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Antioxidants: Extraction and application in food industry

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Abstract

A class of various compounds, 'antioxidants' are the ones which possess an ability to protect the human body against the deleterious effects of oxidation reactions. The concept of good health and well-being revolves around the fact of maintaining a balance of antioxidants and reactive oxygen species (ROS) in the human body. Antioxidants provide protection against the free radicals responsible for cell damage which otherwise are accumulated and leads to a pathological condition known as 'oxidative stress' and is a causative factor for cancer. Formation of free radicals is an inevitable phenomenon but their neutralization by antioxidants is indispensable for the body. Antioxidants are like bodyguards which prevent our body from damage and diseases and have a wide range of applications. In this review, the concept of antioxidants, identification, extraction and their specific applications in the food industry will be covered.

Keywords: antioxidants, oxidative stress, free radicals, ROS

1. Introduction

Oxygen, no doubt is an essential component of living. Proper utilization of oxygen is needed in the production of energy through the metabolism of nutrient components of food. Thus, oxygen is regarded as a pre-requisite for the survival of living beings. On the other hand, it is hard to believe that oxygen, a crucial element for life may have pernicious effects on the human body under certain conditions. A number of chemical reactions continue to occur in the human body responsible for its proper maintenance and metabolism. Oxygen play an extremely important role in the occurrences of these reactions. Oxidation and reduction reactions are of significance among the commonly occurring natural chemical reactions. Both of these reactions occur simultaneously. Oxidation cannot occur without reduction and vice versa. Oxidation reactions are thus the basis of survival for living beings and these reactions are inevitable. Several cellular functions are impossible without oxidation reactions. Oxidation also occurs in food which is mainly responsible for quality, flavor and texture loss of food product and rancidity of fats and oils.

Free radicals formed during the metabolic processes or from the external sources are capable of carrying out chain reactions and causing cellular damage. Environmental agents also initiate free radical generation. The toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light, and pollution may all be due to their free radical initiating capability. Reactive oxygen species is a collective term that includes all reactive forms of oxygen, including both oxygen radicals and several non-radical oxidizing agents that participate in the initiation and/or propagation of chain reaction [1].

Oxidative stress is defined as a pathological condition caused due to the accumulation of free radicals in the body. It is an indicator that the free radicals present in the body are not effectively neutralized by the antioxidants. Oxidative stress is the main causative factor of various diseases, aging and cell damage. Occurrences of oxidative reactions, formation of free radicals and ROS in the human body and food systems no doubt is an inevitable phenomenon but their neutralization by the antioxidants is extremely important as they may further lead to cellular damage in case of the human body and may carry out some undesirable changes in the food product with regards to its flavor, texture and organoleptic properties thus rendering it unacceptable by consumers [2].

Antioxidants are a class of chemical compounds that have an ability to stabilize the free radicals by donating their free electrons and thus combat the ill effects of oxidative reactions. They play a vital role in the neutralization of free radicals and contributes much to the health and well-being of an individual and in maintaining the acceptability of a food product. The food industry is majorly concerned with the neutralization of free radicals by incorporating antioxidants in a food product and also has an emphasis on the development of newer products which will extend a helping hand towards the stabilization of free radicals in the body when consumed. Polyphenolic and flavonoid compounds are a major class of antioxidants. As the food industry, today is very much concerned with the incorporation of antioxidants in various food products newer techniques for the extraction, identification, purification, and recovery of antioxidants continue to emerge. With the development of these techniques, antioxidants have found many applications in the food industry today which will be covered further.

2. Free radicals
Free radicals are chemical species that are capable of independent existence that possesses one or more unpaired electrons in the valence shell of their molecule and capture electrons from other substances in order to neutralize themselves [3]. They can be formed by the loss of a single electron from a stable molecule, or by the gain of an electron to a molecule. They have the capability of donating or accepting an electron with an intention of stabilizing themselves [4]. This will initially stabilize a free radical but will give rise to a chain reaction and will lead to the formation of thousands of free radicals within no time [1]. Free radicals are known to exhibit common properties as they possess an unpaired electron in their structure [5]. The energy required to dissociate the covalent bond can be provided by ultra-violet light, heat or ionizing radiation among others. These reactive species are capable of causing damage to the vital biological molecules such as DNA, proteins, carbohydrates, and lipids [6].

2.1 Mechanism for the formation of free radicals
Free radicals can be formed in three ways -

1. By homolytic cleavage of the covalent bond of a normal molecule, with each fragment retaining one of the paired electrons.
   $X^* + Y^* \rightarrow X : Y$
2. By the loss of a single electron from a normal molecule
   $X: Y \rightarrow X_+ + Y_-$
3. By the addition of a single electron to a normal molecule
   $X + e- \rightarrow X-$

A radical might donate its unpaired electron to another molecules. It might take an electron from other molecules in order to pair or it might simply join to the molecule. When radical gives one electron or takes one electron or simply adds on to the anion to become a radical. Thus the future of the reactions that usually proceed as chain reaction [3].

2.2 Sources of free radicals
- **Internal Sources:** Biological reactions serve as a source of free radicals. Some internal sources of generation of free radicals are mitochondria, xanthine oxidase, phagocytes, and reactions involving iron and other transition metals, peroxisomes, exercise, inflammation.
- **External Sources:** Some external sources of free radicals are cigarette smoke, an environmental pollutant, radiations, ultraviolet light, ozone, certain drugs, pesticides, anesthetics, and industrial solvents.
- **Physiological Factors:** Mental status like stress, emotion etc. and disease conditions are also responsible for the formation of free radicals.

3. Reactive Oxygen Species (ROS)
Molecular oxygen as such is of no harm but is infect essential for living cells to obtain energy. However, some reactive species are derived from oxygen during aerobic metabolism or from environmental conditions (pollution, smoke, radiation, drugs). These are termed reactive oxygen species (ROS) and they exhibit two unpaired electrons in different orbitals at their highest energy level, which influences them to the form free radicals [7]. ROS is a collective term that describes free radicals derived from oxygen superoxide anion (O2), hydroxyl radical (OH), peroxyl radical (RO2), an alkoxy radical (RO), as well as hydrogen peroxide, a non-radical species resulting from oxygen metabolism (H2O2). All are capable of causing cellular damage. Figure 1 shows a diagrammatic representation of various sources of ROS:

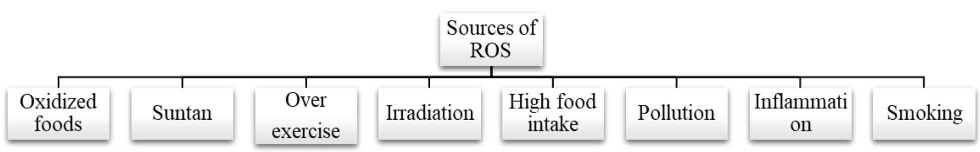

Fig 1: Sources of Reactive Oxygen Species

4. Oxidative stress
The term 'Oxidative stress' refers to a pathological condition responsible for damaging action on cells and tissues of the body. Oxidative stress is basically a state of imbalance between the production of free radicals and the ability of the body to detoxify their effects through neutralization by antioxidants. Oxidative stress is a consequence of the accumulation of free radicals in the body and reduced activity of antioxidants to neutralize them. Oxidative stress is a harmful condition that occurs when there is an excess of ROS and/or a decrease in antioxidant levels, this may cause tissue damage by physical, chemical, psychological factors that lead to tissue injury in human and causes different diseases. There are a variety of different defense mechanisms by which the human body works against oxidative stress induced by free radicals as tissue repair, anticipatory mechanism, physical resistance, and antioxidant defense. Reactive oxygen species of various free radicals are involved in cardiovascular diseases, renal disorders, autoimmune disorders, neurodegenerative disorders, peptic ulcer and lung cancer [8, 9]. Figure 2 shows some of the consequences of oxidative stress.

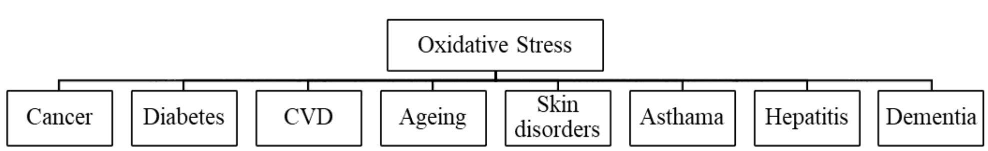

Fig 2: Consequences of oxidative stress

5. Antioxidants

Antioxidants are compounds of many different chemical forms, grouped together as they are capable of compensating the ill effects of free radicals and oxidation reactions in the food system and the human body. An antioxidant can be defined as: "any substance that, when present in low concentrations compared to that of an oxidizable substrate, delays or inhibits the oxidation of that substrate" [10]. Antioxidants were found attractive by the researchers as they were capable of inhibiting rancidity in oils and fats. Dietary sources like fruits, vegetables, tea, etc are rich in antioxidants [11]. Fruits can add important vitamins, minerals, and other bioactive compounds to the human diet. Natural antioxidants of commercial use are tocopherols (vitamin E), ascorbic acid (vitamin C) and rosemary extract [12]. In an easier terminology, antioxidants are the chemical substances which possess an extra electron in their structure, they look for attaining stability by donating the electron to free radicals and thus stabilizes them. As a consequence of which the damage due to free radicals is prevented. Several natural phenolic compounds have been reported to possess high antioxidant properties, but only a few of them are found to be commercially applied in foods. Antioxidants prevents both enzymatic and non-enzymatic reactions protect the body against oxidative damage. Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation.

6. Classification of antioxidants

Antioxidants can be classified into two major types: ie: natural and synthetic antioxidants. Representation of classification of antioxidants is shown in figure 3.

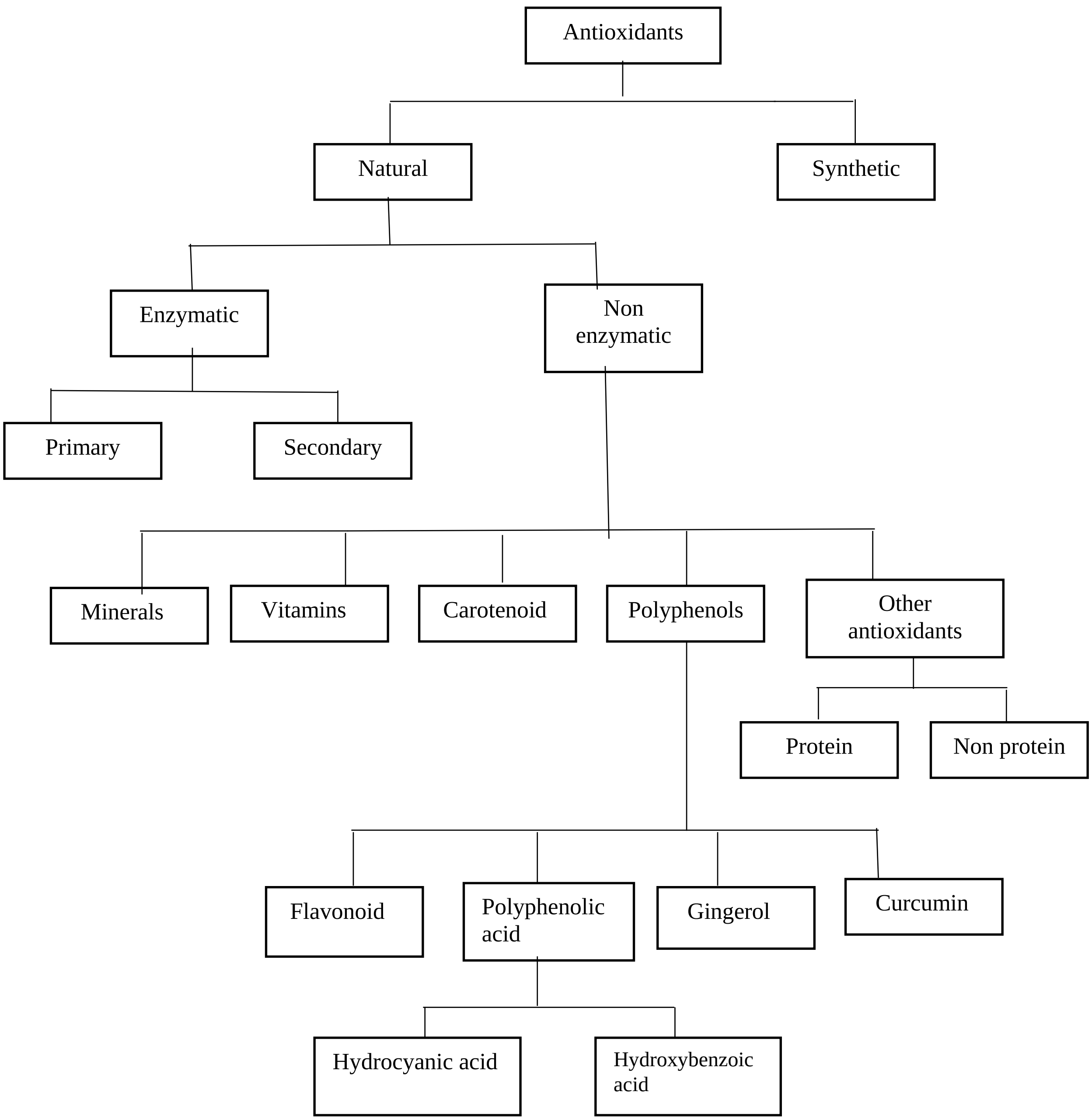

Fig 3: Classification of antioxidants

6.1 Natural Antioxidants
Natural antioxidants are synthesized in human body through metabolic process or are supplemented from other natural sources. Their activity depends on their properties and mechanism of action. Natural antioxidants are further divided as Enzymatic and Non enzymatic antioxidants.

6.1.1 Enzymatic antioxidants
Enzymatic antioxidants are produced in human body and are subdivided as primary and secondary antioxidants. Primary antioxidants are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Secondary antioxidant includes glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH).

6.1.2 Non enzymatic antioxidants
They represent a category of antioxidants which are not produced in the body but are required to be supplied externally for the proper metabolism of the human body. These include minerals, vitamins, carotenoids, polyphenols, and other antioxidants.

6.2 Synthetic antioxidants
The phenolic group compounds are included in synthetic antioxidants and these finish the oxidative stress, free radicals and various biological reactions associated with the free radical negative effect. e.g.; Nordihydroguaiaretic acid (NDGA), esters of gallic acid (propyl gallate), tertiary butyl hydroquinone (TBHQ), butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT).

7. Mechanism of action of antioxidants
Two principle mechanisms of action have been proposed for antioxidants:
The first is a chain-breaking mechanism by which the primary antioxidants donate electrons to the free radicals present in the system. The second mechanism involves removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst.
Chain reactions of free radicals

Initiation stage (Free radical formation)
(1) $RH \rightarrow R^{\cdot} + H^{\cdot}$
(2) $R^{\cdot} \rightarrow R^{\cdot} + O2 \rightarrow ROO^{\cdot}$
(3) $2ROOH \rightarrow ROO^{\cdot} + RO^{\cdot} + H2O$

Propagation stage
(1) $R^{\cdot} + O2 \rightarrow ROO^{\cdot}$
(2) $ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$
(3) $RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$

Termination stage
(1) $R^{\cdot} + R^{\cdot} \rightarrow R - R$
(2) $R^{\cdot} + ROO^{\cdot} \rightarrow ROOR$
(3) $ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O2$
(4) Antioxidants + O2 → Oxidized antioxidants [13].

Oxidation of fats leads to formation of unsaturated fatty acids which give forms free radicals and starts chain reaction forming more free radicals The antioxidants added to it will neutralize the free radicals by donating one of their own electrons ending the reactions.

8. Polyphenolic compounds as antioxidants and their sources
Some of the common types of polyphenols found in food are given in Table 1.

Table 1: Most common types of polyphenols found in foods and plant-derived products

Polyphenols	Source
Phenolic acids	
Hydroxycinnamic acids	Cereals, coffee, cherries, citrus fruits and juices, peaches, plums, spinach, tomatoes, wheat flour, corn flour, rice flour, potato.
Hydroxybenzoic acids	Oilseeds, cereals, coffee, cowpeas, wheat flour, black currant, blackberry.
Flavonoids	
Anthocyanins	Grapes, red wine, grape seeds, grape skins, winery by-products, fermented grape pomace, strawberries, back and red currants.
Chalcones	Apples and apple juices.
Flavones	Apples, grapes, leeks, tomatoes, curly kale, onions, lettuces, berries, beans, red grapes, black and green tea.
Flavanones	Citrus fruits, citrus juices, orange peels and seeds wastes.
Flavonols	Apples, apple peels, beans, leeks, lettuce, onions, tomatoes, olive leafs, chestnut.
Flavones	Spinach, citrus fruits, celery, pepper, capsicum.
Isoflavones	Soybeans, soy flour, soy milk, soy processing waste.
Stilbenes	Red grapes, grapes skins, grape seeds, red grape fermented pomaces.
Xanthones	Mango fruits and mango peels fermented pomaces.
Tannins	
Condensed tannins	Apples, grapes, peaches, pears, chestnut, hazelnuts, nuts.
Hydrolyzable tannins	Pomegranate, raspberries.

9. Extraction of antioxidants
Extraction is basically a separation process. Range of methods have been developed in recent years in order to separate the antioxidants from its original source, their identification, recovery and analysis. All these methods at first requires extraction process to be carried out. Extraction process is used for separating solutes, i.e. bioactive constituents from solutions using specific solvents by adopting standard procedures [14]. The main purpose of this extraction method is to separate the soluble solutes for efficient extraction process. The crude extract obtained also contain several byproducts which require further purification and recovery to get an improved yield of desired antioxidants.

9.1.1 Conventional methods for extraction
Classical/conventional extraction techniques are generally used at small scale to extract antioxidants from several materials. These techniques are usually based on the extraction efficiency of different solvents, which are being

used. Water bath is a popular conventional technique. Conventional extraction and concentration of polyphenols using a water bath is performed at temperatures ranging from 20 to 50°C, temperatures above 70°C are not favorable as they may cause polyphenol degradation rapidly. Temperature is directly proportional to the efficiency of the extraction. However, the use of temperatures higher than 50°C decreases the total polyphenols and proanthocyanidins yield due to their degradation [15].

Conventional extraction is often performed as a three-process approach, which consists of:

i) Soxhlet extraction
ii) Maceration and
iii) Hydro distillation (HD).

i) Soxhlet extraction

Soxhlet extraction is a well-known technique used for the extraction of bioactive compounds. Dry sample from which the bioactive components are to be extracted is kept in the thimble. The thimble is then placed in the distillation flask, which contains selective solvent when overflow level of solvent is reached; the solution of the thimble-holder is aspirated by a siphon. Siphon unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. Solute is remained in the distillation flask and solvent passes back to the solid bed of plant. The process runs repeatedly until the extraction is completed. Figure 4 shows the soxhlet extraction apparatus:

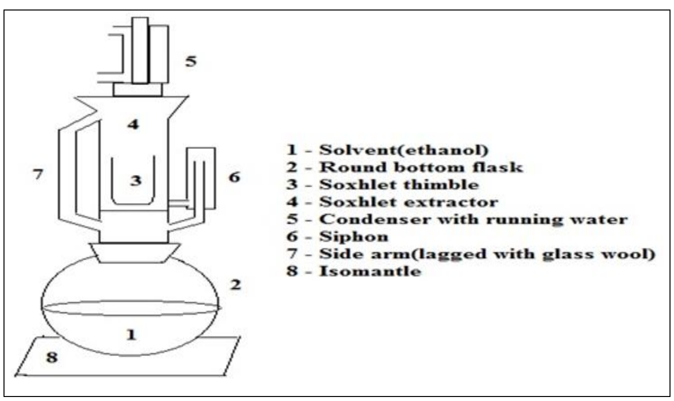

Fig 4: Soxhlet extraction

ii) Maceration

In order to carry out maceration, firstly the materials are ground which increases their surface area. Secondly, appropriate solvent is added in a closed vessel. Thirdly, the liquid is strained off. The solid residue after extraction (Marc) is pressed to recover large amount of solution. Impurities are removed by the process of filtration. Occasional shaking in maceration process promotes extraction by means of two ways: firstly, it will increase diffusion and second, it will remove concentrated solution from the sample surface thus helping to increase the extraction yield.

iii) Hydrodistillation (HD)

HD, which is one of the traditional method used for extracting bioactive compounds and essential oils from several plant materials. This process does not involve organic solvents and it can be performed before dehydration of any plant materials. There are three types of HD: water distillation, water and steam distillation, and direct steam distillation [16].

9.1.2 Non-conventional extraction techniques

Some of the problems encountered while employing the conventional extraction are longer extraction time, requirement of costly and high purity solvent, evaporation of the huge amount of solvent and low extraction selectivity compounds [17]. In order to overcome these limitations of conventional extraction methods, new extraction techniques are emerging. These emerging techniques are categorized as non-conventional extraction techniques.

Methods/Techniques for extraction of antioxidants: (Non-Conventional)

i) Supercritical fluid extraction: (SFE)

Supercritical state is a distinctive state and can only be attained if a substance is subjected to temperature and pressure beyond its critical point. Critical point is defined as the characteristic temperature (Tc) and pressure (Pc) above which distinctive gas and liquid phases do not exist. Supercritical fluid cannot be liquefied by modifying temperature and pressure. Supercritical fluid possesses gas-like properties of diffusion, viscosity, and surface tension, and liquid-like density and solvation power. These properties make extraction easier and helps to give higher yield of desired components [18]. SFE uses solvent in their supercritical states. CO2 is often regarded as an ideal solvent for SFE. The main drawback of CO2 is its low polarity making it suitable for lipid, fat, and non-polar substance, but unsuitable for polar materials. The extraction efficiency of polar compounds with CO2 can be improved by the addition of small quantities of polar organic solvents used as modifiers.

A basic SFE system consists of the following parts: a tank of mobile phase, usually CO2, a pump to pressurize the gas, co-solvent vessel and pump, an oven that contains the extraction vessel, a controller to maintain the HP inside the system, and a trapping vessel. Usually different type of meters such as flow meter, dry/wet gas meter could be attached to the system. A symmetric diagram of typical SFE instrumentation is given in Figure 5.

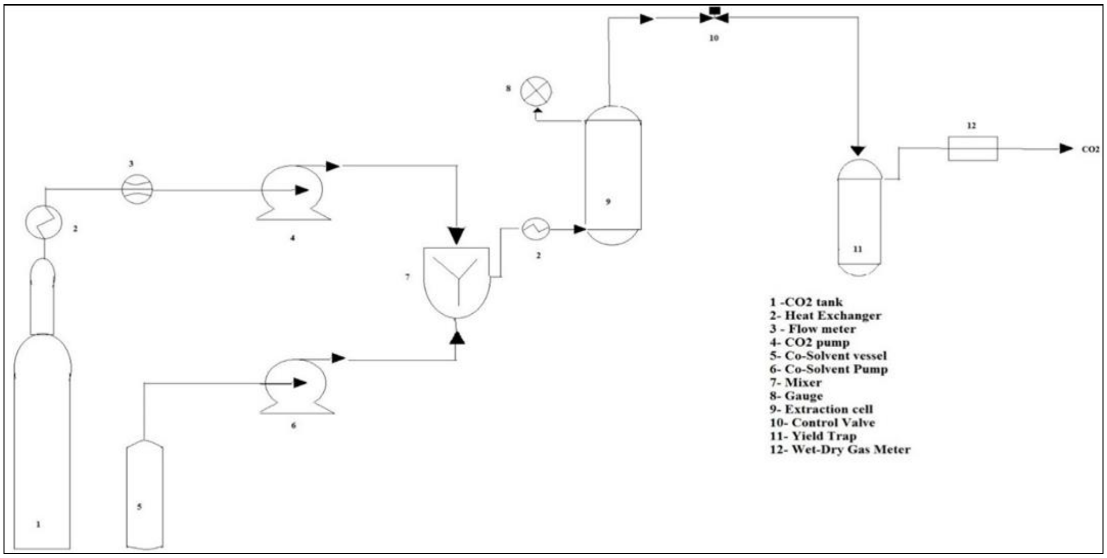

Fig 5: Diagramatic representation of Supercritical fluid extraction

Process system

The SC-CO2 fluid extraction process consist of four steps: extraction, expansion, separation, and solvent conditioning. The steps are accompanied by four primary components: extractor (HP vessel), pressure and temperature control system, separator, and pressure intensifier. Raw materials are usually ground which increases their surface area and are charged into a temperature-controlled extractor forming a fixed bed, in case of a batch and single-stage mode [19]. SC-CO2 technology is available in the form of single-stage and can be upgraded to multistage semi-continuous batch operations coupled with a multi-separation process.

ii) Microwave assisted extraction

Microwave-assisted extraction is a good technique used for extraction in multiple fields. Microwave-assisted extraction (MAE) has been used as an alternative to conventional techniques for the extraction of antioxidants. Microwaves are electromagnetic fields in the frequency range from 300 MHz to 300 GHz. They consist of two oscillating fields that are perpendicular such as electric field and magnetic field. Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms [20].

Process system

In a closed MAE system, the extractions are performed in a sealed vessel with different mode of microwave radiations. Uniform microwave heating is practiced for extractions. High working pressure and temperature of the system allow fast and efficient extraction. The pressure inside the extraction vessel is controlled such that it would not exceed the working pressure of the vessel, while the temperature is regulated above the normal boiling point of the extraction solvent. Recent advancements in the closed system have led to the development of high pressure MAE [21]. One major drawback is that the closed system offers fast and efficient extraction with less solvent consumption, possibilities of losses of volatile compounds with limited sample throughput. Figure 6 shows closed type MAE. Open system is developed to overcome the drawbacks of closed system such as the safety issues. This system has higher sample throughput, and more solvent can be added to the system at any time during the process. Figure 7 represents open type MAE.

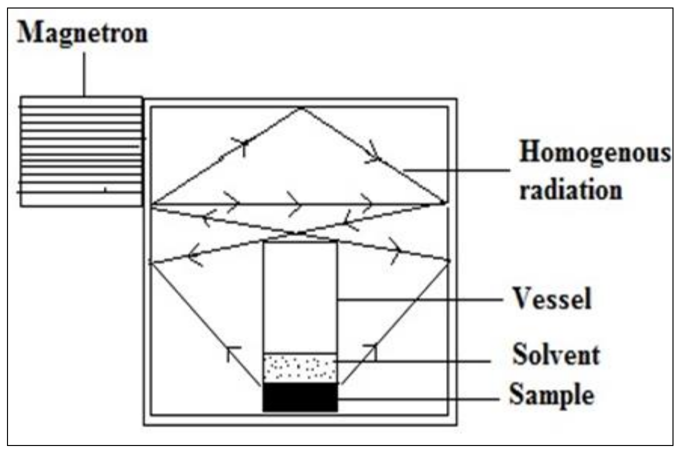

Fig 6: Closed type MAE

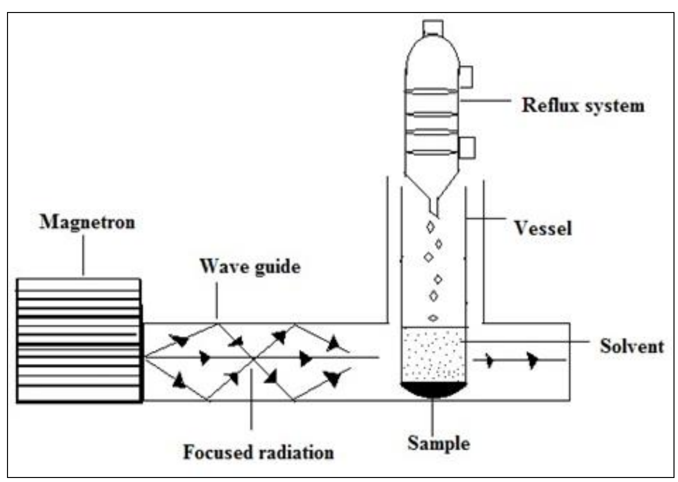

Fig 7: Open type MAE

iii) Pressurized liquid extraction (PLE)

This method is now known by several names: pressurized fluid extraction, accelerated fluid extraction, enhanced solvent extraction, subcritical water extraction (SWE), and HP solvent extraction [22]. The basic concept of PLE is the application of high pressure to remain solvent liquid beyond their normal boiling point. High pressure promotes the extraction process. Decreased extraction time, less solvent requirement and automation in instrumentation are some of the advantages of this technique. PLE can enhance extraction rates of bioactive compounds. A lower temperature range between 75 and 125°C is suitable. Figure 8 shows PLE set up.

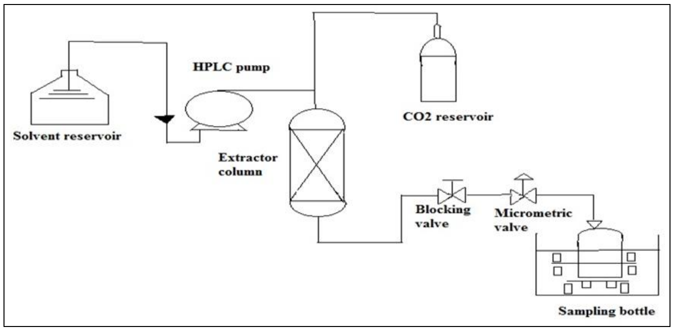

Fig 8: The PLE set-up

Process system

The PLE set-up is shown in Figure 9. The solvent was pumped into the extraction cell, which was placed in an electrical heating jacket at a desired temperature, until the required pressure was obtained. Extraction samples were placed in a 6.57 cm extraction cell containing metal filter at the bottom and upper parts. The cell containing the sample was heated, filled with extraction solvent, and then pressurized. The sample was placed in the heating system for 5 min to ensure that the extraction cell would be at the desired temperature (313–393 K) during the filling and pressurization procedure. After pressurization, the sample with pressurized solvent was kept at the desired pressure (5–10 MPa) for the desired time (3–15 min). After PLE, the extracts were rapidly cooled to 5°C in ice water using amber flasks to prevent degradation of antioxidants [22].

iv) High hydrostatic pressure

The use of HHP is known to improve mass transfer rates increasing cell permeability as well as increasing secondary metabolite diffusion as per changes in phase transitions. During HHP extraction the liquid partially fills the air gaps present in fruit tissues. When the pressure is released, the occluded air in the pores exits damaging plant cell membrane damage [23].

v) High voltage electric discharge

This technique introduces energy directly into an aqueous solution through a plasma channel formed by a high-current/high-voltage electrical discharge between two submerged electrodes. HVED is based on the electrical breakdown in water. Water vapor bubbles that are initially present in water formed due to local heating accelerate the process. If the electrical field is intense enough, the avalanche of electrons becomes a starting point of streamer propagation from the high voltage needle electrode to the grounded one. The electrical breakdown is accompanied by a number of secondary phenomena (high-amplitude pressure shock waves, bubble cavitation, creation of liquid turbulence, etc.). These secondary phenomena cause particle fragmentation and cell structure damage [24]. The HVED method requires short treatment times and low energy consumption [25].

vi) Ultrasonic assisted extraction

Ultrasound is a sound wave beyond human hearing, frequency of these waves ranges from 20 kHz to 100 MHz. Like other waves, it can pass through a medium by creating compression and expansion. Therefore, this process produces a phenomenon known as cavitation, which leads in production, growth, and collapse of bubbles. A large amount of energy can be produced and thereby, it helps in heating the contents of the bubble [26]. UAE is seemed to be an effective extraction technique for bioactive compound extraction from by-products of fruit and vegetable processing industries [26]. The extraction mechanism by ultrasound process involves two main types of physical phenomena: 1. the diffusion across the cell wall and 2.rinsing the contents of cell after breaking the walls [27]. Moisture content of sample, milling degree, particle size, and solvent are very important factors for obtaining efficient and effective extraction. Temperature, pressure, frequency, and time of sonication were the governing factors for the action of ultrasound. Figure 9 shows the batch ultrasonic assisted extraction process.

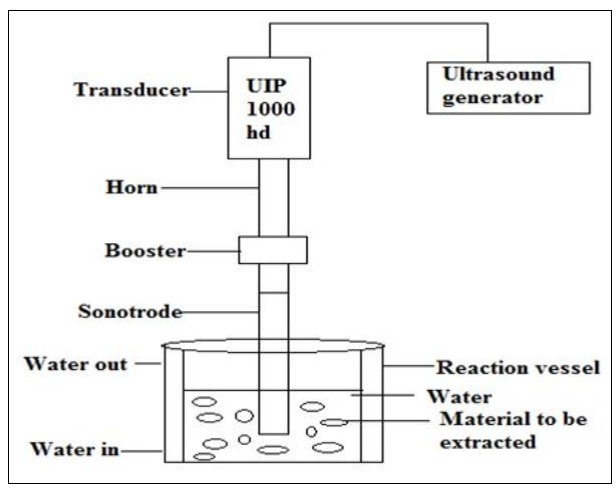

Fig 9: Batch ultrasonic-assisted extraction

Process system

UAE experiments are conducted with a sonotrode and a glass reaction tank [28]. The double-layered mantle of the reactor, allows the control of extraction temperature with a cooling system by means of water circulation. The transducer is connected to the horn with a ‘booster’ installed in amplification mode and finally the sonotrode, which is immersed into the middle of the liquid and samples have to be filled in the tank (Figure 9). Continuous UAE is carried out with an apparatus which is made up of a circulatory pump and the inlet is placed in a large beaker, which contains water and samples [29].

vii) Pulsed electric field (PEF)

The PEF extraction method is the commonly used technique for improving the drying, extraction, and diffusion processes [30]. The basic principle of PEF extraction is to disintegrate the structure of cell membrane for increasing the rate of extraction. The electric potential passes through the cell membrane when it is suspended in an electric field, and this electric potential separates membrane molecules based on dipole nature, i.e. according to their charge in the cell membrane. After exceeding a critical value of approximately 1 V of transmembrane potential, there is a repulsion, which may occurs between the charge carrying molecules that form pores in weak areas of the membrane, and it causes drastic increase in permeability [31]. For PEF treatment of plant materials, a simple circuit with exponential decay pulses is used. The materials were placed in a treatment chamber consisting of two electrodes. Based on treatment chamber design, the PEF process can be operated in either continuous or batch mode [32]. The effectiveness of PEF treatment depends on the process parameters, which includes field strength, specific energy input, pulse number, treatment temperature, and properties of the materials to be treated [33].

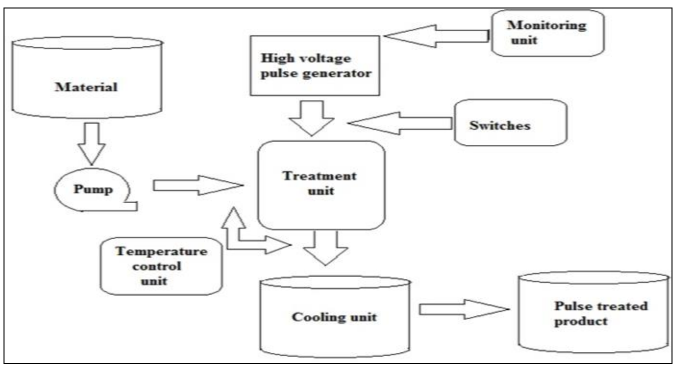

Fig 10: Pulsed electric field extraction

Process system

The PEF system consists of high current generator, treatment unit, fluid handling system, and controlling equipment. The generator of high current supplies electrical pulses of voltages. The system consists of power supplier, charging resistor, capacitor, switches, inductors, and resistors. Power generator converts high voltage (50–60 Hz frequency) alternating current (AC) power to the high direct current power (DC). The energy produced by the generator (5–80 kV DC) is stored at capacitors and used to generate electric fields. Systems possess a switch that is used to discharge high energy through the food materials in treatment chamber. It acts as a bridge between high-energy suppliers and treatment unit [34]. Many different waveforms are being applied in PEF treatment. The PEFs generated by a generator are used in treatment unit or chamber. Treatment units are designed to operate either batch or continuous manner. The components of PEF treatment and flow chart of process are given in Figure 11.

viii) Enzyme assisted extraction

Enzyme-assisted extraction (EAE) is an eco-friendly extraction technologies. Enzymes can effectively catalyze the degradation of vegetable cell walls, promoting the release of bioactive components contained inside the cells. The targeted use of enzymes can increase the yield of extractable compounds. The primary cell wall of plants is mainly composed of cellulose, hemicelluloses (xyloglucans), pectin and proteins [35]. Therefore, cellulases, hemicelullases, pectinases as well as other enzymes can be used to disrupt the cell wall structure, break down complex interior storage materials, thereby promoting the release of intracellular bioactive compounds [36]. The breakdown of cell walls is the critical step for the extraction of many bioactive compounds, which are existing inside the cell walls. Enzyme pretreatment followed by a solvent extraction process is referred to as enzyme-assisted extraction. To improve the enzyme assisted extraction it can be combined with ultrasound extraction (UAEE). Another combination used is enzyme assisted extraction with microwaves (MAEE) [37].

The process of EAE method from the natural products is shown in Figure 11.

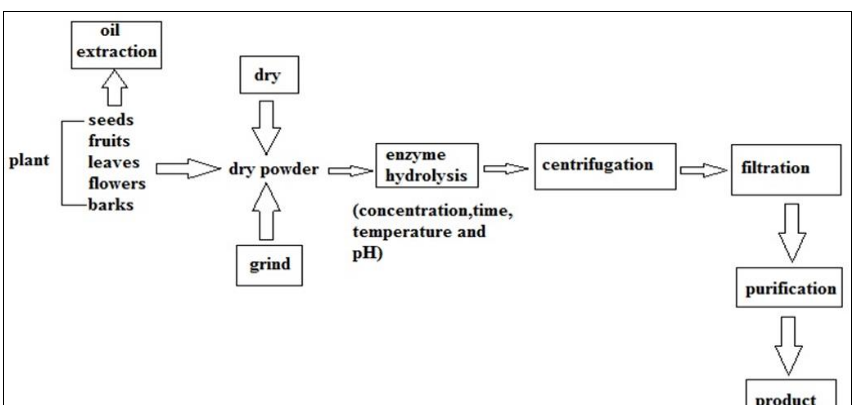

Fig 11: Enzyme assisted extraction

ix) Application of antioxidants in food industry

Food is an essential requirement for mankind and its preservation has become a challenge today. Preservation encounters a lot of problems especially shelf life. The shelf life of food is very limited and is prone to varieties of spoilage due to its composition viz. moisture, lipid, protein, carbohydrates, etc. As lipids are one of the major components of food, autooxidation of lipids and the generation of free radicals is one of the natural phenomena in biological and food systems [38].

Antioxidants have become necessary group of food additives mainly because of their unique properties of enhancing the shelf life of food products without any damage to sensory or nutritional qualities. Various natural and synthetic antioxidants are developed and successfully used in food industry for the purpose of preservation and extending shelf life. The synthetic approaches have been targeted for developing nontoxic, nonabsorbable compounds and their incorporation in a wide range of food products [38]. Some of the commonly used antioxidants in different food industries with their uses are represented in the Table 2.

Table 2: Commonly used antioxidants in different food industries

Industry	Antioxidants	Uses
Lipid industry	**Natural:** Sesamol, Gossypol, Tocopherol, Tocotrienol, Ginistein,Flavonoids **Synthetic:** Butylated Hydroxy Toluene (BHT) Butylated Hydroxy Anisole (BHA) Propyl Gallate (PG) Tert- Butyl hydroquinone(TBHQ)	These antioxidants slow down the oxidation but slow it down, thereby extending the induction period and shelf life of fat containing foods.
In packaging	Butylated Hydroxy Toluene (BHT) Low density polyethylene(LDPE) Butylated Hydroxy Anisole (BHA) etc	Maintain freshness, releases essential components into package and extend shelf life.
As preservatives	**Natural:** Ascorbylpalmitate,tocopherol tocopherol acetate **Synthetic:** Butylated Hydroxy Toluene (BHT) Butylated Hydroxy Anisole (BHA) Propyl Gallate (PG) Tert- Butyl hydroquinone(TBHQ) etc	Guard against oxidation, extend shelf life and help in preservation.
Dairy industry	Carotenoids, VitaminE, Vitamin A, vitamin D3, Conjugated linoleic acid (CLA) etc.	Prevents against autoxidation and helps in improved shelf life of dairy products.
Bakery industry	Butylated Hydroxy Anisole (BHA) Tert- Butyl hydroquinone(TBHQ) Ascorbyl palmitate, Butylated Hydroxy Toluene (BHT) etc.	Preventing oxidation due to the presence of fats and oils in bakery products and extending their shelf life.
Meat industry	Propyl Gallate (PG),Ascorbic acid Butylated Hydroxy Anisole (BHA) etc.	Meat colour development, flavor development and prevents oxidation
Beverage industry	Vitamin A,Vitamin C,Sorbic acid, potassium sorbate, sulphur dioxide, sodium sorbate etc.	Helps in preventing oxidation, prevents development of off flavor and contributes to extend shelf life.
Neutraceutical industry	Carotenoids, Vitamin E, Ascorbic acid, Lipoic acids and polyphenols	These antioxidants play a very important role in neutraceutical industry. The use of neutraceutical extend a helping hand in health maintenance by stabilizing free radicals
Functional food industry	Polyphenols, Vitamin C, Vitamin E etc.	Probiotics and prebiotics are important products of this industry.

10. Conclusion

Polyphenols represent a large and diverse group of secondary plant metabolites, therefore they are abundantly present in a majority of fruits and vegetables, even in larger quantities than vitamins. Possessing several biological effects, they represent important human nutrients. Recent research carried out in the field of natural antioxidants is increasing knowledge about naturally healthy compounds that are available in food. New methodologies of extraction, purification, identification and quantification of antioxidants using ecofriendly techniques need to be developed to improve the extraction yields. Developing new techniques in this field is necessary for the efficient extraction and utilization of antioxidants in order to use them further on an industrial level.

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