid matrix and their separation into the supercritical solvent are the two main phases in the process of supercritical extraction (da Silva et al. 2016). As the solvent moves through the packed bed during extraction, it solubilizes the chemicals already present in the organic matrix. The solvent then leaves the extractor bearing the solubilized chemicals, and by lowering the pressure and/or raising the temperature, the extract becomes solvent-free (da Silva et al. 2016).

3.2.7. Liquid-liquid extraction:

A technique for separating bioactive molecules from natural resources including plants, herbs, and fruits is solvent extraction (Ofoedum et al. 2023). In this procedure, the target chemicals are extracted and dissolved from the source material using an appropriate organic solvent. The polarity of the bioactive molecules that are being targeted determines the appropriate solvent, with nonpolar substances like essential oils being handled by hexane and polar compounds being handled by ethanol or methanol. A yield of 82% was noted when using solvent extraction method was used to extract bioactive components from pineapple peel powder. Antioxidant activity and total phenolic content was observed to be 32 % and 1.287 mgGAE/g of extract (Madhumeena et al. 2021). Combinations of water and alcohol are more effective in extracting phenolic chemicals than equivalent mono-component solvent solutions. In particular, several water-ethanol ratios were examined, and the polyphenolic yield's extracted with 50% ethanol (vol.) at different temperatures (20, 40, and 60°C) were nearly twice as high as those obtained with pure water (Hidalgo and Almajano. 2017). Using methods like evaporation or distillation, the solvent is normally removed from the extract after extraction, leaving behind a concentrated extract full of bioactive components. Solvent extraction also known as liquid-liquid extraction. The solvent used for extraction process is known as "Menstruum". Hexane and Dicholoromethane(DCM) are used to extract non-polar compounds.

4.Methods of estimating polyphenols, flavonoids, proanthocyanidin (condensed tannin and hydrolysable tannin):

4.1.Method to determine Total phenolic content (TPC):

Polyphenols, often known as phenolic compounds, are a class of hydroxylated molecules that are highly oxidation-sensitive. According to several studies, they possess a variety of biological qualities, including those that are anti-proliferative, anti-diabetic, anti-cancer, anti-microbial, anti-inflammatory, and antiviral (Cosme et al. 2022). Among the phenols are simple phenols, phenolic acids (derivatives of benzoic & cinnamic acids), coumarins, flavonoids, hydrolyzable and condensed tannins, lignans, and lignins. These substances are the most prevalent secondary metabolites in the plants, serving primarily as phytoalexins, pollinator attractants, pigment-contributing agents, antioxidants, and UV light blockers, among other functions. Phenolics are divided into two categories hydroxybenzoic acid derivative and hydroxycinnamic acid derivatives (Fig 8). According to (Boulila et al. 2015), the Folin-Cieucalteu (FC) test was used to measure total phenolic (TP). To sum up, 500 µL of newly diluted 10-fold FC reagent in water, 1 mL of 20% sodium carbonate solution, and 100 litres of extract were combined. 760 o V-630 UV-vis spectrophotometer was used to measure the absorbance following a one-hour dark incubation period. Micrograms of gallic acid equivalents (g GAE/g) are employed to express the results, with gallic acid serving as the benchmark.

4.2.Determination of Total Flavonoid Content (TFC):

Flavonoids are the most prevalent and extensively dispersed class of plant phenolics and are found in a broad variety of foods; the most often consumed flavonoids are rutin and quercetin (Rao. 2016). The AlCl3 colorimetric technique was used to measure the total flavonoid (TF) concentration (Boulila et al. 2015). 1.5 mL of methanol, 0.1 mL of a 10% AlCl3 solution, 0.1 mL of potassium acetate (1 M), and 2.8 mL of DW (distilled water) were combined with a 500 L sample. Following a 30-minute incubation period at room temperature, 415 nm was used to quantify the absorbance. As a reference standard, quercetin was utilised, and the TF content was given as micrograms of quercetin equivalents (~g QE/g) (Boulila et al. 2015).

4.3.Determination of Total Tannin Content(TTC):

Using the technique outlined in (Nurdalilah et al. 2018), the total tannin content was calculated. In a test tube, an addition of 0.1 ml methanolic extract, 0.5 ml Folin-Ciocalteu reactive, 7.5 ml distilled water, and 1 ml of a 35% aqueous solution of Na2CO3 were made (Nurdalilah et al. 2018). Next, 0.9 millilitre of purified water was introduced. At room temperature, the combination was left for half an hour in the dark. a UV/Vis spectrophotometer to determine the absorbance at 725 nm (A.Ramlee and Sembok. 2021). For the purpose of creating the standard curve, gallic acid (20–100 ppm) was utilised. The results were given in g of gallic acid equivalents (GAE)/100 g of extract (Nurdalilah et al. 2018).

Table 1. Chemical composition of the different waste fraction that constitutes pineapple waste (Roda and Lambri. 2019)

As % Dry Basis	Ensiled	Fresh	Dry	Peel	Whole	Skin	Crown	Pulp
Moisture	72.49	71.07	27.43	92.2				
Total solid	27.51	29.03	72.57	7.80				
Volatile solids	87.12	96.12	95.90	89.40				
pH	4.00	4.70	4.70					
Ash	12.88	3.88	4.10	10.60	0.70	0.60	0.40	0.20
Cellulose	9.00	11.20	12.00	19.80	19.40	14.00	29.60	14.30
Hemicellulose	4.70	7.00	6.50	11.70	2240	20.20	23.20	22.10
Pectin	5.10	6.70	7.10					
Ether soluble solids	4.00	6.10	6.70					
Protein	0.91	3.13	3.30		4.40	4.10	4.20	4.60
Reducing sugar	5.00	25.80	27.80		6.50			
Non- reducing sugar	1.70	5.70	4.90		5.20			
Total sugar					11.70			
Lignin	9.00	11.52	11.00		4.70	1.50	4.50	2.30

Table 2. Different bioactive compounds extracted by different solvents (Salve and Ray. 2020)

Water	Ethanol	Methanol	Chloroform	Dichloromethanol	Ether	Acetone
Anthocyanins	Tannins	Anthocyanin	Terpenoids	Terpenoids	Alkaloids	Flavonoids
Tannins	Polyphenols	Terpenoids	Flavonoids		Terpenoids	
Saponins	Flavonol	Saponins				
Terpenoids	Terpenoids	Tannins				
	Alkaloids	Flavones				
		Polyphenols				

Table 3. Characteristic of solvents used in SFE obtained from (Herrero et al. 2006)

Bioactive compound	Retention time (min)
Gallic acid	9.58
Chlorogenic acid	20.54
Caffeic acid	24.32
Ferulic acid	18.01
Catehin	23.02
Quercetin	43.80
P-coumeric acid	31.26
Epicatechin	25.32

Table 4. Different bioactive compounds separated and quantified using HPLC obtained from (Rivera et al. 2023)

Solvent	Temperature(°C)	Pressure(atm)	Density$_{\rho SCF}$ (g/mL)	Solubility$_{\sigma SFC}$(cal$^{-1/2}$ cm$^{-3/2}$)
Carbon Dioxide	31.2	72.9	0.470	7.5
Ethane	32.4	48.2	0.200	5.8
Ethene	10.1	50.5	0.200	5.8
Methanol	-34.4	79.9	0.272	8.9
Nitrous oxide	36.7	71.7	0.460	7.2
n—Butene	-139.9	36.0	0.221	5.2
n-Pentane	-76.5	33.3	0.237	5.1
Sulfur hexafluoide	45.8	37.7	0.730	5.5
Water	101.1	217.6	0.322	13.5

5.Spectrophotometric assays of determining antioxidant activity:

5.1.Antioxidant activity by DPPH Radical Scavenging Activity:

The chemical 2,2-diphenyl-1-picrylhydrazyl, or DPPH (Fig 10), is crystalline and purple. It is light-sensitive and stable free radical. While DPPH is soluble in organic solvents such as methanol and ethanol, it is insoluble in water. Because methanol is more harmful than ethanol, ethanol is preferred (Gulcin and Alwasel. 2023). If there are any antioxidant molecules present in a plant extract, they will react with the free radical and reduce it. DPPH will be lowered due to the antioxidant molecules giving an electron to the free radical, which possesses an unpaired electron in its outermost shell. Radical scavenging method by DPPH can be determined following the protocol described by (Rivera et al. 2023). Raw pineapple waste showes a higher antioxidant content compared to dried pineapple waste as heat sensitive components degrades due to application of heat (Saraswaty et al. 2017). In a test tube 100 µl of plant extract is taken and 2900µl of DPPH solution were added. The solution is agitated in a vortex and kept it for incubation for 30 minutes. Then the absorbance is taken at 517nm in UV-vis Spectrophotometer. Antioxidant activity can be calculated using the following equation.

$$\text{Antioxidant activity}(\%) = (A_c - A_s)/A_c \times 100 \quad (1)$$

Where A_c is the absorbance of the control and A_s is the absorbance of the sample (Tan et al. 2015).

5.2.Antioxidant activity by FRAP method:

Ferric Reducing Ability Power can be expressed by the method expressed by (Nurdalilah et al. 2018; Smita et al. 2021). In a test tube 2.5ml of plant extract at various concentration (31.5-1000ppm) is taken. 2.5ml of sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide are added to the test

tube containing plant extract. The mixture is placed in water bath at 50°C for 20 min, after that 2.5ml of 10% trichloro acetic acid (TCA) is added to the mixture and centrifuged at 3000 rpm for 10 min (Nurdalilah et al. 2018). After that 2.5 ml of distilled water and 0.5ml of 0.1% ferric chloride is added to the mixrure and take absorbance at 700 nm after 5 min incubation (Nurdalilah et al. 2018). The antioxidant activity is calculated from the ascorbic acid calibration curve (Serbessa and Bikila. 2019). Ferric reducing activity is calculated using following equations

AEAC(µgAA/g)= [(activity)(dilution factor)($V_{extract}$ (ml)] / g(sample) (2)

Where AEAC = ascorbic acid equivalent antioxidant capacity; µgAA = Microgram of ascorbic acid; activity = calculated from the calibration curve of the equation y = ax + C

$$\text{Activity (X}\mu\text{g/ml)} = y - c / a \quad (3)$$

Where y = absorbance of the sample; c = y – intercept and a = slope

Now the percentage of ferric reducing activity is calculated by

$$\%FRAP = [1 - (1 - A_s / A_c)] \times 100 \quad (4)$$

Where A_c = Absorbance of standard (ascorbic acid) at max concentration and A_s = Absorbance of sample (Serbessa and Bikila. 2019). Ferric reducing power is different for different fruit waste. A study shows pineapple peel has the lowest ferric reducing power compared to apple peel extract(Afsharnezhad et al. 2017)

5.3.Antioxidant activity by ABTS method :

2,2′-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid) shortly ABTS is a spectrophotometric method that measures the antioxidant activity of plant extract. ABTS radical cation which is blue-green in appearance is produced when ABTS reacts with potassium persulfate. ABTS radical cation is a stable compound. The stable radical is scavenge by the antioxidant molecules present in the plant extract and reduces its absorbance(Hidalgo and Almajano 2017). In the method described by (Hidalgo and Almajano. 2017), an aliquot of sample is mixed with the ABTS solution. After that the mixture is incubated for some amount of time and then absorbance is measured at 734nm. This can be calculated using equation-

%ABTS radical cation scavenged = [($A_{control}$ – A_{sample})/ $A_{control}$] × 100 (5)

Where '$A_{control}$' is the absorbance of ABTS radical cation solution and 'A_{sample}' is the absorbance of ABTS radical cation with the plant sample.

6.Extraction of Bromelain from pineapple waste fraction:

One of the major class of protease enzyme present in the pineapple is Bromelain, complex mixture of protein digesting enzymes. It is present in stem, fruit, crown, core, leaves of pineapple (Mohan et al. 2016). The enzyme extracted from stem is called stem bromelain (EC 3.4.22.32) and from fruit is known as fruit bromelain (EC 3.4.22.33). Similar proteases like Polyphenol oxidase (PPO) an also be found in different pineapple waste fraction's such as peel, core, stem, crown and leaves (Ketnawa et al. 2011). Bromelain has a wide range of therapeutic uses and may be used to treat a number of diseases, including platelet aggregation, fibrinolysis, anti-inflammatory action, cell adhesion, cytokine induction, cardiovascular use, and digestive help (Benefo and Ofosu, 2018). It is also used as dietary supplements. A research done by (Abbas et al. 2021) focuses on removing bromelain from the pineapple's core, crown, fruit, peel, and stem, among other elements. Using ammonium sulphate with 40% saturation, the isolated enzyme was precipitated, and dialysis was then performed. For peel, crown, core, fruit, and stem, the corresponding fold of purification was determined to be 1.948, 1.536, 1,027, 1.989, and 1.232. The Azocasein test was used to measure the bromelain activity; peel had the maximum activity, measuring 3.417 U/lg. The test organisms were used to determine antimicrobial activity and minimum inhibitory concentration (MIC) of the crude and purified bromelain fractions. *Staphylococcus aureus* was most inhibited by the crude and purified extract of peel, followed by *Propionibacterium* acne (Abbas et al. 2021). The formation of melanin in animals is regulated by the enzyme polyphenol oxidase (PPO; EC 1.1.4.18.1 and 1.10.3.1), which is abundantly present in nature. PPO is used in the manufacture of black tea since traditional techniques for the purification of proteins and enzymes have a number of drawbacks, including scale-up issues and high production costs. Bromelain may be extracted and purified utilising a variety of methods, modern as well as conventional. Conventional techniques include the following procedures. Centrifugation, ultrafiltration, lyophilization, and (Ramli et al. 2017). Global industrial bromelain production has expanded recently as a result of the advent of more contemporary purification methods for example; filtration, ion exchange chromatography, affinity chromatography, aqueous two-phase extraction, and reverse micelle chromatography. Additionally, recombinant DNA technology has become a substitute method for creating vast quantities of ultrapure bromelain (Ramli et al.

2017). According to (Ketnawa et al., 2011a) bromelain can be extracted using an aqueous two-phase system(ATPS). In order to separate and purify mixtures of proteins and enzymes, the aqueous two-phase system (ATPS) is a practical and affordable technique. The ATPS is made up of two incompatible polymers, such as polyethylene glycol (PEG) and dextran, or one polymer combined with a salt, such as PEG and a phosphate salt, in an aqueous solution. The two phases split if the solubilization of these phase-forming chemicals in an aqueous solution rises over a threshold concentration. It can eliminate unwanted byproducts present in unidentified polysaccharides and interfering proteins that lowers the activity of enzymes. The crude extract's bromelain was primarily partitioned to the phase that contained a lot of polyethylene glycol. The optimal partitioning conditions for bromelain were determined to be 18% PEG6000-17% $MgSO_4$, which raised purity 3.44-fold and resulted in an activity recovery of 206%. The MW of bromelain was determined to be around 29 kDa by protein patterns and activity staining. At a pH of 8.0 and temperature of 60°C, bromelain had the highest relative activity. Its activity steadily declined with increasing NaCl concentrations (up to 1.5%, w/v). Collagen was hydrolyzed with bromelain (0–0.3 units). When collagen's, 1, 2 components were exposed to bromelain, they were thoroughly broken down into tiny peptides (Ketnawa et al., 2011a). Reverse micellar technique can also be used to extract bromelain from pineapple and has different effects on surfactant structure. This technique is complicated compared to other techniques available for the extraction of bromelain. Several factors effects this techniques that includes nature and composition of target protein, pH, the concentration and species of ions, types and concentration of surfactants, the composition of reverse micelles (Wan et al. 2016). The polar heads of the surfactant molecules that make up reverse micelles face the polar core while the hydrophobic tails protrude into the nonpolar surroundings. The formation of aqueous compartments within these micelles makes them unique. Proteins can be dissolved within these pockets of liquid, where they are shielded from the organic material around them and kept biologically functioning. The liquid-liquid reverse micellar extraction procedure consists of two steps: forward extraction, in which a target protein is selectively solubilized into the organic phase, and backward extraction, in which the target protein is stripped into the aqueous phase by the addition of fresh aqueous buffer, also known as stripping solution(Wan et al. 2016). Bromelain extracted using techniques mentioned above are called the “Crude bromelain” or “Crude Bromelain Extract (CBE)”. CBE is then purified and characterized using various techniques. Ammonium sulphate $(NH_4)_2SO_4$ precipitation is the simple and cost effective method for the partial purification of bromelain(Gul et al. 2021). (Gul et al. 2021) conducted a research where crude bromelain was extracted using phosphate buffer (pH 7), it underwent partial purification using varying ammonium sulphate $(NH_4)_2SO_4$ fractions, including 30, 40, 50, and 60%, and was then desalted and concentrated. The casein digesting unit (CDU) technique was utilized to determine the enzyme activity. The findings indicated crown bromelain had the highest amount of purification, at 30%, at 4.34 times. From the pineapple's peel, core, and crown debris, bromelain was recovered. A straightforward and affordable method for partially purifying bromelain was the precipitation of ammonium sulphate $(NH_4)_2SO_4$. For the crown, core, and peel bromelain samples, respectively, a purification of 4.34, 2.75, and 2.59-fold was attained.

7.Extraction of other bioactive components present in pineapple peel:

The bioactive compounds found in pineapple by-products, such as gallic acid, ferulic acid, epicatechin, and catechin, may be significant because they offer a potential source of naturally occurring antioxidants, antimicrobials, and functional food ingredients with positive health effects (Polanía et al. 2022). The pineapple peel extract contains dominant phenolics such as epicatechin, gallic acid, catechin, epicatechin and ferulic acid. The primary dietary phenolic components with several health advantages include flavonoids, tannins, and phenolic acids. The antioxidant activity of pineapple peel extract is notable and includes DPPH,FRAP, and ABTS scavenging activities (S. Sharma et al. 2022). Bioactive compounds can be extracted using conventional and non-conventional techniques as mentioned in this review however to make bioactive compounds more accessible , such as gallic acid, ellagic acid, citric acid, lactic acid, and others from plant residues solid state fermentation is an effective alternative (Polanía et al. 2022). *Rhizopus oryzae* is used in a wide range of industrial applications because of its capacity to yield a wide range of chemicals, including volatile compounds, cellulases, proteases, tannases, xylanases, pyruvate decarboxylases, and lipases, as well as organic acids like lactic and fumaric acids (Polanía et al. 2022). In a study done by(Rivera et al., 2023) found that solid state fermentation of pineapple peel is a useful bioprocess for releasing phenolic chemicals with antioxidant properties. In comparison to the control sample (unfermented peels), which showed a 176.2% rise in TPC, the solid-state fermentation (SSF) procedure raised the TPC percentage in all treatments. The five phenolic chemicals found in pineapple peel—gallic, chlorogenic, caffein, epicatechin, and p-

coumaric acid could be identified and measured via HPLC analysis (Rivera et al. 2023). Pineapple peel is a rich source of ferulic acid, sometimes referred to as hydroxycinnamic acid. It has several biological advantages and low toxicity (N. Sharma and Borah. 2023). A research done by (N. Sharma and Borah. 2023) shows extraction, purification and identification of ferulic acid from pineapple peel by using UV-Spectrophotometer and HPLC. The result shows improvement in the extraction of ferulic acid, the alkaline hydrolysis of pineapple peel was optimized through the use of Response Surface Methodology in conjunction with Central Composite Design. The variables at play, specifically the temperature (°C) and extraction time (min.). The results showed 46.34% DPPH and 3.34 mgGAE/g for TPC. Using HPLC, the yield of ferulic acid was 0.471%. The ideal temperatures and extraction times found in this investigation are 42 ´C and 200 min, respectively(N. Sharma & Borah, 2023).

8.Separation & quantification of extracted bioactive compounds

8.1.Separation and quantification by High Performance Liquid Chromatography:

HPLC, High Performance Liquid Chromatography is a technique to identify components in a plant extract. To perform the HPLC analysis plant extract is prepared using methanol and water (Madhumeena et al. 2021) and ethanol and water (Rivera et al. 2023) then the extract is filtered and injected into the HPLC column. The column is packed with stationary phase particles. The column is eluted with a gradient of methanol and water (Madhumeena et al. 2021) or ethanol and water (Rivera et al. 2023) which is called the mobile phase and eluted components were detected using UV detector. Bioactive compounds in a plat extract can be identified using by comparing their retention time to the retention time of the standard compounds and can be quantified by comparing the peak areas of the sample to the standard compounds (Rivera et al. 2023; Madhumeena et al. 2021). The following table shows the different compounds separated and quantified from pineapple peel.

9.Characterization of Bioactive compounds

For the structural characterization of bioactive substances, NMR spectroscopy, or NMR, is a potent strategy. It offers details on the kind and quantity of atoms present in a molecule, their connection, and their spatial arrangement, among other aspects of its molecular structure. The foundation of NMR spectroscopy is the idea that by absorbing electromagnetic radiation in the radio frequency range, some atoms' nuclei, such those of hydrogen and carbon, may be stimulated to higher energy levels. Because the kind and quantity of atoms bound to the nucleus define its chemical environment, the frequency of the absorbed radiation is also influenced by these factors (Santos and Silva. 2014). The first step in utilizing NMR spectroscopy to characterize a bioactive chemical is dissolving it in an appropriate solvent. Next, radiofrequency radiation is applied to the solution while it is submerged in a strong magnetic field. The radiation is absorbed by the compound's atoms' nuclei, which are then stimulated to greater energy levels. Radio frequency radiation is released when the radiation is switched off and the nuclei relax back to their ground states. There is no difference in the frequency of radiation absorbed and radiation released (Santos and Silva. 2014). The most adopted type of NMR spectroscopy is 1H-NMR spectroscopy. The type, quantity, and chemical environment of hydrogen atoms in a substance may all be determined using 1H-NMR spectroscopy. Many NMR spectroscopy methods are available to characterize the bioactive substances, such as: Chemical shift: The electron density surrounding an atom in hydrogen is measured by the atom's chemical shift. The NMR spectrum makes it possible to discriminate between different kinds of hydrogen atoms due to their distinct chemical shifts; Coupling constants: A measure of the interaction between two hydrogen atoms is known as the coupling constant. Coupling constants can provide details about a compound's bonding structure; Integration values: An NMR signal's integration value is directly correlated with the quantity of hydrogen atoms that contributed to the signal's formation. It is possible to ascertain the compound's stoichiometry using this information (Jain et al. 2023).

10.Conclusion

The valorization of pineapple waste into value-added bioactive components offers a promising way to reduce waste and promote sustainable agriculture. Significant advancements in this field have been made recently, demonstrating the possibility of converting waste into useful components. This review has highlighted some of the finding recent advances in this area. Various techniques are used to extract bioactive components. Researchers are focusing on sustainable and environmental friendly approaches to convert waste into useful products. As research in this field progresses, It is evident that valorization of of pineapple waste offers numerous economic environmental, and health benefits. However many challenges are there in improving the extraction processes, scaling up of production and making it economically viable. With continued research and investment, we can expect new advances in the valorization of pineapple waste, which will promote a more

sustainable and resource efficient agro-industrial sector.

11. References

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