CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

CURRENT DEVELOPMENTS IN THE VALORIZATION OF APPLE PROCESSING WASTE IN TO VALUE ADDED FUNCTIONAL BIOACTIVE COMPOUNDS: A COMPREHENSIVE OVERVIEW

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https://doi.org/10.34302/crpjfst/2024.16.4.7

Article history:
Received
May 15th, 2024
Accepted
November 10th , 2024

Keywords:
Apple pomace;
Valorization;
Conventional extraction;
Non-conventional extraction;
Bioactive constituents.

ABSTRACT

Apple is a potential fruit and consumed throughout the globe. Processing apple results three fourth fraction of apple juice as a major product and one fourth fraction of apple pomace as by product or waste. Direct disposal of this waste creates environmental problem. However, apple pomace is considered to be a significant source of pectin, carbohydrates, amino acids, protein, essential fatty acids, and phenolic compounds. Effective utilization of apple pomace into food and nutraceutical industries could be a suitable waste management strategy. In order to extract these bioactive constituents, various conventional e.g. Soxhlet extraction (SE), maceration, and hydro-distillation (HD) and novel processing techniques e.g. ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), supercritical fluid extraction (SCFE), pressurized liquid extraction (PLE), pulse electric field extraction (PEF), enzyme assisted extraction (EAE), and liquid-liquid extraction techniques (LLE) are considered. Separation, purification, identification and quantification followed by high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectroscopy are important in order to characterize the bioactive constituents available in apple pomace. This paper reviews the valorisation of apple pomace into high valued bioactive constituents by different extraction strategies as a sustainable waste management approach.

1. Introduction

Apples (*Malus Domestica*), celebrated for their delicious taste and health benefits, stand as a formidable force in global agriculture (Perussello et al. 2017). India produces only 2.05 percent of the world's total apple production. Every time apple juice is produced, leftover apple pomace is generated. Constituting approximately 25% of the weight of fresh fruit, this byproduct poses a substantial challenge for waste management (Waldbauer et al. 2017). Apple pomace is a significant contributor to fruit waste, with up to 12 million tons generated globally each year. It is made up of apple peel, pulp, seeds, cores, stems, and a mixture of these components that are left over after the juicing process. The production involves several steps such as milling, liquefaction, and juice extraction (Hobbi et al. 2023). Addressing this waste has become imperative for environmental and economic considerations. Beyond being more pulp, apple pomace is a valuable repository of nutrients and bioactive compounds. Although it is naturally biodegradable, its sheer volume creates logistical challenges for producers, despite its traditional use as animal feed (Grigoras et al. 2013). Despite this, millions of tons of apple pomace go unused, representing an untapped resource. Apple pomace consists of generally

peel, fleshy part along with a insignificant fraction of seeds and stems. However, several factors e.g. variety, origin and processing techniques influences variation of nutritional ingredients of apple pomace (O'Shea et al. 2015). Typically, it features a nutrient-rich profile, including fiber, carbohydrates, sugars, and essential minerals like calcium, potassium, and magnesium (O'Shea et al. 2015). Realizing the potential of apple pomace involves exploring two main pathways: fermentable and non-fermentable applications. In the fermentable approach, pomace becomes a natural food source for microbes, leading to the development of various high quality products. On the other hand, non-fermentable methods focus on extracting inherent biologically active constituents for implementation in food processing, nutraceuticals, and other industries. Due to its perishability and high biodegradability, the large-scale apple processing industry produces a significant amount of nutrient-rich pomace which can harm the environment. Therefore, it is crucial to find ways to utilize and reduce the amount of pomace produced (Perussello et al. 2017). Repurposing apple pomace goes beyond waste management, it's a strategy to maximize the potential of an abundant resource. By harnessing its natural richness, we can elevate a byproduct into a valuable contributor to a more sustainable and circular economy.

2.Apple pomace constituents

Apples (*Malus domestica*) are a commonly consumed food in many cultures. Therefore, it is important to manage the by-product of apples, called apple pomace, efficiently. Several methods for utilizing apple pomace more effectively have been proposed. These include using it as fodder, producing organic acids, enzymes, bioethanol, and biogas through fermentation by the influence of microorganisms microbiological fermentation, and creating novel materials such as bio composites (Makris and Şahin . 2019). Apple pomace waste refers to the by-products of apple processing, such as peel, core, stem, and seeds. Disposing of this waste is a major environmental issue, as millions of tons are produced every year and often end up in landfills or are burned. This not only leads to wastage but also contributes to discharge of greenhouse gas and air pollution, worsening environmental problems (Perussello et al. 2017).

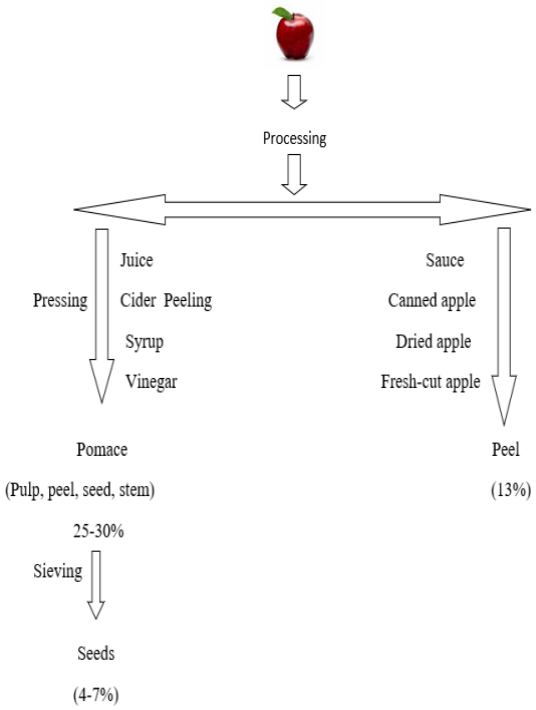

Figure 1. Development of apple derived by-products Adapted from (Rabetafika et al. 2014)

Despite these approaches, there remains a substantial edible portion of this by-product that can serve as a source of essential components. These components include anthocyanins (cyanidin 3-O-galactoside), dihydrochalcones (phloretin 2-O-glucoside and phloretin 2-O-xyloglucoside), hydroxycinnamic acids (chlorogenic acid and p-coumaroylquinic acid), and flavan-3-ols (ranging from monomers like epicatechin to large polymers known as procyanidins) (Fernandes et al. 2019). Research studies have demonstrated that using 70% ethanol as an extraction solvent reveals apple pomace as a rich source of bioactive compounds such as quercetin (1195 μg/g), chlorogenic acid (891 μg/g), phloridzin (678 μg/g), epicatechin (431 μg/g), catechin (314 μg/g), caffeic acid (296 μg/g), and rutin (123 μg/g) (Rashid et al.

2023). Apples, being highly consumed fruits, contain various bioactive compounds such as phenolic compounds, vitamins, dietary fibers, triterpenic acids, oligosaccharides, dihydrochalcones, flavonols, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids. These compounds provide to the strong qualities of free radical scavengers of apples, with a concentration exceeding 20 mmol TE/kg, as recognized by experts (Asma et al. 2023).

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

The extraction of bioactive substances involves carefully removing beneficial and physiologically active molecules from natural sources like plants, fruits, and marine life. There are several procedures used to achieve this, including solvent extraction, supercritical-fluid extraction, and enzyme-assisted extraction. The selection of a solvent and extraction method is based on the properties of the desired components. The utilization of extracted bioactive compounds has contributed to advancements in health, nutrition, and new product development across various industries, e.g. food processing, cosmetics, and pharmaceutical products. This process is crucial for numerous industries due to sustainability and ongoing research initiatives aimed at improving extraction techniques, reducing waste, and discovering new bioactive chemicals. A complete and standardized screening approach must be developed to filter out substances that are good for the development of human health, considering the significant number of plant species and the diverse range of bioactive chemicals (Azmir et al. 2013).

3.1. Conventional Extraction Methods:

Extraction of bioactive constituents from plant resources comprises of several techniques. The majority of these procedures are dependent on the strength of the solvents used, as well as thermal treatment and/or mixing. Bioactive compounds from plant resources can be extracted by using three classical techniques viz. Soxhlet extraction apparatus, Maceration, and Hydro distillation (Azmir et al. 2013).

3.11.Extraction by Soxhlet Apparatus:

The Soxhlet method of extraction uses a pure solvent to extract solid materials in a way that is effective and affordable. It uses minimal solvent and maintains extraction efficiency by repeatedly extracting solid materials. 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 sources. A lot of new techniques uses this old technique to compare the extraction efficiency. This technique follows the following protocol. Dry plant powder is placed inside the porous thimble first and then the thimble is finally introduced inside the distillation assembly 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.

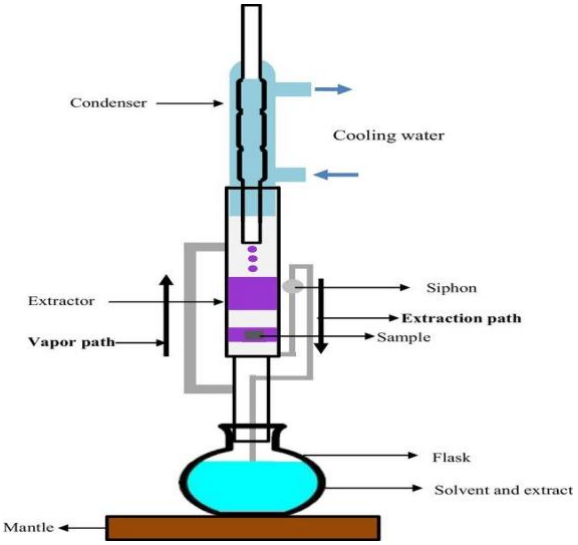

Figure 2. Representation of a Soxhlet extraction apparatus adapted from (Asif et al. 2023)

When the solvent is heated the solvent evaporates and the vapour flows through the extractor and goes to the condensation apparatus 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 an old technique for extracting valuable components from natural resources. It was commonly used in the past to prepare tonics for various health issues and is still used today due to its convenience and easy to follow protocol. This method can also be employed for essential oils extraction, although the yield is lower compared to other available techniques. By modifying some of the parameters of the maceration process, the yield can be increased. Typically, dried and ground plant materials are mixed with a suitable solvent, which is known as the menstruum. The plant material is then combined with the menstruum and left for one to several days for better absorption of the components, while occasional shaking can increase absorption. After this period, the solution is filtered, and the remaining plant material is called marc. Sometimes, more solvent is added to the older menstruum to increase the yield.

3.1.3.Hydro-distillation:

Bioactive constituents e.g. plant based essential oils, can be extracted by using hydro-distillation as is a conventional method without any requirement of organic solvents. There are three different classes of hydro-distillation: distillation induced by direct steam, water based distillation, and distillation induced by water and steam both. 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 extracts the bioactive chemicals from plant are steam and hot water. The vapor combination condenses by indirect cooling, allowing the oil and bioactive chemicals to be separated from the water. Finally, the essential oils and oil-based bioactive substances are typically dried over anhydrous sodium sulfate (Purkait et al. 2023). During hydro distillation, some volatile ingredients, natural colors, and heat-labile bioactive chemicals may be lost as the process is conducted at higher temperatures than water's boiling point.

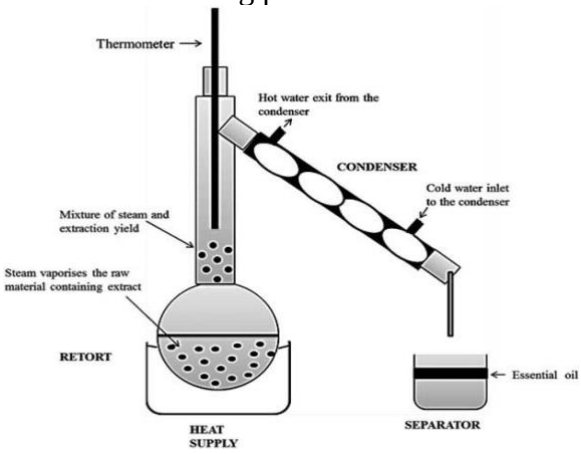

Figure 3. Representation of a hydro-distillation assembly (Cleavenger equipment) (Samadi et al. 2016)

3.2. Novel extraction techniques:

The primary liabilities of traditional extraction techniques include long extraction times, expensive, high-purity solvent requirements, large vaporisation of solvent, low level of selectivity for extraction, and heat degradation of thermostable substances. However, there are novel and intriguing extraction approaches that have been developed to overcome these limitations. These methods are known as novel extraction techniques or emerging processing technologies and the most promising ones include pressurized liquid extraction (PLE), pulsed electric field aided extraction (PFE), microwave assisted extraction (MAE), enzyme assisted extraction (EAE) , supercritical fluid extraction (SCFE), and ultrasound assisted extraction (UAE) (Azmir et al. 2013).

3.2.1.Ultrasound assisted extraction:

The food processing industry utilizes ultrasonic technology for various purposes such as extraction, filtering, freezing, packaging, cutting, and nano-formulation. In addition, significant laboratory research has been conducted to explore its potential in extracting bioactive compounds from plant matrices (Belwal et al. 2020). Ultrasound frequencies

typically range from 20 kHz to 100 MHz. It passes through a medium by causing compression and rarefaction, like other waves. Cavitation, or the implosion of bubbles, is a phenomenon caused by compression and rarefaction that involves the development, expansion, and disintegration of bubbles. Due to the fact that ultrasonic energy facilitates the percolation of both biological and inorganic components from plant matrix, the main benefit of UAE (Ultrasound Assisted Extraction) is observed in plant samples (Purkait et al. 2023). There are two main types of ultrasound applications are high intensity and low intensity.

According to (Putra et al. 2023), the optimum conditions observed for extraction of apple pomace using indirect sonication in an bath type ultrasonicator were liquid-solid ratio of 34.4:1 (v/w), a KOH concentration of 3.3 M, and a 2.5-hour ultrasonic-assisted extraction period.

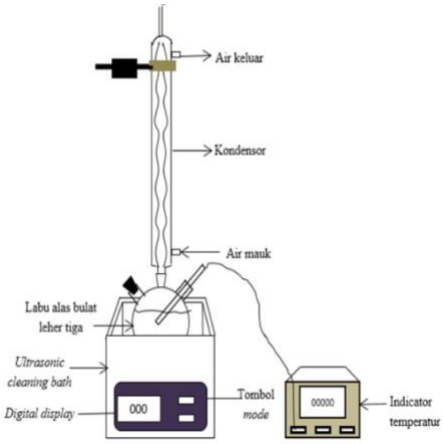

Figure 4. Representation of an ultrasonicator (Qadariyah et al. 2018)

3.2.2.Supercritical fluid extraction:

A CO_2 pump with a pressure cell containing the sample, a mechanism to maintain the system's pressure constant, and a collecting vessel are required to extract substances from a sample. The liquid is heated by pumping it to a heating zone, which boosts the temperature to critical levels. The extracted chemical then dissolves and diffuses quickly into the solid matrix inside the extraction vessel. The recovered component settles out, and the material that has dissolved is carried from the extraction unit into a lower-pressure separator. Finally, CO2 can be released into the atmosphere or cooled, compressed, and reused (Sapkale et al. 2010).

Since carbon dioxide is in gaseous stage at ambient temperature, it is eliminated when the extraction phenomenon is finished and the system is decompressed, producing an extract without any solvent. When carbon dioxide utilization is large on an industrial level, the recycling will be the management strategy. Nevertheless, because of its less polar nature, CO_2 is less successful in removing highly polar chemicals from organic matrices (Da Silva et al. 2020). The dissolution of the chemical components existing on the solid origin and their segregation into the supercritical solvent are the two main phases in the process of supercritical extraction (Da Silva et al. 2020).

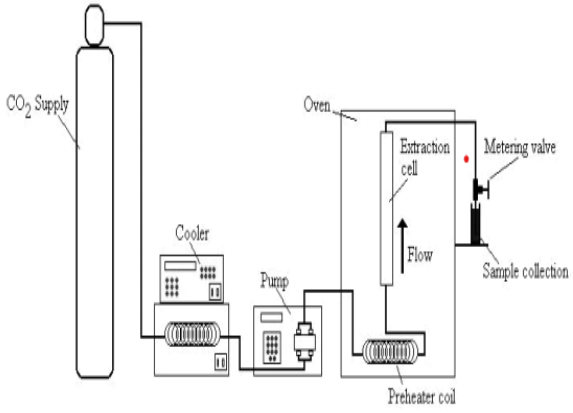

Figure 5. Schematic diagram of SCFE (Sapkale et al. 2010)

According to the experimental observation of (Putra et al. 2023), the effects of SCFE (supercritical fluid extraction) was found at a pressure level of 20 and 30 MPa at 45°C and 55 °C respectively in presence of ethanol (5%) as co-solvent. The highest level of extraction yield was obtained as 5.63 mg/g at 30 MPa pressure, 45 °C temperature and a period of two hours using ethanol (5%) as a co-solvent. However, conventional extraction methods such as Soxhlet technique and boiling water maceration achieved higher levels of extraction yield viz.2.05 mg/g of and 1.14 mg/g respectively.

3.2.3.Microwave-assisted extraction:

MAE is an automated and eco-friendly extraction method that offers numerous benefits. Compared to traditional methods, MAE reduces extraction time and solvent usage, can extract up to 40 samples simultaneously, and significantly increases sample throughput. It provides a highly appealing alternative for extracting organic and organometallic compounds from a broad range of resources while meeting the minimal requirements for sample preparation processes (Kataoka . 2019).

MAE is a standard technique for recovering active compounds from therapeutic plants. It entails heating of both solvents and samples with the application of microwave energy and segregating analytes from a sample matrix into the solvent (D. Sinha et al. 2022). The fundamental advantage of this technique is its ability to accelerate the heating of sample solvent mixtures, making it suited for the rapid extraction of analytes, especially thermally fragile fraction (Kataoka et al. 2019). The beneficial effects of MAE are determined by a variety of factors, which includes the solvent, sample, and extracted elements, particularly their dielectric properties.

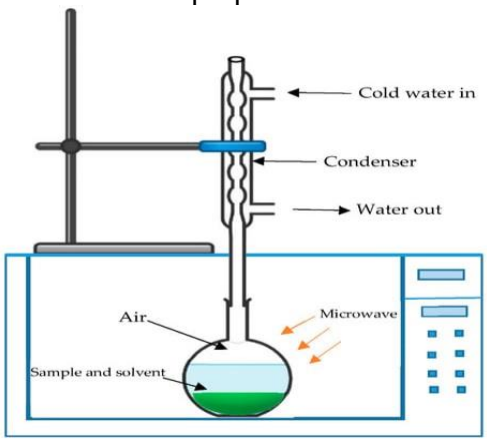

Figure 6. Representation of Microwave-assisted extraction technique (Saifullah et al. 2021)

When developing procedures for extracting medications from plants, various MAE factors, such as size of the sample, temperature of extraction and duration, power of microwave oven, and the polar character and volume of the solvent being extracted, should be optimized (Kataoka. 2019).

According to (Putra et al. 2023), it was found that a solid-liquid ratio of 0.069, a pH of 1.01, a microwave power of 499.4 W, and an extraction period of 20.8 minutes are the optimal parameters for extracting pectin. The use of MAE significantly minimized the time of extraction for dehydrated apple pomace.0.315 grams of pectin were predicted to be extracted optimally from 2 grams of dehydrated apple pomace.

3.2.4.Pressurized-Liquid extraction:

Pressurized-Liquid extraction (PLE) is an economical approach of extracting substances using liquid solvents under high pressures and temperatures. This method yields better results compared to those obtained using room temperature and atmospheric pressure methods (Mustafa and Turner.2011). In PLE, solvents are used at high temperatures and pressures, which keeps them liquid even when they would normally boil. To select appropriate operational factors, the key components must be considered theoretically. The fundamental ideas of PLE for solid samples are included as well. However, since commercial equipment has its limitations, the only way to handle liquid samples is by adding an absorbent to turn them into solids.

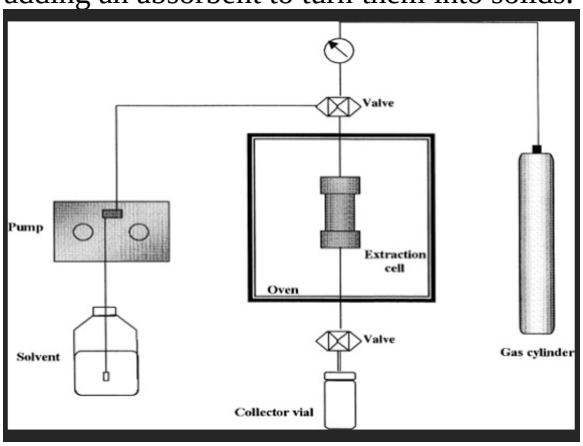

Figure 7. Schematic diagram of instrument used for extraction of honey carbohydrates using PLE (Soria et al. 2012)

3.2.5.Pulsed electric field aided extraction:

The PEF treatment is a non-thermal food processing technique, due to its extremely low temperature rise upon application. A refrigeration device, a high-voltage source of power, a device with energy storage capacity, a sterilization chamber with a pump to transport food through it, and a computing device to manage the system's controls are all part of the test system. PEF technology works on the principle that cells undergo a transformation when exposed to a strong external electric field, which increases the permeability and electrical conductivity of the cellular material (Korma et al. 2016). PEF technology has gained interest in recent years as a way to extract valuable components from food waste and by products. PEF removes the negative effects of traditional heating methods and has been used accurately to stabilize, separate, intensify and dehydrate essential substances while retaining their nutritional qualities. It is a promising alternative to conventional procedures like boiling, microwave and ultrasound-assisted extractions. In addition to enhancing the extraction process, PEF can also stress plant cells and promote the production of active components (Ranjha et al. 2021).

According to the observation of (Putra et al. 2023), apple pomace was suitably utilized for the extraction of antioxidants and polyphenolic compounds using Pressurized Liquid Extraction (PLE) process. Two different temperature ranges, i.e., 160 to 193 °C and 75 to 125 °C was considered for the extraction process. The highest antioxidant activity was attained at a temperature of 200 °C. Therefore;75 to 125 °C was considered the recommended temperature range for extraction. This spectrum of temperature was used to determine the maximum antioxidant activity of 60% ethanol and 102 °C.

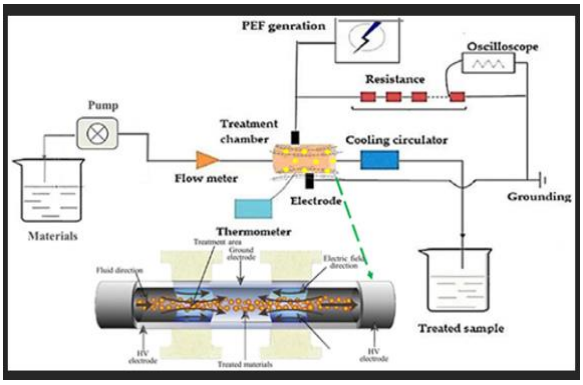

Figure 8.Schematic diagram of PEF system (Fan et al. 2022)

3.2.6.Enzyme assisted extraction:

Several chemical compounds found in plant's bases are distributed throughout the cytoplasm of cells, whereas other substances are bound in the matrix of polysaccharide molecules and complex lignin by hydrophobic or hydrogen bonding and are insoluble in a standard extraction procedure. Pre-treatment with enzymes has been seen as a unique and efficient method of releasing bound molecules and raising total yield (Azmir et al. 2013). In example, treatment of plant material with enzyme is followed before extraction using standard protocol. A versatile type of enzymes are required frequently e.g. cellulases, pectinases, and hemicellulase, to compromise the strength of the plant cell wall and improve the extraction of bioactive constituents from plants. These enzymes increase the permeability of the cell wall by decomposing its constituent parts, which raises the yields of bioactives that may be extracted (Puri et al. 2012). Enzyme assisted aqueous extraction (EAAE) and enzyme assisted cold pressing (EACP) are considered as combined enzymatic method. Generally speaking, EAAE techniques were created primarily for the different oilseed based oil extraction (Azmir et al. 2013).

3.2.7.Liquid-liquid extraction:

Solvent extraction technique used to separate biologically active molecules from natural resources such as plants, herbs, and fruits. In this process, an appropriate organic solvent is used to extract and dissolve the target

chemicals from the source material. The polarity of the bioactive molecules determines the type of solvent used, with nonpolar substances like essential oils being handled by hexane and polar compounds being handled by ethanol or methanol.

The source material is combined with the solvent, which helps the bioactive chemicals transfer into the solvent phase. Combinations of water and alcohol are more effective in extracting phenolic chemicals than mono-component solvent solutions. Particularly, water and ethanol in different proportions were examined, and it was found that the yield of polyphenolic extracted with 50% ethanol (v/v) at various temperature levels (20, 40, and 60°C) was nearly twice as high as that obtained with pure water (Hidalgo &Almajano, 2017). After the completion of extraction process, the solvent is typically eliminated from the extract using methods like evaporation or distillation, leaving behind a concentrated extract full of bioactive components. Solvent extraction is also known as liquid-liquid extraction, and the solvent used for the process is known as "Menstruum". Hexane and Dichloromethane (DCM) are used to extract non-polar compounds.

4.Methods of estimating polyphenols, flavonoids, and tannin content

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

Phenolic substances, also known as polyphenols, are molecules containing hydroxyl groups that are prone to oxidation. Numerous studies have shown that polyphenols possess various biological properties, including anti-inflammatory, antiviral, anti-diabetic, anti-cancer, and anti-microbial activities (Cosme et al. 2022). Apple pomace is considered to be a huge resource of polyphenolics and is therefore considered valuable.

The total phenolic (TP) content was measured using the Folin–Ciocalteu (FC) test. To do this, 500 µL of newly diluted 10-fold FC reagent in water and 1 mL of 20% sodium carbonate solution were mixed with 100 L of extract. The absorbance was then measured at 760 nm following a one-hour dark incubation period using a V-630 UV–vis spectrophotometer. The data were expressed as micrograms of gallic acid equivalents (g GAE/g), with gallic acid being used as the benchmark (Boulila et al. 2015).

4.2.Determination of Total Flavonoid Content (TFC):

The most widely distributed and common family of plant phenolics are flavonoids, which may be found in a wide range of foods. Rutin and quercetin are the flavonoids that are most often ingested.

As per the study conducted by (Boulila et al. 2015), the total flavonoid (TF) concentration was measured using the AlCl3 colorimetric technique. To prepare the solution, 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 distilled water were mixed with a 500 L sample aliquot. The mixture was incubated for 30 minutes at room temperature and then the absorbance was measured at 415 nm. Quercetin was used as a reference standard, and the TF content was expressed in micrograms of quercetin equivalents (g QE/g).

4.3.Determination of Total Tannin Content (TTC):

To calculate the total tannin content, we followed the methodology described in (Nurdalilah et al. 2018). First, we combined 0.1 ml of methanolic extract, 0.5 ml of Folin-Ciocalteu reactive, 7.5 ml of distilled water, and 1 ml of a 35% aqueous solution of Na2CO3 in a test tube. Then, we added 0.9 ml of purified water and left the mixture in the dark for half an hour at room temperature. We used a UV/Vis spectrophotometer to measure the absorbance at 725 nm and created a standard curve using gallic acid (20-100 ppm). The results were expressed in g of gallic acid equivalents (GAE)/100 g of extract (Purkait et al. 2023).

5.Spectrophotometric assays of determining antioxidant activity

5.1.Antioxidant activity by DPPH Radical Scavenging Activity:

DPPH, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a stable free radical characterized by its purple crystalline appearance. This compound is light-sensitive and not soluble in water, but it can be dissolved in organic solvents such as methanol and ethanol. Due to the lesser harm associated with ethanol compared to methanol, the latter is the preferred solvent (Gulcin and Alwasel . 2023).

The study represented by (Saikia et al. 2016) was used to determine antioxidant activities. Through testing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical's ability to scavenge free radicals, the total antioxidant activity of Ajwain seed extract was examined. The DPPH free radical seeks to rob antioxidants of their hydrogen. The presence of its free radical gives the methanolic DPPH its purple colour. First, 4 mg of DPPH was dissolved in 100 ml of methanol to make DPPH solution. In test tube add with a 400 µl solution of the methanolic extract. The mixture included 5600 µl of DPPH solution. 30 minutes were given for the mixture to remain at room temperature after it had been mixed and kept in the dark. Using a spectrophotometer, the solution's absorbance was calculated at 515 nm.

The percentage inhibition for each sample was determined, and the 50% inhibitory concentration (IC50) for each sample was calculated based on the concentration versus percentage inhibition graph. The IC50 value represents the concentration at which the sample scavenges 50% of the DPPH free radical. The radical scavenging activity was expressed as a percentage of inhibition. Graphical methods were employed to determine the IC50 values for both the extracts and standards, and measurements were conducted in triplicate. The IC50 of the extract signifies the concentration at which the radical scavenging potential is 50% (Phuyal et al. 2020). The percentage of inhibition was calculated using the following formula:

$$I\% = [(AC - AO)/AC] \times 100\%. \quad (1)$$

Where, AC = absorbance of the control (1 mL methanol + 1 mL DPPH solution) and AO = absorbance of the sample solution.

5.2.Antioxidant activity by FRAP method:

This method involves using antioxidants to reduce a ferric-tripyridyl triazine complex to a ferrous state, which is colored. To measure the reducing power of food extracts, their ferric reducing activity was determined. The FRAP reagents were freshly made by combining 300 mM of acetate buffer (pH 3.6), 20 mM FeCl3, and 10 mM TPTZ (2,4,6-Tripyridyl-S-triazine) in a 1:1:10 (v/v/v) ratio. After mixing 1 mg of the apple pomace extract with 2 ml of FRAP reagent, the mixture was incubated for 30 minutes in the dark at 37 °C. The production of the TPTZ-Fe2 + combination in the presence of antioxidant chemicals was measured at 593 nm using a spectrophotometer. The absorbance was calculated using the µM Fe2 + equivalent from the aqueous solution of ferrous sulphate ($FeSO_4 \cdot 7H_2O$) calibration curve (100 ~ 1,000 µM) based on triple analyses. After determining the Trolox methanolic solution's FRAP values, they were represented as TEACs, or Trolox equivalent antioxidant capacity (Moni Bottu et al. 2022). To calculate the Ferric Reducing Activity, the following equations were used:

Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) is measured in micrograms of ascorbic acid per gram of sample. The formula for AEAC is:

$$AEAC(\mu gAA/g) = [(activity) \times (dilution\ factor) \times (V_{extract}\ (ml))] / g(sample). \quad (2)$$

The activity is calculated from the calibration curve of the equation y = ax + C. The formula for calculating activity is:

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

Where y = absorbance of the sample, c = y - intercept, and a = slope.

Finally, we calculate the percentage of Ferric Reducing Activity with the following formula:

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

Where A_c is the absorbance of the standard (ascorbic acid) at maximum concentration, and $A_s$is the absorbance of the sample.

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 it's 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-

$$\%ABTS \text{ radical cation scavenged} = [(A_{control} - A_{sample})/ A_{control}] \times 100 \quad (5)$$

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

6.Extraction of Pectin from apple pomace

Apple pomace is a significant source of pectin, and extracting pectin is a reasonable approach to utilizing pomace. Apple pomace contains protopectin, which is a type of acid-soluble polysaccharide that has pectin in it. Pectin is an important constituent that can be extracted from citrus and apple waste. Pectin is a soluble fiber found in fruits and vegetables. It is also used as a gel forming agent, surfactant and thickener in various processed foods such as confectionery, bakery, jellies, yoghurts, and beverages. Pectin has numerous applications in the pharmaceutical and food processing industries. The most well known methods for obtaining pectin from raw materials include aqueous extraction, which involves direct boiling, microwave heating, ultrasonication, autoclaving, and electromagnetic induction. All of these pectin extraction techniques contribute to a certain degree of pectin quality deterioration. The temperature, duration of the extraction process, pH level, and source material all affect the production of pectin. In an acidic solution, pectin is extracted using inorganic acids such as phosphoric acid, hydrochloric acid, or sulfuric acid. Commercial pectin extraction often involves the use of extraction by acid and precipitation by alcohol on an industrial level. The basis for pectin extraction using acid is the higher temperature at which protopectin hydrolyzes. Using strong acids to extract pectin has several benefits, the primary ones being a high pectin production and a shorter period of extraction. However, there are drawbacks as well, including the disposal of acidic wastewater and significant energy and chemical costs (Chandel etal.2022). Pectin extraction from various sources using different acids was compared. Citric acid emerged as the most promising option due to its economic and environmental advantages, yielding the highest pectin output (13.75%) from apple pomace. Additionally, the effect of extraction conditions on pectin production was demonstrated by optimizing temperature (50, 75, 100°C) and duration (30, 50, 80 min) with 5% citric acid on apple pomace. It is important to remember, however, that the presence of residual chelating agents during the extraction process might reduce the final pectin's functionality (Chandel et al. 2022).

The first step in the pectin extraction process involved cleaning and finely chopping apples using an electric grinder. The crushed apple pomace was then pressed to achieve a consistent weight, and the resulting mash was dried at room temperature and then at 50℃ to produce apple flour (Kumar and Chauhan.2010). The apple flour served as the starting material for all pectin extraction experiments. To extract pectin, a reflux method was used in a condensation system at 97°C for 30 minutes, with a solute/solvent ratio of 1:50. The extractant was diluted hydrochloric acid with a pH of 2.5, and

a known weight of apple flour was used as the raw material. The same process was repeated using diluted citric acid with a pH of 2.5, and both acids were diluted with de-ionized water. The procedures used to extract pectin from the flour made from the other type of apple were also repeated (Kumar and Chauhan.2010).

7.Extraction of other bioactive components present in apple pomace

Apple pomace is considered to be a significant resource of biologically active constituents such as quercetin (1195 μg/g), chlorogenic acid (891 μg/g), phloridzin (678 μg/g), epicatechin (431 μg/g), catechin (314 μg/g), caffeic acid (296 μg/g), and rutin (123 μg/g) (Rashid et al. 2023). Apples are a highly consumed fruit and are packed with various bioactive components such as phenolic component, vitamins, dietary fibers, triterpenic acids, oligosaccharides, dihydrochalcones, flavonols, anthocyanidins, hydroxycinnamic acids, and hydroxybenzoic acids. These compounds give apples strong antioxidant qualities, with a concentration of more than 20 mmol TE/kg, as recognized by many experts (Asma et al. 2023). Table 1 lists the major functional biologically active constituents found in apple pomace and their corresponding bioactivities. Apple pomace is composed of lignins (15.30–23.50%), hemicelluloses (4.26%–24.40%), and fibers, with a high concentration of both soluble (14.6%) and insoluble (36.5%) dietary fiber. These fibers make up 35–60% of the total quantity of non-starch polysaccharides in the material. The primary ingredients in this diet are pectins (5.50%–11.70%), cellulose (7.20%–43.60%), and other fibers. Lignin is generally insoluble in water, Pectin, cellulose (depending on its form), and some hemicelluloses are partially soluble in water. Galacturonic acid, being a monomeric sugar acid, shows its maximum solubility in water. The layer of cutin found in apple pomace also contains triterpenoids, with ursolic and oleanolic acid being the primary ones along with their alcoholic derivatives. Other derivatives include those with extra hydroxyl groups and p-coumaroyloxy- or cinnamoyloxy-moities. Although the number of identified apple triterpenoids is growing, we still don't know their overall abundance in apple pomace (Waldbauer et al. 2017).

Apple pomace is a great resource of hemicelluloses and cellulose, containing up to 18% of the polysaccharides found in apple cell walls, mainly fucogalactoxyloglucans (xyloglucan). Many industrial compounds like methylcellulose, hydroxypropyl cellulose, and carboxymethylcellulose are produced using cellulose, which has extensive industrial applications. However, the extraction of xyloglucan from apple pomace is still in its early stages.

8.Separation and quantification of extracted bioactive compounds

8.1. Separation and quantification by High Performance Liquid Chromatography:

The extract components were separated and detected using HPLC-UV-ELSD analysis in reversed phase. A Varian Pursuit XRs C18 analytical column (150 x 4.6 mm, 5 μm) connected to an RP-C18 protective column was used. A gradient elution program was applied which involved using water as solvent A and methanol as solvent B, both acidified with 0.1% formic acid, at a flow rate of 1 mL/min (Lyu et al. 2020). According to (Da Silva et al. 2020) At 1.1 mL/min, the gradient profile looked like this: 0.05 minutes, 10% B; 0.5 minutes, 15% B; 2.0 minutes, 12.5% B; 3 minutes, 15% B; 4 minutes, 80% B; 5 minutes, 100% B; 6 minutes, 100% B; 7 minutes, 5% B. The injection volume was 5 μL, and there was a 5-minute equilibration period in between runs. Every molecule was identified using a profile that was found by UHPLC-MS/MS tests, co-elution with genuine standards, retention durations and UV spectra comparisons, and other methods. Each compound's standard curve (7 points, 0.1–100.0 mg L−1) was created by graphing the concentration against peak area. These compounds were quantified using the calibration curves of related compounds, and the

results were reported as equivalents (M. A) (Da Silva et al. 2020).

9.Characterization of Bioactive compounds

Nuclear magnetic resonance (NMR) spectroscopy is an advanced analytical technique used extensively in the fields of materials science, biology, and chemistry. It works by using radiofrequency radiation and external magnetic fields to interact with certain atomic nuclei, such as protons and carbon-13. To obtain the 1H NMR spectra, a high-resolution Bruker AVANCE-400 MHz NMR spectrometer running at 9.4 T and 400 MHz frequency was used. According to (Gabriel et al. 2013), the analysis was carried out using a 5.0 mm coil with a 90º pulse, 16 cumulative scans, acquisition points at 64 K, and a spectral window of 10 ppm. The 90º pulse width was 7.5 µs, and the acquisition duration was 7.5 s. The recycle delay was 10 s, and the total time for the process was 17.5 s. These parameters were also applied to other projects (Gabriel et al. 2013). An NMR spectrometer with a frequency of 20 MHz and a 1H nucleus (manufactured by Resonance Instruments, Whitney, U.K.) was used to examine apple powders that had been adjusted to different relative humidity levels. The samples were quickly transferred to glass NMR tubes with an 18 mm diameter and lifted to a height of approximately 20 mm from the controlled humidity chambers. Subsequently, the tubes were sealed to prevent outside moisture from getting in (Lavelli and Kerr.2012). To calculate the T2 timings, the samples were analyzed using free induction decay. A single 90° pulse was used with a recycle delay of 2 seconds at 4.1 µs. All analyses were performed at 22°C (Lavelli and Kerr. 2012)

Table 1. Major biologically active constituents from apple pomace fraction adapted from (Lyu et al. 2020)

Class	Concentration (mg/kg DW)	Major Constituents	Bioactivity and Therapeutic Potential
Carbohydrates	48–62	Pectin and pectin oligosaccharides	Fiber and dietary fiber of properties e.g. solubility, viscosity and fermentable, significant prebiotic characteristics and hypo-cholesterolemic impacts
Phenolic acid	523-1542	Chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid sinapic acid, p-coumaroyl-quinic acid	Antioxidant, antimicrobial, anti-inflammatory, anticancer and cardio-protective effects
Flavonoids	2153-3734	Isorhamnetin, kaempferol, guercetin, rhamnetin, glycoconjugates, procyanidinB2, epicatechin	
Anthocyanins	50-130	Cyanidin-3-O-galactoside	
Dihydrochalcones	688-2535	Phlorizin, phloretein	Antidiabetic, potential in treating obesity, promoting bone-forming, blastogenesis

10.Conclusion

Apple pomace stands as a substantial reservoir of bioactive elements, offering a sustainable remedy to both environmental and economic challenges through the efficient utilization of waste from apple processing. Diverse extraction techniques have been examined to unlock the bioactive potential harbored within apple pomace. Novel approaches, categorized as non-conventional extraction strategies, have demonstrated superior efficiency and sustainability compared to traditional methods involving high temperatures, acidic conditions, and organic solvents. Among these innovative techniques, such as ultrasonic and microwave extraction, have proven effective, potentially supplanting conventional methods. While these advanced strategies show promise in extracting valuable bioactive components, challenges persist in terms of productivity and economic feasibility. Thus, further advancements are warranted to address these challenges and pave the way for environmentally friendly solutions in the future.

11.References

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Acknowledgment

Authors are grateful to the Department of Food Engineering and Technology Kokrajhar, Kokrajhar, Assam, India or providing the necessary assistance to carry out this review work.