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Comprehensive study of different extraction methods of extracting bioactive compounds from pineapple waste - A review

Ravindra R Salve and Subhajit Ray

Abstract
Pineapple (Ananas Comosus) is the most important and delicious fruit among other fruits cultivated worldwide and India is one of the leading producer of pineapple as well as different processed product e.g. juice, jam, jelly, marmalade etc. Now during processing a huge quantity of pineapple waste has been generated every year. Pineapple waste consisting of Crown, Peel, stem and Core contributes more than fifty percent waste of the whole fruit. Interestingly waste fraction contains a significant quantity of valuable nutrients and bioactive substances. Though conventionally pineapple waste management deals with dumping or burning in the field, cattle feed or conversion of biogas but exploitation of high-value nutritional and bioactive components are essential for effective waste utilization. Therefore both the conventional e.g. soxhlet extraction, maceration, hydrodistillation etc. and nonconventional methods e.g. ultrasound assisted extraction, pulsed electric field extraction, enzyme assisted extraction, microwave assisted extraction, pressurized liquid extraction, and supercritical fluid extraction (SCFE) are employed to extract bioactive compounds like anthocyanin, polyphenols, flavonoids, flavones, terpenoids, alkaloids, bromelain, pectin etc. and nutritional components e.g. protein, some insoluble fibres, carbohydrate and micronutrients e.g. Vitamin and minerals e.g. Fe, Ca, Mn, Zn, Cu, Cd, and Na from pineapple waste. Nowadays non-conventional extraction techniques also termed as green extraction technology has gained significant importance over the conventional techniques due to requirement of less time, utilisation of less solvent, reduction of the consumed energy and subsequent purification steps. Moreover extracted bioactive compounds from pineapple waste can be utilized to develop functional food and nutraceuticals

Keywords: Pineapple waste, bioactive compounds, extraction, conventional methods, non conventional techniques

1. Introduction
Fruits contain all types of essential supplements which are required to maintain good health, most of the nutritional values of fruits are still not utilizing properly and while consuming and processing such fruits nutritional value will be lost as a generation of waste. One such important fruit is pineapple, Pineapple fruit is delicious to eat as a whole or else preparing juices, jams, and making dry snakes by reserves. Botanical name of Pineapple is Ananas comosus and Pineapple belongs to Bromeliaceae family, it's a perennial plant of tropical fruit and only fruit of bromeliaceae family which can be edible or consume directly. Majorly four varieties of Pineapple available worldwide: Smooth Cayenne, Red Spanish, Queen, and Abacaxi (Shweta Saloni *et al*. 2017) [68]. All four varieties have distinguished characteristics, Smooth Cayenne variety has a smooth external appearance and most suitable variety for canning. Red Spanish is purple-tinged fruits and leaves. Queen pineapple has a rough, compact, and dwarfish appearance. Lastly, Abacaxi has long fruits with while translucent and juicy flesh, Abacaxi pineapple is the most delicious variety but is not appropriate for canning because of its fragility.Costa Rica (3056445 tonnes), Philippines (2671711 tonnes), Brazil (2253897 tonnes), China (2129936 tonnes), Thailand (2123177 tonnes), and India (1861000 tonnes) are the major pineapple producing countries in the world. The major state producing pineapple in India are West Bengal, Assam, Karnataka, and Meghalaya. However, India is one of the leading country to produce the Pineapple and consequently, leading county to produce pineapple waste by increasing the production of Pineapple processed products industries. In India, apart from pineapple processed industries from rough handling of pineapple at various levels from farm to industry and exposure to adverse environmental conditions during transportation and storage can cause up to 55% of product waste (i.e. pineapple waste) (Nunes

et al., 2009) [51]. These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further wastes can be sorted into two classes: POFW (Pineapple on Farm Waste) and PPW (Pineapple Peel Waste). POFW consists of leaves, stem, and roots leftovers in the farm once pineapple harvesting is done. In the pineapple product industry, Pineapple processed in many ways like slicing, pulping, and juicing. Before, processing pineapple product, pineapple underwent various steps are removal of crown, thereafter removal of peels and core. Every step generates a different volume of PPW. Pineapple juice making industry produces liquid wastes and pomace followed with peels, crown, and core. Reaming flash produce 30% approx Pomace and juice or pulp recovery is considered to be 70% (Sreenath, Sudarshanakrishna, & Santhanam, 1994) [72]. Researcher finds out that Pineapple produced 9.12% of a core, 13.48% of peels, 14.49% of pulp, 14.87% of the top, and 48.04% of finished products (J.F. Ayala-Zavala, *et al*, 2010) [30]. Therefore, as a whole pineapple producing or processing residuals would be 45% and 65%. It's directly representing the huge challenge of disposal of pineapple waste, which will further cause environmental pollution. If, not utilized successfully. Studies also have shown the comparison of some physicochemical constituents present in pineapple pulp and pineapple waste. (R. Hemalatha and S. Anbuselvi, 2013) [60].

Table 1: Physical and chemical constituents of pineapple pulp and waste

S.No	Parameters	Pineapple pulp	Pineapple waste
01	Ascorbic acid (mg/100g)	21.5	26.5
02	Ash content (mg/100g)	1.8	0.04
03	Crudefibre(g/100g-fw)	0.41	0.60
04	Moisture (%)	87.3	91.35
05	Non reducing sugars (%)	7.4	8.8
06	Protein(mg/100g)	7.2	10
07	Reducing sugars (%)	10.5	8.2
08	Titratable acidity (%)	2.03	1.86
09	Total soluable solids (%)	13.3	10.2
10	Total sugars (%)	8.66	9.75

Adapted from - R. Hemalatha and S. Anbuselvi, 2013 [60].

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Several studies have been carried out since decades to utilized the pineapple waste and trying to explore the new possibility of using these wastes because pineapple wastes can be utilized for extraction of bromelain (enzymes have lots of therapeutic importance), preparation of ethanol, extraction of phenolic antioxidants, preparation of organic acids (Citric acid, lactic acid, and ferulic acid), used as an energy and carbon source, and anti-dyeing agent, (Atul Upadhyay *et al* 2010) [5] Similarly, studies have also confirmed that bioactive compounds like antioxidants can present higher in fruit waste or residues of waste than the pulp of the fruit (Gorinstein *et al*., 2001) [23]. An antioxidant is a polyphenolic compound that has a property to inhibit the oxidation process in human beings and prevent the oxidative stress and free radical generated in the human body for various reasons (Diaz *et al*., 1997) [17]. Fruits and fruits generated waste contains an ample amount of ascorbic acid, phenolics, and flavonoids compounds of Antioxidant. Which usually discarded as fruits wastes, these bioactive compounds could be used as an alternative or important source of nutrients to enhance the nutritive value of under nutritive foods or may be useful to help to improve diets of economically poor peoples. Polyphenolic compounds considered as an antioxidant, anti-carcinogenic, anti-microbial, anti-allergic, anti-mutagenic, and anti-inflammatory, also minimize cardiovascular diseases (Kima *et al*., 2003) [34]. Flavonoids are the polyphenolic compound, responsible for the coloring pigments in fruits and plants and also act as antioxidants at different levels. Studies also showed that flavonoids also protect membrane lipids from oxidation. (Terao *et al*., 1994) [77].Pineapple wastes generated at different levels are one of the most valuable and economical, suitable and easily available raw material from which phenolic compounds, bromelain, fiber, and organic acid can be derived (Larrauri *et al*., 1997; Tanaka *et al*., 1999; Kumar *et al*., 2003; Imandi *et al*., 2008) [38, 75, 36, 28]. Apart from the bioactive compounds pineapple waste can be used as a fermentable substrate. Since, pineapple waste also having a recognized amount of carbon which can be the source for acid fermentation (Abdullah & Hanafi, 2008) [1]. Moreover, bioactive compounds present in different fruit processing were identified as an essential source for food and other industries to offer or create novel, alternative and innovative food products for enrichment nutrition and health enhancement (Cheok *et al*., 2018) [11]. These bioactive compounds identified or utilized by using extracting methods.

Utilization of Pineapple Waste

The major challenge for a farmer or the fruit process industries from a very long time is to proper disposal of processed waste and on-farm pineapple waste. However, farmers usually prefer to dump, burn or use as animal feed, and use as compost. Some farmer produces biogas or bioethanol since pineapple waste content prominent amount of carbohydrate. And it gives a much better yield comparison to cow dung (0.2–0.5m^3 per kg of dry matter) (Tauseef, Premalatha, Abbasi, & Abbasi, 2013) [76]. Again, the digested leftover slurry of biogas will be utilized as a useful soil conditioner. Importantly, pineapple waste consists of a crown, core, and peels. Therefore, each portion of waste is disposed of differently and the majority of the portion of pineapple solid waste is generated from the crown of pineapple, it could be approx. 25-30%. Reused or replanted of Crown usually takes a long time to bear fruit, thus farmer avoids or occasionally reused or replanted for the next corp. The core of pineapple contributes 15% of total waste and core is not specifically discarded it can be a dump or used for compost. But the core is a rich source of one of the most important enzymes. i.e. bromelain. (Tochi, Wang, Xu, & Zhang, 2008) [78, 87]. Whereas, pineapple peels are only utilized for the cattle feed (Tran, 2006) [80]. It also provides supplementary fiber to feed dairy animals (Sruamisri, 2007) [73]. In the conventional approach, pineapple waste was not utilized properly because in this process all-important and valuable bioactive compounds were lost and the scope of utilization of bioactive compounds are restricted. Due to inappropriate waste management, valuable Bioactive components such as Bromelain, Carbohydrate, and polyphenols which are available as a suitable substrate for the extraction were fully un-utilized. (Arianna Roda & Milena Lambri 2019) [4] studies showed that these un-utilized pineapple wastes also contain some main compounds like pectin, proteins, some insoluble fibers, and simple sugars, it has also contained some micronutrients like vitamin, minerals phenolic compounds (Abdullah & Hanafi, 2008) [1]. Pineapple wastes compositional analysis is shown in Table 2. The total protein present is nearly about 5%, and total polyphenols are 4% (Sepulveda *et al*., 2018) [64]. Microelements like Ferrous (Fe), Calcium (Ca), Magnesium (Mn), Zinc (Zn), Copper (Cu),

Cadmium (Cd), and Sodium (Na) are found in very small amounts and High Potassium (Na) found in the pineapple waste. The concentration of ion chlorine is greater than sulphate and nitrate, but the phosphate ion is null (Hemalatha & Anbuselvi, 2013) [60]. On liquid waste analysis, it is reported that the main sugars present in the wastes are sucrose, glucose, and fructose. The concentration of fructose and glucose are the same. (Abdullah & Hanafi, 2008) [1]. The sugars composition of pineapple waste reported 82%. Where reducing sugar is 55% and non-reducing sugar is 27%

Table 2: Chemical composition of the different parts of pineapple waste

	Ensiled	Fresh	Dry	Peel	Whole	Skin	Crown	Pulp
Moisture %	72.49	71.07	27.43	92.20	-	-	-	-
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
As % dry basis								
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	22.40	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

Adopted from Arianna Roda & Milena Lambri 2019 [4]

Therefore, it is directly indicated that improper utilisations or un-utilization is mainly responsible for the loosing valuable nutritional bioactive compounds along with the chemical composition present in it. However, proper procedure requires establishing to utilize such a bioactive compound. Extraction is one of the most effective methods to handled and utilize pineapple waste. Pineapple waste produce at a different level while processing or handling the fruit can be further utilized as a very economical source of a bioactive compound that also contain chemical substance will be extracted by various methods *viz*. Conventional Methods and Non-conventional Methods.

Extraction Techniques

Extraction is a process, which involved in separating desirous solutes by using specific solvents with the help of following standard operating procedures. (Handa *et al*., 2008) [24]. The intension or role of the extraction method is to separate the soluble solutes from the sample by using the effective extraction process or procedure. The initial raw/crude extracts recovered by using these processes which also contain complex mixtures of different metabolites namely phenolics, alkaloids, glycosides, flavonoids, and terpenoids. There are several extraction methods i.e Conventional and Non - Conventional extraction is available to efficiently extract bioactive compounds from the sample or plant by-products.

Conventional extraction techniques

This technique is an ancient extraction technique mostly used in small scale level to extract solute/bioactive compounds from the sample. Conventional techniques are mainly dependent on the extraction efficiency of different solvents. These Extraction further techniques further classified into 1. Soxhlet Extraction, 2. Maceration, and 3. Hydrodistillation (HD).

Soxhlet Extraction

It is one of the most important and extensively used techniques at the primary level to extract solute / bioactive compounds from various sample i.e plant and fruit materials. Soxhlet extraction is one of the first known extraction technique which was used for the extraction and also called as solid-liquid extraction technique (Luque de Castro MD, García-Ayuso LE, 1998) [41] The Soxhlet is an apparatus and the process or procedure of Soxhlet technique is very simple, a known amount of sample which need to be treated (i.e plant/fruit) require to be placed in the thimble. Later on, the thimble is placed properly in the distillation flask, distillation flask filled with suitable or selective solvent, and when overflow mark level of solvent is filled; the solution of the thimble-holder is aspirate by a siphon. Siphon flow the solution return back into the distillation flask. However, this solution carries extracted solutes from the sample into the bulk liquid, and the solute remains in the distillation flask and the solvent again passes back to the solid bed of the plant. Perform this process repeatedly until the extraction is completed. Literature provided a huge amount of practical examples for the optimization of the extraction system and suitable conditions. (Cravotto *et al*., 2011; Xhaxhiu, Korpa, Mele, & Kota, 2013) [14, 88]. One of the disadvantages of this technique requires a more extensive-time period and large amounts of solvent. (Heleno *et al*., 2016) [26].

Maceration

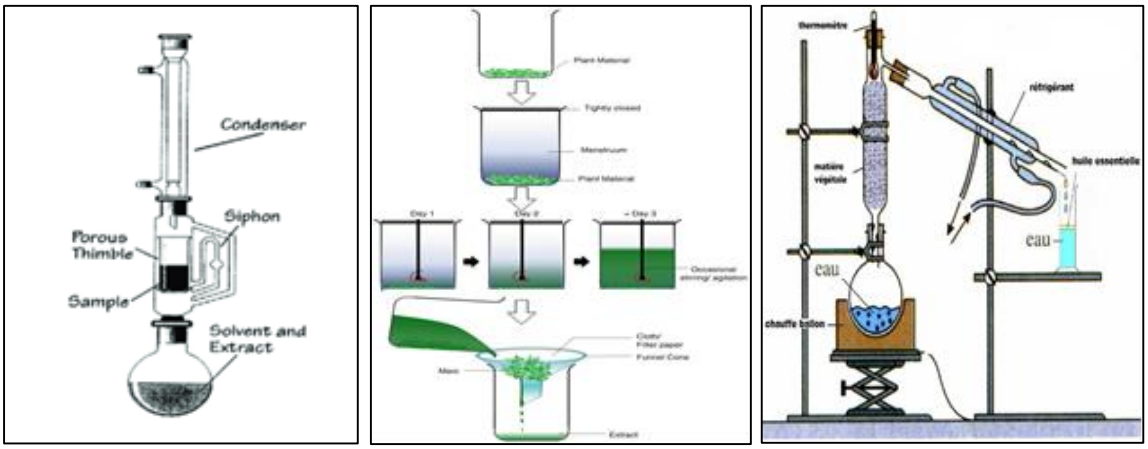
The maceration process involves grinding the sample (i.e.plant / fruit) and makes possibly smaller particles so that it can increase the surface area of the sample along with the suitable solvent. This technique becomes popular and the cheapest way to obtain essential oils and bioactive compounds. The principle of the maceration technique makes extraction simple in two different ways: 1 it increases the diffusion and 2. It removes the concentrated solution from the surface of the sample. (Azmir *et al*., 2013) [6]. The maceration process performed in a different stage at a small scale extraction level as follows:- 1. Material needs to grind properly so that the sample size is reduced and it will enhance the surface area with a suitable solvent. 2. The ground sample and suitable solvent called menstruum and menstruum is placed in a closed sealed vessel and leave it for the stipulated time. 3. One the stipulated time is over, the solution is strained out. Further, the obtained strained solution and the left press out liquid are mixed, impurities will be filtrated by filtration.

Hydrodistillation

Hydrodistillation is a common and traditional method to obtain several bioactive compounds and essential oils from different plant samples. In this method, organic solvents are not required. Hydrodistillation is performed only with distilled water and it usually takes 6–8 hrs to complete the extraction, The hydrodistillation work on three different types of principles i.e water distillation, water, and steam distillation, and direct steam distillation (Vankar, 2004) [82]. The principle of hydrodistillation is 1. Sample packed or placed in still compartment 2. A sufficient amount of water will be added and then water is allowed to boil. Additionally,

steam can be injected directly into the plant sample. Both boil water and steam act together which is the main influencing factor for the release of bioactive compounds from the sample. 3. Now, a Condensed mixture of water and sample flows from condenser to a separator, in this process oil and bioactive compounds automatically separated from the water (Silva *et al*., 2005) [70].

Hydrodistillation has undergone mainly three physicochemical properties: 1. hydro diffusion, 2. hydrolysis, and 3. Decomposition by heat. During hydrodistillation there is a possibility that some volatile compounds may be lost because of higher extraction temperature, some of the volatile components will be lost. However, the disadvantage of hydrodistillation is that it consumes high levels of energy and time. (M. Selvamuthukumaran *et al*. 2017) [44].



(A) (B) (C)

Fig 1: Conventional Techniques: (A) Soxhlet Extraction, (B) Maceration, (C) Hydrodistillation

The quality and extraction yield mainly depends on the solvent which has been selected for any conventional method. The selectivity of the right solvent is very important to get better extraction (Cowan, 1999) [13]. Table 3 represents a certain example of selective solvent and different bioactive components that have been extracted by using these solvents.

Table 3: Example of some extracted bioactive compounds by different solvents

Water (1.000)	Methanol (0.762)	Ethanol (0.654)	Acetone (0.355)	Chloroform (0.259)	Ether (0.117)
Anthocyanins	Anthocyanin	Alkaloids	Flavonoids	Anthocyanin	Alkaloids
Saponins	Saponins	Flavonol		Flavonoids	Terpenoids
Tannins	Terpenoids	Polyphenols		Flavones	
Terpenoids		Terpenoids		Polyphenols	
				Terpenoids	
				Tannins	

Source: Adapted from Cowan (1999) [13].

Non-conventional extraction techniques

As studies indicated that the conventional extractions methods having lots of limitations and disadvantages such as require long extraction time, a requirement of expensive and good quality of solvent, a huge solvent is evaporated during the extraction, the selectivity of extraction is low, and decomposition of thermolabile components (Luquede- Castro and Garcia-Ayuso, 1998) [41]. New and emerging extraction techniques are now coming up to replace or to minimize these limitations of conventional extraction methods. And these techniques are called as non-conventional extraction techniques, and some of the important non-conventional extraction techniques are Ultrasound-Assisted Extraction (UAE) technique, Pulsed Electric Field (PEF) Extraction technique, Enzyme-Assisted Extraction (EAE) technique, Microwave-Assisted Extraction (MAE) technique, Pressurized Liquid Extraction (PLE) technique, and Supercritical Fluid Extraction (SFE) technique. Few among these techniques are referred to as 'green techniques' as they fulfil the standards set by the Environmental Protection Agency (2015) [18].

Ultrasound-assisted extraction

Ultrasonic-assisted extraction (UAE) is the combination of the selective solvents and disturbance of energy which passes through matter called acoustic energy to extract desirous extraction from the various samples (i.e. plant/fruit) (Wang *et al*. 2015) [86]. Ultrasound is a sound wave, its frequency ranges from 20 kHz to 100 Mhz, and beyond the hearing capacity of human being, it has generated compression and expansion when it passed through a medium. However, when a sample containing solvent exposed to an ultrasound wave it breaks down the cell wall due to this effect, cell hydration degree will increase and enhance the swelling of cell and these combine impact help to improve the diffusion and mass transfer (Vinatoru M, 2001) [83]. This phenomenon referred to as cavitation. And cavitation again helps to generate or develop and collapse of bubbles, in another way the process from which vapour bubble develops, rapidly grown and then collapse also known as cavitation. These bubbles attain temperature up to 5000K, the pressure is around 1000 atmosphere, and heat exchange i.e heating and cooling rate is more than 1010 K/s.

(Suslick and Doktycz (1990) [74], UAE technique is completely based on this principle. The cavitation effect applies only to liquid and liquid containing solid materials. Moreover, it is a combined effect of pressure, heat, and turbulence which help to increase mass transfer in the extraction process (Patist and Bates, 2008) [53]. The principle of extraction in the ultrasound process is based on two important physical phenomena: 1. diffusion across the cell wall and 2. Increase the contents of a cell once the cell was broken (Mason *et al.*, 1996) [45]. Ultrasound device should be placed in an appropriate position because it helps to give a better yield of the extraction and accelerate the extraction in a solvent extraction unit, (Vinatoru *et al.*, 1998) [84]. The advantages of UAE are it will be reduced extraction time, energy, and solvent.

Process system

Sonotrode (a tool that creates Ultrasonic vibration) and a glass reaction tank are required to perform the UAE experiment (Hielsher, 2013). For controlling the other parameter like extraction temperature can be handled with a cooling system that is maintained by using water circulation and is controlled by the double-layered mantle of the reactor. The Ultrasound transducer is connected to sonotrode, which is placed into the middle of the liquid, and samples need to be filled in the glass reaction tank. With the help of the apparatus which is made up of a circulatory pump for the continuous UAE (Del-Valle *et al.*, 2005) [16].

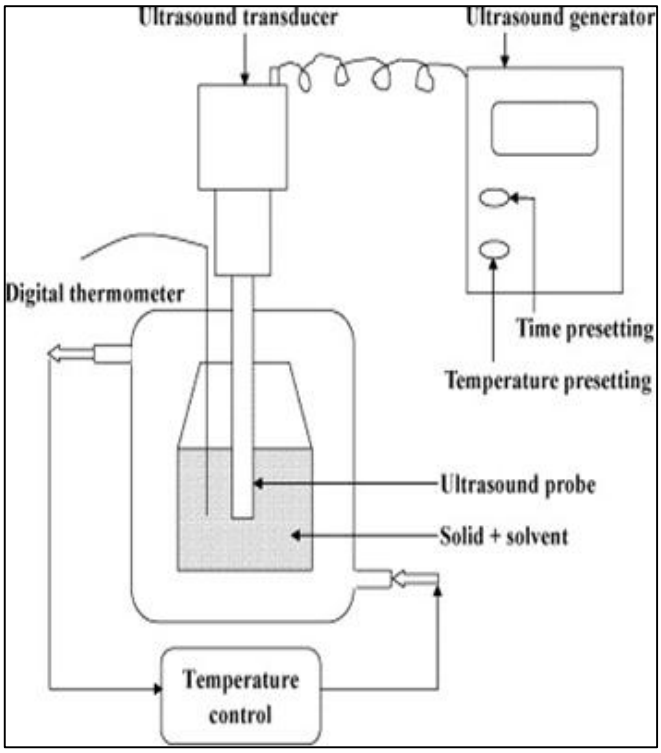


Fig 2: Ultrasound-assisted

Factors Influencing UAE Efficiency

Operational Temperature and frequency

The extraction temperature will influence the UAE efficiency. It has been observed that temperature change adversely affects on the extraction time and these will impact on extract recovery, therefore temperature must be regulated cautiously during the optimization of an experiment. (Wang and Weller 2006) [85]. Sometimes, the nature of sample i.e porous nature of plant matrices will be affected by the frequency set for the sonication, because the correlation of lower frequency will increase the cavitation during the experiments.

Pulsed electric field extraction

Pulsed electric field extraction (PEF) technique is emerging, low energy consuming, non-thermal technology that can be performed in both organic and aqueous media to extract bioactive compounds. The basic principle of the technology is to apply electroporation (the electric field is applied) on the cell membrane, to enhance the extraction rate. Bioactive compounds will be extracted according to their charge present on the biomolecules when electric charge passes through the cell membrane, it has also created repulsion effect due to different charge molecules present in a sample, repulsion effect also creates pores and will enhance the permeability, (Azmir *et al.*, 2013; Rajha *et al.*, 2015) [6, 61]. PEF treatment chamber consists of two electric electrodes, and the sample was placed into this electric chamber, based on the design of the electric chamber mode of operation i.e. whether continuous or batch extraction process will be decided. (Puertolas *et al.*, 2010) [58]. Because, the PEF target to increase the cell permeability by the distraction of cell membrane structure and help to enhance mass transfer during the extraction process, this effect also helps to reduce the extraction time. (Toepfl *et al.*, 2006) [79]. Moderate electric field range from 500 and 1000 V/cm is used for conduction of PEF extraction (Fincan and Dejmek, 2002) [20].

Process system

The PEF system is assembled with the high current generator, controlling equipment, fluid handling system, and treatment unit. The generator along with the pulse forming systems mainly responsible for producing electrical pulses of voltage, shapes, and application time. Systems consist of a switch that is mainly used for the discharge of high energy through the sample in the treatment chamber. The switch performs an important role in PEF because switch help to decide the require pulse current and application time, basically switch acts as a bridge between high-energy suppliers and treatment unit (Mohammed and Ayman, 2012; Vallverdu-Queralt *et al.*, 2013) [46, 81]. Studies have shown that many different kinds of waveforms used in the PEF technique, either square wave or exponentially wave pulse shape are being used. Again treatment units designed as per the requirement of operation whether i.e batch or continuous manner. The assembly of the PEF process shown in Figure 3.

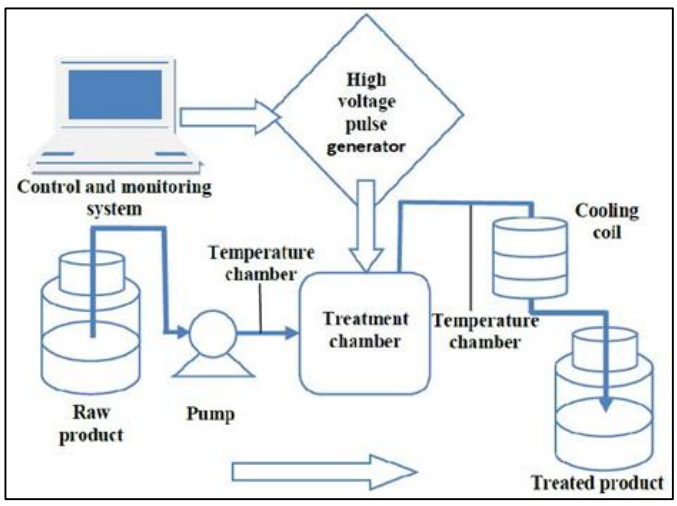


Fig 3: Pulsed electric field extraction

Factors Influencing PEF Efficiency

Electric Field Strength

Electric field strength is one of the factor influence the efficiency of PEF is a breakdown of a dielectric electrode, because the presence of bubbles in the chamber may responsible for the decreasing uniformity of the electric field strength. (Góngora-Nieto *et al*. 2003) [22]. The electric field strength also leads to the destruction of cell walls therefore impaired electric field strength will not be able to destroy the cell wall which directly affected the extraction yield.

Enzyme-assisted extraction

Enzymatic extraction is very useful in different technologies, an enzyme also useful in the extraction of some special bioactive compound by hydrolyzation of certain polysaccharides and lipids compound present in the cell wall, therefore, it has been considered as a novel technology which recover surface-bound compounds and help to enhance their overall yield (Rosenthal and others 1996) [63].

Extraction Process

Based on the extraction process or approach enzyme assisted extraction mainly divided into two classes 1. Enzyme-assisted cold pressing (EACP) and 2. Enzyme-assisted aqueous extraction (EAAE) (Latif and Anwar 2009) [39]. EACP technique mainly hydrolyzes the seed cell wall, since in the technique polysaccharide-protein colloid is missing (Concha *et al.*, 2004) [12]. Wherein, EAAE techniques have been formulated for the extraction of oils from different seeds (Hanmoungjai and others 2001; Sharma and others 2002) [25, 65]. The main extraction factor depends upon the catalyst type, the molecular size of sample materials, water proportion present in it, and the time taken to hydrolysis which again completely depends on the moister contain present in the sample (Niranjan and Hanmoungjai 2004) [50]. In the enzyme assisted extraction, the critical step is to the breakdown of the cell wall and extract the bioactive compounds present in it. And the enzyme assisted extraction technique mainly depends on the enzyme's ability to hydrolyze the cell wall disrupt the structural integrity that allows the better extraction and release of bioactive compounds (Pinelo *et al.*, 2006; Gardossi *et al.*, 2010) [56,, 21]. As usual catalysis of the enzyme directly proportionate to the kinetic rate of the enzyme and the substrate concentration (Sowbhagya and Chitra, 2010) [71] apart from all these enzymatic reactions depends on several other parameter temperatures of reaction, time of extraction, pH of the system, enzyme concentration, and particle size of the substrate. Bhattacharjee *et al.* (2006) [7] and the optimization of these parameters will accelerate the extraction. EAE is referred to as an eco-friendly technique because only water is used as the solvent in place of organic solvents to extract oil and bioactive components (Puri and others 2012) [59].

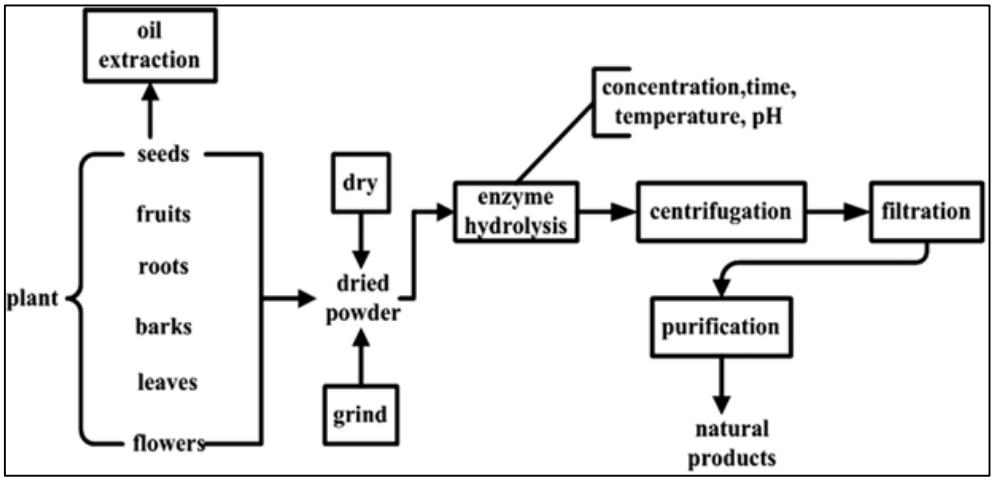


Fig 4: Enzyme-assisted extraction

Factors Influencing EAE Efficiency

Optimal Requirement (Time, Temperature, pH, enzyme Concentration, etc)

As per the literature review, the optimum time for suitable extraction time for the EAE is 40 min. However, some physical parameter influences the efficiency of extraction. Therefore, before enzymes are employed for extraction the optimal working environment for the enzyme should be optimized, the physical parameter mainly is pH, temperature, and enzyme concentration they should be optimized to get better yield in any enzymatic reaction. Other factors affecting conventional extraction (that is, agitation, solid/liquid ratio, and particle size) also perform an important role in enzymatic extraction (Camila A. Perussello, 2017) [8].

Microwave-assisted extraction

Microwave-assisted extraction (MAE), developed in the 1980s, now this technique considered as one of the best novel technique to extract the soluble from the sample by using microwave energy, is a process which involves the heating of solvent by in contact with sample with microwave (Pare *et al.*, 1994) [52]. Microwaves are formed from two oscillating fields and they are perpendicular, like electric field and magnetic field and commonly called as electromagnetic fields and frequency range from 300 MHz to 300 GHz. (Letellier and Budzinski, 1999) [40] The principle behind the extraction is to produce heat using a microwave that is electromagnetic energy is converted to heat followed by ionic conduction and dipole rotation of molecule (Jain, 2009) [31]. In the process of ionic conduction mechanism, heat is generated due to resistance from the medium to restrict the flow of ion. On the other way, ion keeps their direction of flow on frequently changing signs field, this change of direction of signs field results in a collision among the molecule, and heat is generated. The optimal heating completely depends upon the dielectric constant of the solvent. Lager, the dielectric

constant generates more heat. (Kaufmann and Christen, 2002) [33]. The advantage of microwave in the extraction process is that microwave generates heat and this heating effect target or interruption the weak hydrogen bonds present in the sample and it promotes dipole rotation in the molecule. This molecule rotation is depending on the viscosity of the medium, Higher viscosity of medium lower the rotation of the molecule. (Kaufmann and Christen, 2002) [33]. MAE mechanism is involved in three sequential steps suggested by Alupului *et al.* (2012) [3]: 1. solutes separation from active sites of sample matrix under increased temperature and pressure; 2. solvent diffusion across sample matrix 3. Solutes release from sample matrix to solvent. Certain advantages of MAE have been suggested by Cravottoa *et al.* (2008) [15] it is very fast to generate heat for the extraction of bioactive components from the sample, decreased thermal gradients; equipment size has been reduced; and finally more important it increased extract yield. Hence, MAE is quick and more rapidly to in terms of extraction of bioactive components, comparison to conventional extraction process MAE has a best possible or better recovery of bioactive compounds, However, MAE also consider as a best green technology because it reduces the use of an organic solvent (Alupului *et al.*, 2012) [3].

Process system

The MAE systems are two types of multi-mode system and focused-mode system also called mono-mode. In a multi-mode system, it will allow random scattering of microwave radiation in the cavity. Whereas, focused system (mono-mode) allows focused microwave radiation on a particular zone in the cavity. Importantly, the multi-mode system is allied with high pressure (HP), whether, the mono-mode system is performing under or below atmospheric operating pressure. To take that advantage, the mono mode system will also perform at high pressure and low atmospheric pressure. To simplify to this phenomenon both the types are classified as 'Closed system' that perform above atmospheric pressure and 'Open System' that perform under atmospheric pressure (Dean and Xiong,2000; Garcia and Castro, 2003). The schematic diagrams of both the system shown in Fig 5a and 5b

Closed MAE system

To performing closed system extraction, there has certain or specific requirement 1. extraction has to be carried out in a sealed vessel in the presence of different modes of microwave radiation, 2. Extraction generally performs under uniform microwave heating. 3. Better and fast extraction require High working pressure and temperature 4. pressure inside the extraction vessel should be controlled in such a way that it should not exceed the working pressure of the vessel, 5. temperature always regulate or maintain above the normal boiling point of the extraction solvent. Both the factors that are increased in temperature and pressure and solvent that absorb the microwave energy is responsible for the good extraction (Wang *et al.*, 2008) [87]. Apart from all advantages that closed system provides fast and efficient extraction with less solvent consumption, but this system has susceptible to losses of volatile and thermolabile compounds.

Open system

Open system is not a complex system like a closed system and open system counter all the shortcomings of the closed system mainly the safety issues, it is more suitable to extract thermolabile compounds also. During the operation of an open system, more solvent can be added at any time and it has higher sample throughput. Since an open system can be operated out in more mild and safe conditions. This system widely used in analytical chemistry to extract active compounds. This system operates at standard atmospheric conditions, and only part of the vessel is directly exposed to the propagation of microwave radiation (mono mode).

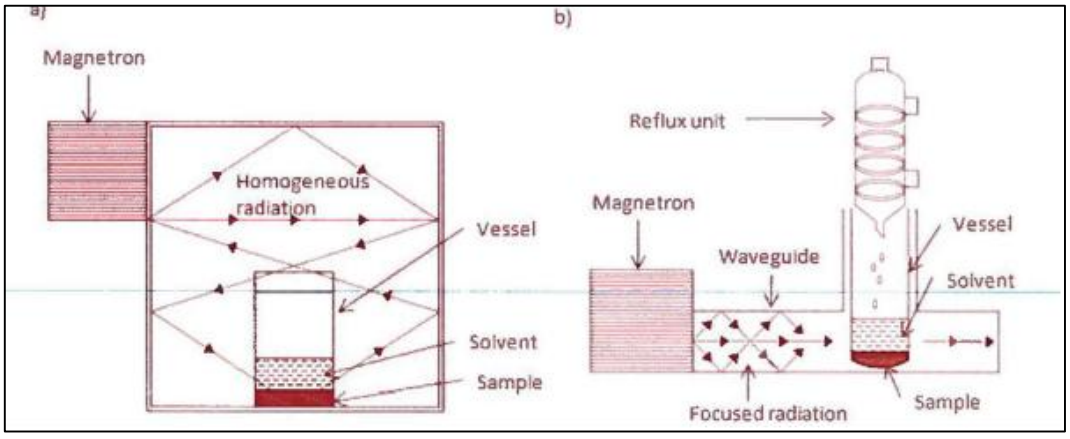


Fig 5(a): Closed type microwave system and **(b)** open type microwave system (modified from Mandal *et al.*, 2007).

Factors Influencing MAE Efficiency

Microwave power

High microwave power can lead to poor or low recovery of compounds because of all thermolabile components may get degraded.

Extraction time

Extraction time also important and influencing factor of MAE along with the power and temperature, when microwave radiation exposes for a longer time of period it will decrease overall extraction yield because long exposure of microwave leads to disrupt of the structural integrity of chemically active compound present in the sample.

Features of the sample matrix

The desired sample must be in particular forms because very small-sized sample particles may difficult to separate in the form of extraction and require additional cleaning step after extraction. (Chan and others 2014) [10]. Moreover, pre-treated powdered samples require 90 min. before extraction with extractant for better MAE efficiency, Similarly, the dried sample matrix is required pre-treatment with water to

accelerate the heating effect of microwaves.

Stirring effect

Concentrated bioactive compounds like polyphenols attached or bound as the active compound in the sample which may create a barrier for mass transfer in the case of depletion of extractant, stirring process help to minimize this barrier effect.

Effect of additives and solvent choice

To get a desirable impact on the efficiency of extraction, Binary solutions of organic solvents with water have played an important role, it is also noted in binary solution (presence of water) has a property to enhance penetration in the sample matrix rather simple solvent. And this property promotes microwave heating which positively enhances the efficiency of extraction (Alfaro and others 2003; Wang and Weller 2006) [2]. Another important factor is solvent toxicity, solvent toxicity must evaluate while selective suitable solvent for extraction.

Pressurized liquid extraction

This method is comparatively a new method, which will be carried out at high temperature (50 to 200°C) and pressure (3.5 to 20 MPa), these both the factor allow the enhance efficiency of extraction as compared to conventional methods which were carried out in room temperature and standard atmospheric pressure (Mustafa A, Turner C, 2011) [47]. this method firstly describes by Richter *et al.* (1996) [62]. PLE technique is now a very popular technique and knows as different names: pressurized fluid extraction, accelerated fluid extraction, enhanced solvent extraction, subcritical water extraction (SWE), and HP solvent extraction (Nieto *et al.*, 2010) [49].

The principle of PLE is to apply High Pressure to maintain or remain solvent liquid beyond their normal boiling point. This technique requires the least amounts of solvent because of the combined effect of High pressure and temperature accelerate extraction. High temperature plays an important role to promote solubility of analyte which not only increases the solubility but also enhances the mass transfer rate. And also helps to decrease the viscosity of solution and surface tension of solvents, these all affect directly Influence to improve extraction rate (Ibanez *et al.*, 2012) [27]. PLE was found to dramatically decrease extraction time and solvent in comparison to the traditional conventional soxhlet extraction technique (Richter *et al.*, 1996) [62]. PLE is a very useful technique to extract different molecules or compounds e.g. Polar compound (Kaufmann and Christen, 2002) [33], Highly temperature stable Organic pollutants from environmental (Wang and Weller, 2006) [85]. Bioactive compounds from marine sponges (Ibanez *et al.*, 2012) [27]. Applications of the PLE technique for obtaining natural bioactive compounds are commonly available in the literature (Kaufmann and Christen, 2002) [33]. Additionally, PLE performs in the least amount of organic solvent get the recognition as a green extraction technique (Ibanez *et al.*, 2012) [27].

Process system

The PLE set-up is shown in Figure 6. Extraction cell placed in electrical heating jacked at the desired temperature till the required pressure was not attained and the solvent was pumped into the extraction cell. The extraction cell is containing a sintered metal filter at the bottom and upper portion, and extraction sample ware placed in it. As the PLE process starts the cell-containing sample was heated, it will be filled with extraction solvent and later on the pressurized. Approx. 5 Min the sample was placed in a heating system to ensure the desired temperature will attain up the range of 313-393k during the solvent filling and pressurization procedure. Once pressurization is done, the sample with pressurized solvent was kept statically ideal at the desired pressure (5–10 MPa) for the desired time (3–15 min). After completion of PLE, the extracts were rapidly cool down to 5°C in ice water using amber flasks to prevent anthocyanin degradation.

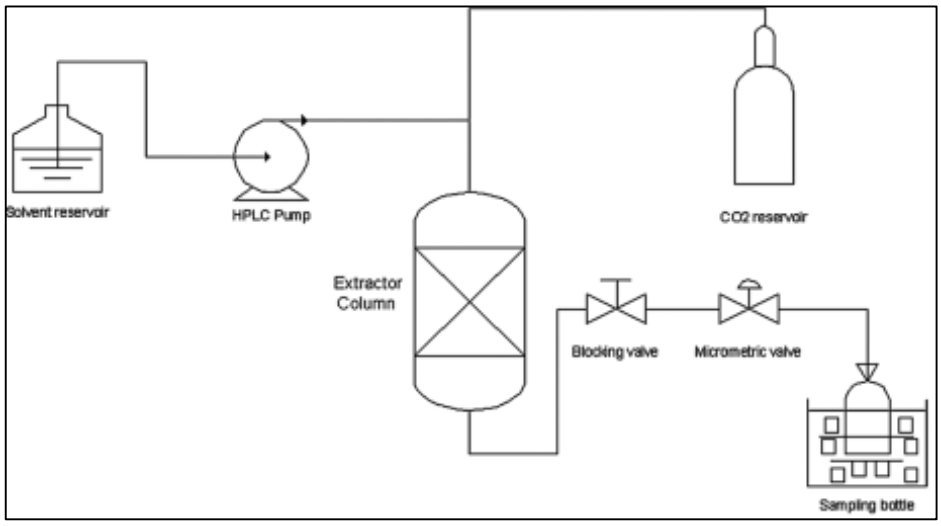


Fig 6: Pressurized liquid extraction set-up (modified from Santos *et al.*, 2012).

Factors Influencing PLE Efficiency

The selection of solvent and toxicity, matrix characteristics, and extraction temperature are the main factors that influence PLE extraction efficiency.

Solvent choice and toxicity

While selecting a suitable extractant, the choice is always preferred by famous notion "like dissolves like." Therefore, to achieve maximum extraction an appropriate solvent and analyte combination that can have a higher diffusion rate and have a property to increase mass transfer quickly is necessary. On the other hand safety of solvent and toxicity of solvent along with the economic aspect of the solvent must be taken into consideration (Pineiro and others 2004; Plaza and others

2013) [55, 57]. A non-harmful solvent which is having low toxicity and the quantity of solvent is required in a very low volume that solvent termed as "green" and best fitted and suitable for the extraction In PLE, various binary (mixture) solvents that methanol-water or ethanol-water are have been used for extracting (Luthria and others 2007; Mustafa and Turner 2011) [42, 47].

Matrix characteristics of the sample

The characteristic of sample i.e Molecule bonding behavior, moisture contents, and particle size are unique and different for every sample, therefore, the ability to mass transfer of sample which accelerate the extraction yield is different for every sample. (Carabias-Martınez and others 2005) [9].

Extraction temperature

Operational conditions such as temperature and pressure can influence PLE selectivity and efficiency, as elevated temperatures under the reduced pressure and use of thermal energy are allowed to disrupt the sample matric structure by overcoming the molecular bonding force (Luthria 2008) [43]. Elevated temperatures are responsible for minimizing surface tension among samples, solvent, and solute interfaces during PLE. However, reduction in surface tension is initiated the formation of solvent cavities and elevated temperature helps to reduce the viscosity of extractant, which leads to improved penetration inside the sample matrix (Wang and Weller 2006) [85].

Supercritical fluid extraction

The SFE technique is one of the most important and widely used technique, it has grabbed attention in scientific interest, it is mainly used in pharmaceutical, polymer, and food applications industries (Zougagh *et al*., 2004) [90]. Apart from this industry, decaffeinated coffee preparation industries using this technique for many years (Ndiomu and Simpson, 1988) [48]. The technique is based on the Supercritical state of liquid, the Supercritical is a typical state that can be only attained or possible when a substance is subjected to temperature and pressure beyond its critical point and can only be attained if a substance is subjected to temperature and pressure beyond its critical point. Further, Critical point is defined as the characteristic temperature (*T*c) and pressure (*P*c) above which distinctive gas and liquid phases do not exist (Inczedy *et al*., 1998) [29]. In a supercritical state, the change of the specific properties of gas and/or liquid, commonly referred is supercritical fluid cannot be liquefied by applying additional or modifying temperature and pressure. Supercritical fluid behaves or shows gas-like properties of diffusion, viscosity, and surface tension, and liquid-like density and solvation power. Therefore, these properties make it suitable for extracting compounds in a short time with higher yields (Sihvonen *et al.*, 1999) [69]. The SFE system comprises of the following components: A Mobile phase tank mainly CO2, a pump required to pressurize the gas, co-solvent vessel and pump, an oven that holds the extraction vessel, a controller to maintain the High Pressure inside the system, and a trapping vessel. Certain types of meters such as flow meter, dry/wet gas meter could be attached to the system. A systematic diagram of typical SFE instrumentation is shown in Figure 7. CO2 is considered as an ideal or perfect solvent for SFE because the critical temperature of CO2 (31°C) is near to room temperature, and the low critical pressure (74 bars) which offers the possibility to operate at moderate pressures, range between 100 and 450 bar (Temelli and Guclu-Ustundag, 2005). The disadvantage or drawback of CO2 is its low polarity, which makes it ideal for lipid, fat, and non-polar substance, but unsuitable for polar materials. Using chemical modifier this limitation of low polarity could be overcome successfully. (Lang and Wai, 2001) [37]. For example, 0.5 ml of dichloromethane (CH2Cl2) can enhance used as a chemical modifier and it will help in the extraction process (Hawthorne *et al.*, 1994). The other common variables also affecting the extraction efficiency that are temperature, pressure, particle size, and moisture content of the material, time of extraction, the flow rate of CO2, and solvent-to-feed ratio (Temelli and Guclu-Ustundag, 2005; Ibanez *et al.*, 2012) [27]. The advantages of using supercritical fluids for the extraction of bioactive compounds brief as: (Lang and Wai, 2001) [37]: 1. The supercritical fluid has scientific properties and they are higher diffusion coefficient and lower viscosity and lower surface tension than a liquid solvent, which allows more penetration to sample matrix and enhances mass transfer. As a result extraction time will be reduced drastically by SFE in comparison with conventional methods. 2. It gives complete extraction due to the repeated reflux of supercritical fluid to the sample. 3. The efficiency of supercritical fluid is higher than liquid solvent as liquid solvent efficiency can be altered by changing temperature or pressure. 4. It is a time saving method bypassed by the depressurization. 5. It is the most suitable technique for thermolabile compound extraction since it is operated at room temperature. 6. In SFE, the least amount of sample can be extracted in comparisons to solvent extraction methods where the requirement of the sample is more. 7. It importantly referred to as environment friendly because SFE does not use an organic solvent. 8. Generate minimum waste since the recycling and reuse of supercritical fluid is possible. 9. SFE provides a wide scale for different purposes from milligram samples in the laboratory to tons of samples in industries.

SC-CO_2 fluid technology Process system

The SC-CO_2 fluid extraction process is based on extraction, expansion, separation, and solvent conditioning. Further, this process requires four scientific primary components: extractor (High-Pressure vessel), pressure and temperature control system, separator, and pressure intensifier. SC-CO2 fluid technology process is semi-batch continuous processes where, raw sample materials are generally ground and charged into a temperature-controlled extractor forming a fixed bed, for a batch and single-stage model (Shi *et al*., 2007a, 2007c; Kassama *et al.*, 2008) [66, 67, 32]. As mention, the processes are semi-batch continuous processes where the SC-CO2 flows in a continuous mode, whereas, in batches, the extractable solid feed is charged into the extraction vessel. Therefore, multiple extraction vessels are sequentially used to enhance process performance and output at a commercial level. Although its continuous process and it is interrupted at the final stage of the extraction period, and again the same process is switched to another vessel which is prepared for extraction, and so on. The loading and/or unloading of the spent vessels can be carried out while extraction is in progress, it will reducing the downtime, and improving production efficiency. On a commercial scale, a semi-continuous approach is used where the multistage extraction process involves the running system by harnessing a series of extraction vessels. In this process, the system will not be interrupted at the final stage of the extraction, because the process automatically switched to the

next already prepared vessel by control valves. Having the advantages of this system the SC-CO2 technology can be convertible to a single-stage batch to multistage semi-continuous batch with the multi separation process. SC-CO2 fluid extraction could be cost-effective under large scale production.

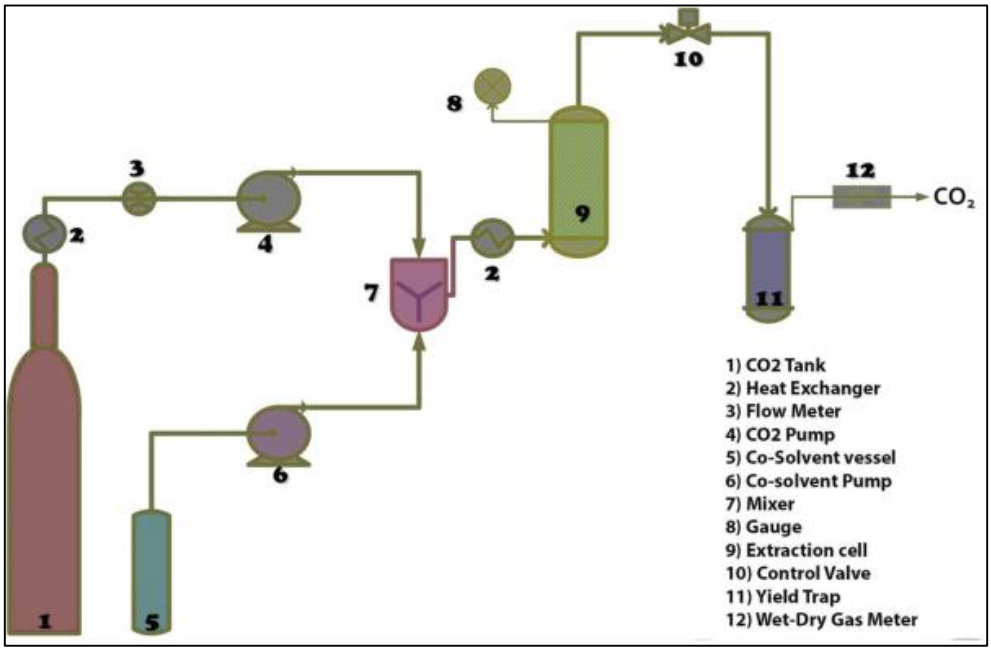


Fig 7: A symmetric diagram of SFE apparatus (modified from Yi *et al.*, 2009) [89].

Factors influencing SFE efficiency

Correct SC fluid selection.

For extraction of bioactive compounds (i.e polyphenol) through SFE the correct SC fluid selection is a very crucial factor. Because this class of bioactive compounds is thermolabile and because of this nature of water it cannot be a good choice as SC, although waster has high extraction yield if the compound is polar. Polyphenols are having a low degree of solubility in SC-CO2 and selection of CO2 usage alone unfavorable for such bioactive compounds (King 2014) [35]. Therefore, to overcome this problem, modifiers have been added to SC-CO2 to improve solubility and recovery rates of phenolic compounds. And some effective modifiers are Acetonitrile, acetone, methanol, ethyl ether, and ethanol, (Pereira and Meireles 2009; Azmir and others 2013) [54, 6]. Ethanol has been reported to be the best suitable modifier compared to others by virtue of its lower toxicity and enhanced extraction of polyphenols. As already mentioned that Water has very low solubility in SC-CO2 and is normally not used as a modifier alone. In order to increase water solubility, binary mixtures of ethanol and water are used (Wang and Weller 2006; King 2014) [83, 35]. There are certain other commonly used supercritical fluids, mentioned in table 4. which can be used in SFE as per there critical properties.

Table 4: Critical properties of commonly used supercritical fluids

Fluid	Molecular weight (g/mol)	Critical temperature (K)	Critical pressure (MPa)
Carbon dioxide	44.01	304.1	7.38
Water	18.02	647.3	22.12
Methane	16.04	190.4	4.60
Ethane	30.07	305.3	4.87
Propane	44.09	369.8	4.25
Ethylene	28.05	282.4	5.04
Propylene	42.08	364.9	4.60
Methanol	32.04	512.6	8.09
Ethanol	46.07	513.9	6.14
Acetone	58.08	508.1	4.70
Ammonia	17.031	405.6	11.3
Chlorotrifluoromethane	104.46	302.0	3.92
Diethyl ether	74.12	467.7	3.64
n-Pentane	72.15	469.6	3.37

Source: Adapted from Liong *et al.* (1991).

However, at last the comparative summary of entire extraction methods i.e. conventional and Non-Conventional are mention in the table 5 along with the advantages and disadvantages of methods and recommended compound will be extracted from using these methods.

Table 5: Comparative summary of entire extraction methods

	Conventional methods			Non-conventional methods					
	Solvent extraction	Maceration	Hydro distillation	Ultrasound-assisted extraction	Pulsed electric field	Enzyme assisted extraction	Microwave assisted extraction	Pressurized solvent extraction	Supercritical fluid extraction
Brief description	Solvent is heated by a conventional oven and passed by the sample	Increase the diffusion or surface area of grind sample	Based on water and stem distillation	Immersion of the sample in solvent and submission to ultrasound using a US probe or US bath	Pulses of high electric voltages are applied to the sample placed in between two electrodes	hydrolysation of polysaccharides and lipids of cell wall by enzymatic reaction	Immersion of the sample in solvent and microwave energy is submitted	Heat of the sample by a conventional oven and crossed by the extraction solvent under pressure	Immersion of the sample in solvent and microwave energy is submitted
Extraction time	6-8 hours	6-8 hours	6-8 hours	10–60 min	10–60 min	40 min	3–30 min	10–20 min	10–60 min
Sample size	50-100 g	50-100 g	50-100 g	1–30 g	1–30 g	1–30 g	1–10 g	1–30 g	1–5 g
Solvent volume				50–200 ml			10–40 ml 2–5 ml (solid trap)	15–60 ml	30–60 ml (liquid trap)
Cost	Low	Low	Low	Low	High	High	Moderate	High	High
Advantages	Rapid and easy to handle	Maceration based on solubility in Solvents and extraction	Best suited for small-scale industries	Easy to use	Rapid and non-thermal process	Significantly suitable to extract bound compounds	Rapid Easy to handle Moderate solvent consumption	Rapid No filtration necessary Low solvent consumption	Rapid Low solvent consumption Concentration of the extract No filtration necessary Possible high selectivity
Disadvantages	High solvent consumption, long treatment time and thermal degradation	Raw material is not fully exhausted	Not suitable for heat-labil compounds	Large amount of solvent consumption Filtration step required	Mechanism not well known and process intensification is difficult	Not feasible at industrial level due to the behaviour of enzymes	Extraction solvent must absorb microwave energy Filtration step required	Possible degradation of thermolabile analytes	Many parameters to optimize
Recommended Compound	Lipid/fat extraction	Alkaloids, Glycosides, Tannins and Steroids	Oil and bioactive compounds	Phenolic compounds, lipids, chlorophyll, carotenoids	Best for phytosterols and various polyphenols	For the extraction of oil and bounded phytochemicals	For the rapid extraction of bioactive compounds (especially polyphenols)	Agro-industrial by-products for phytochemical extraction	Best suited for volatile compounds

Conclusion

Waste generated from Pineapple fruit and exploitation of those wastes to extract important nutritional and bioactive components by suitable extraction technologies is an important concern in context to effective waste management perspective. Various waste generated from pineapple e.g. crown, peel or skin, core etc. contains significant amount of high-value bioactive compounds. It could be used for production biopolymers which have immense potential applications in the food industry as artificial sweeteners, prebiotic supplements, and food packaging material. The utilization of waste approach not only addresses the environmental issues but also creates an opportunity to build a multi-million-dollar industry to manufacture products required for food and pharmaceutical industries and therefore there is a blooming market of pineapple waste. Nowadays novel scientific and technological methods generally termed as nonconventional extraction methods are employed for the extraction of valuable products e.g. various bioactive and nutraceuticals from pineapple waste due to high extraction efficiency. Therefore, the increasing economic significance of bioactive compounds derived from pineapple waste may lead to finding out more high yielding sophisticated extraction methods in the future.

Conflict of Interest

The authors declare no conflict of interest.

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