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A Review on Antioxidant from Natural Origin

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Abstract: *A variety of horrible health diseases, including diabetes, rheumatoid arthritis, cataracts, Alzheimer's disease, cardiovascular diseases, and many more, are linked to deficiency in antioxidants, which are nutraceuticals. by preventing the production of reactive oxygen species (ROS) or by directly scavenging free radicals, phytochemicals may have an antioxidant effect in diet or in vivo. Synergists are substances that, when taken alone, have little antioxidant effect but can increase the effect of true antioxidants by reacting with heavy metal ions that catalyze auto- oxidation.*

Keywords: reactive oxygen species

I. INTRODUCTION

A variety of horrible health diseases, including diabetes, rheumatoid arthritis, cataracts, Alzheimer's disease, cardiovascular diseases, and many more, are linked to deficiency in antioxidants, which are nutraceuticals. by preventing the production of reactive oxygen species (ROS) or by directly scavenging free radicals, phytochemicals may have an antioxidant effect in diet or in vivo. Synergists are substances that, when taken alone, have little antioxidant effect but can increase the effect of true antioxidants by reacting with heavy metal ions that catalyze auto-oxidation. These compounds may act as antioxidants in vivo through elevating the level of indogenous antioxidant defenes by up-regulating the expression of the genes in coding synergists. [1]

Natural antioxidants compound can be classified as Vitamins, Carotenoids, hydroxycinnamates and flavanoids. Among all the above, flavanoids is the largest group of antioxidants which are almost ubiquitous in nature in most of the fruits, vegetables and plant. The various types of natural antioxidant and their dietary source are given in tablesuperoxide dismutase (SOD), catalase or glutathione peroxidase.

Antioxidant can be broadly derived into three categories

(a) True antioxidants

(b) Reducing agents

(c) Antioxidant synergist.

True antioxidant react with free radicals and block the chain reaction of the free radicals. Reducing agent have a lower redox potential and readily get oxidized and are found effective against oxidizing agents.[2]

Oxygen is an essential chemical element in the metabolism of aerobic organisms. However, it may trigger unfavorable reactions, and there has been a growing interest in studying the role of its reactive species. Reactive oxygen species (ROS) include free radicals like the superoxide anion, singlet oxygen, lipid peroxides and the hydroxyl radical. These reactive species are by-products of the normal cellular energy production and functional activities, presenting an important role in cell signaling, apoptosis, gene expression and ion transportation. Nevertheless, if ROS level increase intensely, it can results in damage of many molecules, including proteins,lipids,RNA and DNA, since they are highly reactive. Furthermore, the production of free radicals is not only associated with the normal metabolic processes in the human body (endogenous sources), but can also be due to environmental factors (exogenous sources) such as stress, ozone radiation, pollution, pesticides and industrial chemical.[3,4,5,6,7,8] When higher production of ROS in relation to their removal by biological systems (antioxidant defenses) d ion transportation. Nevertheless, if ROS levels increase intensely, it can result in damage of many molecules, including proteins, lipids, RNA and DNA, since they are highly reactive. Furthermoreoccurs,it is called oxidative stress.[9]

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Antioxidants

Vitamins C or ascorbic acid is often claimed to be an important antioxidant in be due to free radical scavenging by ascorbate and dehydroascorbate radical. Vitamin E or -tocopherol delays lipid peroxidation by reacting with chain-propagating peroxy radicals, faster than these radical can react with proteins or fatty acid side-chains. B-carotene has remarkable antioxidant properties by interacting with a free radical to form B-carotone-derived radical which in the presence of oxygen forms a peroxyl radical.[10,11,12] Antioxidants act at different levels in the oxidative sequence, involving lipids and the extent to which oxidation of fatty acids and their esters occurs depends on the chemical Nutraceuticals are the medicinal or nutritional components that includes a food, plant or other naturally occurring material that is used for improvement of health by prevention or treatment of disease. Their prominent health benefits can be found in the domain of cardiovascular, mental, joint, skin and womwns health some of the nutraceuticals are prominently used for prevention of cancer and others for enhancement of sport performance and weight management. Some of these substances can be isolated, purified, concentrated and formulated in variety of dosage form, However some are used directly as functional food. Some of these isolated naturally nutraceuticals substances and herbs which are used as functional foods has been discuss below. [13,14,15]

Lycopene	Tomatoes
Beta-carotene	Carrots, sweet potato, green vegetables
Xanthophylls	
Beta-Cryptoxanthin	Mango, papaya, oranges
Lutein	Banana, egg yolk, greenvegetables
Zeaxanthin	Paprika
Hydroxycinnamates	
Ferulic acid	Cabbage, spinach, grains
Caffeic acid	White grapes, olive, spinach
Flavanoids	
Flavone	
Rutin	Buckwheat, tobacco, *Eucalyptus* Spp.
Luteolin	Lemon, red pepper, olive
Flavonols	
Quercetin	Onion, apple skin, black graps
Kaempferol	Grapefruit, tea
Flavonone	
Naringin	Citrus peel
Taxifolin	Citrus fruit
Chalcones	
Liquiritin	Liquorice
Anthocyanidins	
Cyanidin	Grapes, strawberry
Delphnidin	Aubergin skin
Catechins	
Epicatechin gallate	Green tea polyphenols
Epigallocatechin gallate	Green tea polyphenols

Table No.1 Naturally Occurring Antioxidants

Classification of antioxidants

Antioxidants are groupedinto two namely.

(1) Primary or naturalantioxidants.

(2) Secondaryor synthetic antioxidants

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(1) Primary or natural antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products Antioxidants of this group are mainly phenolic.

Antioxidants minerals

These are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such carbohydrates. Examples include solenium, copper, iron,as zinc and manganese

Anti oxidants vitamins it is needed for most body metabolic functions. They include vitamin C (Figure 1). vitamin E, vitamin B

Phytochemicals - These are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids:

These are phenolic compounds that give vegetables, fruits, grains, seeds leaves, flowers and bark their colours, Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin A when the body lacks enough of the vitamin, Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source include Diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives.

(2) Secondary or synthetic antioxidants

These are phenolic compounds that pedormthe function of capturing free radicals and stopping the chain reactions.z

1. Butylated hydroxyl anisole (BHA)
2. Butylatedhydroxyrotoluene (BHT)
3. Propylgallate (PG) and metal chelating agent (EDTA).
4. Tertiary butyl hydroquinone (TBHQ)
5. Nordihydroguaretic acid (NDGA).
6. Primary or naturalantioxidants. [21,22,23]

Extraction Methods of Antioxidants from Foods and Medicinal Plants

Extraction is the first and crucial step for studying the natural antioxidants from plants (Figure 1). Many extraction factors play important roles in the extraction efficiency, such as type and concentration of extraction solvent, extraction temperature, extraction time, and extraction pH. Among them, the solvent is one of the most influential factors. Numerous solvents have been used for the extraction of antioxidants from food and medicinal plants. The selection of solvents is based on the chemical nature and polarity of antioxidant compounds to be extracted. Most of the phenolics, flavanoids and anthocyanins are hydrosoluble antioxidants. The polar and medium polar solvents, such as water, ethanol, methanol, propanol, acetone and their aqueous mixtures, are widely used for extraction. [24,25,26,27] Carotenoids are lipid-soluble antioxidants, and common organic solvents, such as the mixtures of hexane with acetone, ethanol, methanol, or mixtures of ethyl acetate with acetone, ethanol, methanol, have been used for extraction. [28,29,30]

Various extraction procedures, including conventional extraction methods and non-conventional extraction methods, can be chosen to extract antioxidants from food and medicinal plants. The conventional extraction methods are mainly hot water bath, maceration and Soxhlet extraction, which are very time-consuming and require relatively large amounts of organic solvents with low extraction yields . Furthermore, the long heating process such as hot water bath and Soxhlet extraction may lead to the degradation of the thermolabile compounds. To obtain antioxidants from plants in an energy-efficient and economically sustainable way, ultrasound, microwave, pressurized liquid, enzyme hydrolysis, supercritical fluids, high hydrostatic pressure, pulsed electric field, and high voltage electrical discharges have been studied as non-conventional methods. [31,32,33]

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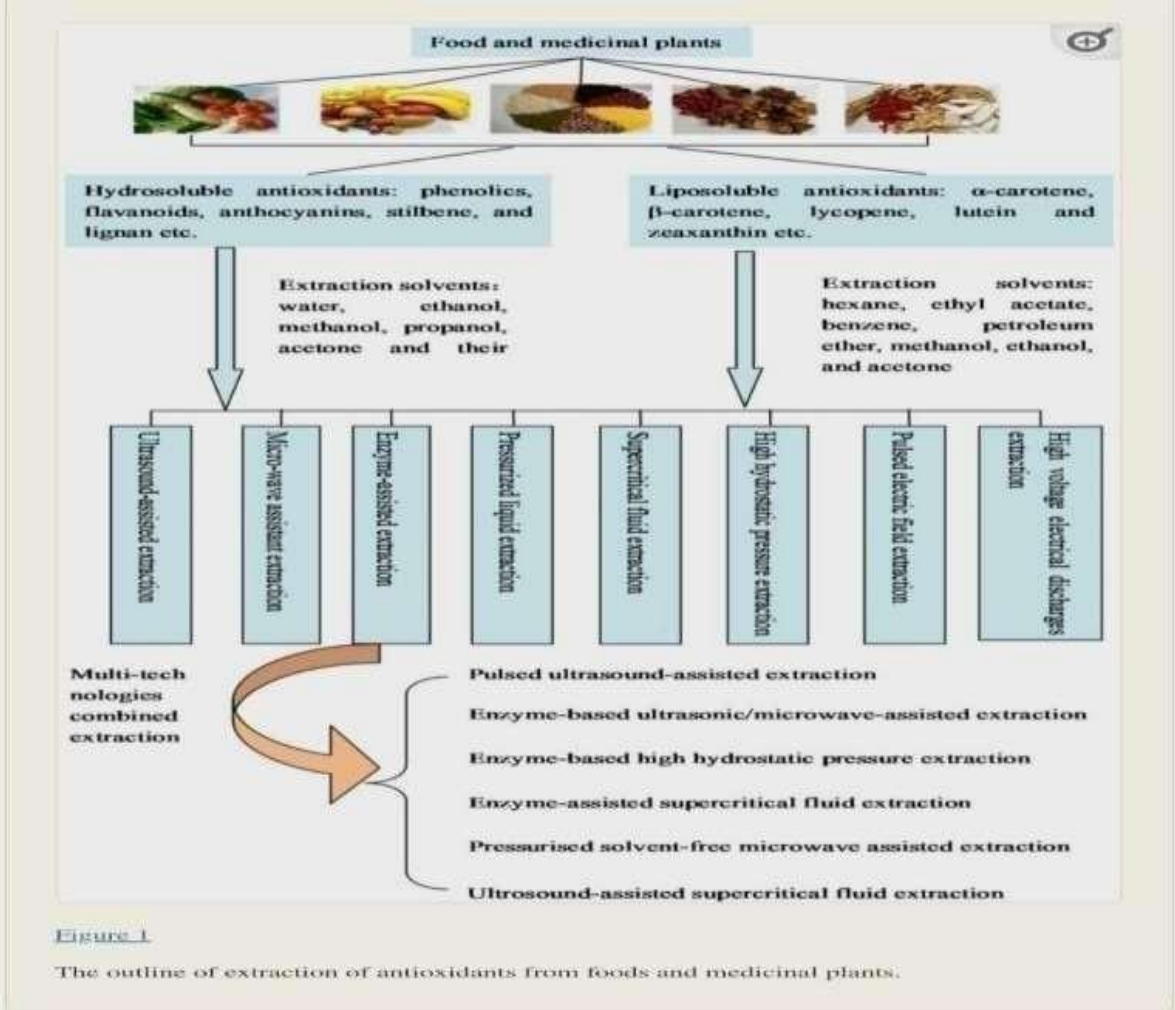

Fig NO.1 The Outline Of Extraction

1. Ultrasound-Assisted Extraction (UAE)

Ultrasound assisted extraction (UAE) has been applied widely in the last three decades as an efficient extraction method in the food and pharmaceutical industries. [34] The mechanism is based on the cavitation phenomenon. The spread of ultrasound in liquid systems is via a series of compression and rarefaction waves, which can induce the production of cavitation bubbles within the fluid. [35] The size of these bubbles grow over the period of a few cycles until reach a critical point, then these bubbles collapse and release a great quantity of energy, which would generate extreme temperatures (5000 K) and pressures (1000 atmospheres) at room temperature. During the ultrasound assisted extraction of bioactive components fromplant materials, the high temperature and pressure would destroy the cell walls, facilitate the release of bioactive compounds from plant cell walls and enhance the mass transport. The heat transfer of UAE is from outside of the plant cell to the inside, which is in the oppositedirection of microwave assisted extraction. [36]

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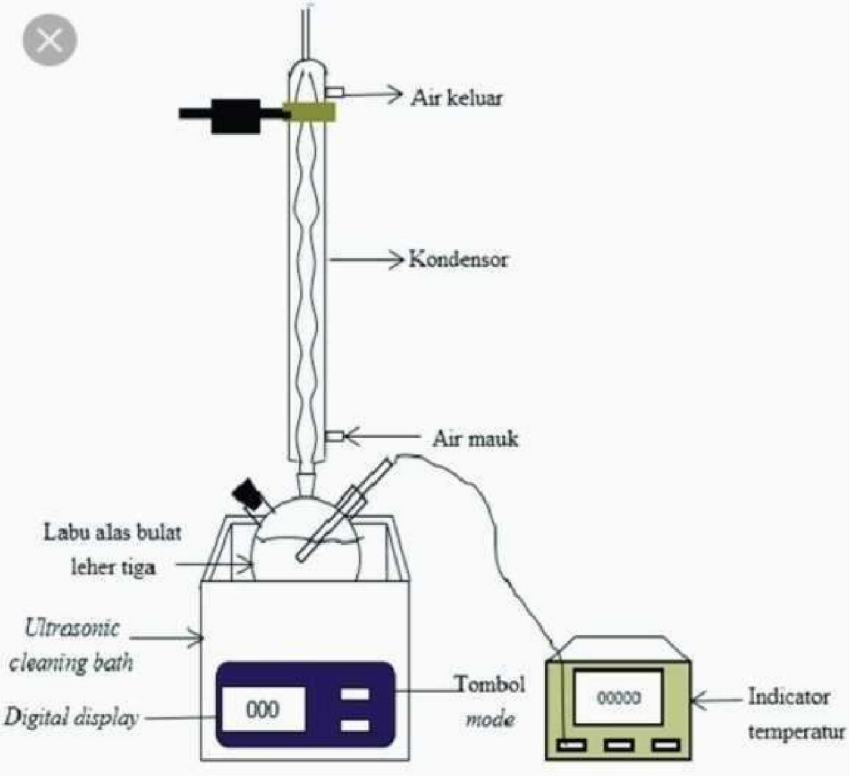

Fig No.2 Ultrasound Assisted Extraction

2. Microwave-Assisted Extraction (MAE)

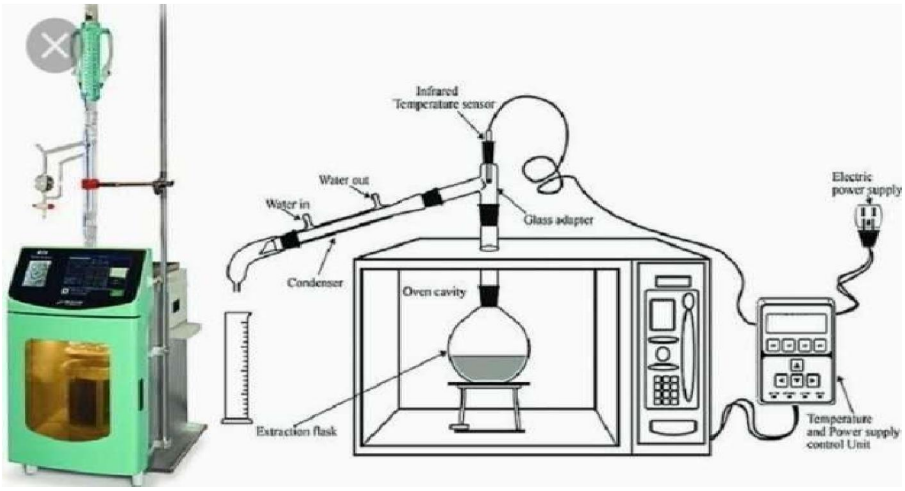

Fig No.3 Microwave-Assisted Extraction (MAE)

Microwave is an electromagnetic radiation. During MAE, microwave can deliver energy to solvent and plant matrix and the energy can be absorbed by molecules inside plants, particularly the polar molecules. The severe thermal, localized pressures and mechanical stress caused by microwave significantly change the physical properties of the cell walls and finally result in rupture of cell walls and release of target components. [37,38] Since microwave irradiation was applied for the first time in 1986, there have been various studies on MAE in the recovery of the antioxidants from

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plant materials. [39,40] MAE is not adaptable for the extraction of the thermally labile antioxidants due to the thermal effect from microwave irradiation, which might result in the reduction of extraction yield. In addition, MAE is only applicable to the extraction solvents that must be able to absorb microwaves. [41,42]

3. Enzyme-Assisted Extraction (EAE)

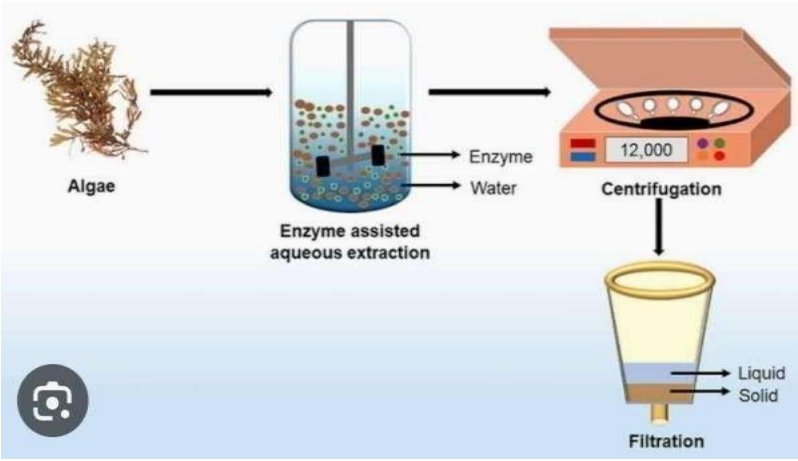

Fig No.4 Enzyme-Assisted Extraction (EAE).

Enzymes have the properties of high specificity and high efficiency. Enzyme-assisted extraction (EAE) is a potential green extraction method because of the mild extraction conditions and barely any effect on the environment. [43] The enzymes could degrade the compositions and destroy the structural integrity of plant cell wall, which enhance the release of bioactive compounds. Cellulase, pectinase, hemicellulase and β-glucosidase are extensively used in the EAE. These enzymes can be obtained from various materials such as bacteria, fungi, vegetable and fruit extracts, or animal organs. [43,44] EAE techniques have been shown to improve the extraction efficiencies for antioxidants including phenolics, flavonoids, anthocyanins, and carotenoids. [45,46]

4. Pressurized Liquid Extraction (PLE)

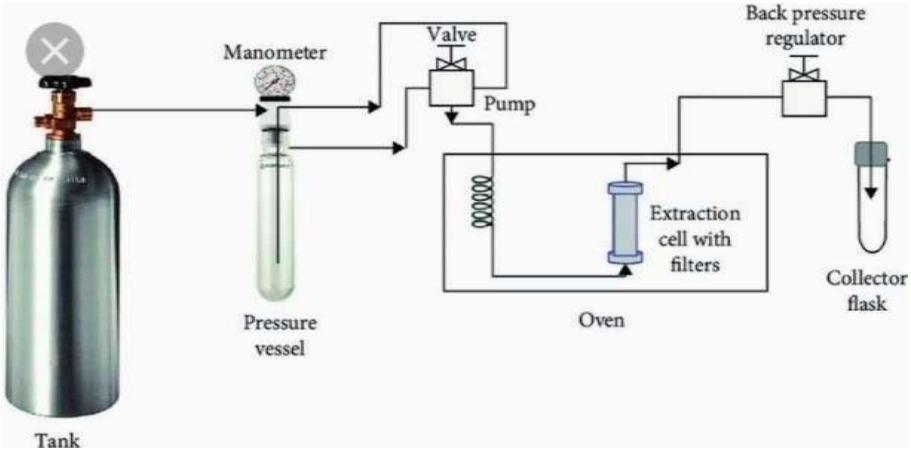

Fig No.5 Pressurized Liquid Extraction (PLE)

PLE is based on the use of solvents at elevated temperature and pressure to extract target components from various matrices. [47,48] By elevating the pressure, the temperature of solvent under liquid state can be above its boiling point at normal temperature, which can enhance mass- transfer rate and promote the solubility of the analytes. The wide ranges of temperature from room temperature to 200 °C and pressure from 35 to 200 bar can be applied in PLE. When the extraction solvent is water, PLE is also called sub-critical water extraction (SWE). When the water is heated to 200–

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250 °C in SWE, it can be maintained in liquid state, while the dielectric constant (ε) of water is decreased from 80 to 30–25, which is close to the dielectric constant of some organic solvents such as ethanol or methanol. The closed dielectric constants mean the similar polarity of the organic solvent. Although not viable for every application, the use of SWE can be regarded as an effective alternative to organic solvents in some applications. Due to free of organic solvents, SWE is perceived as the "greenest" of the PLEs. [48]

5. Supercritical Fluid Extraction (SFE)

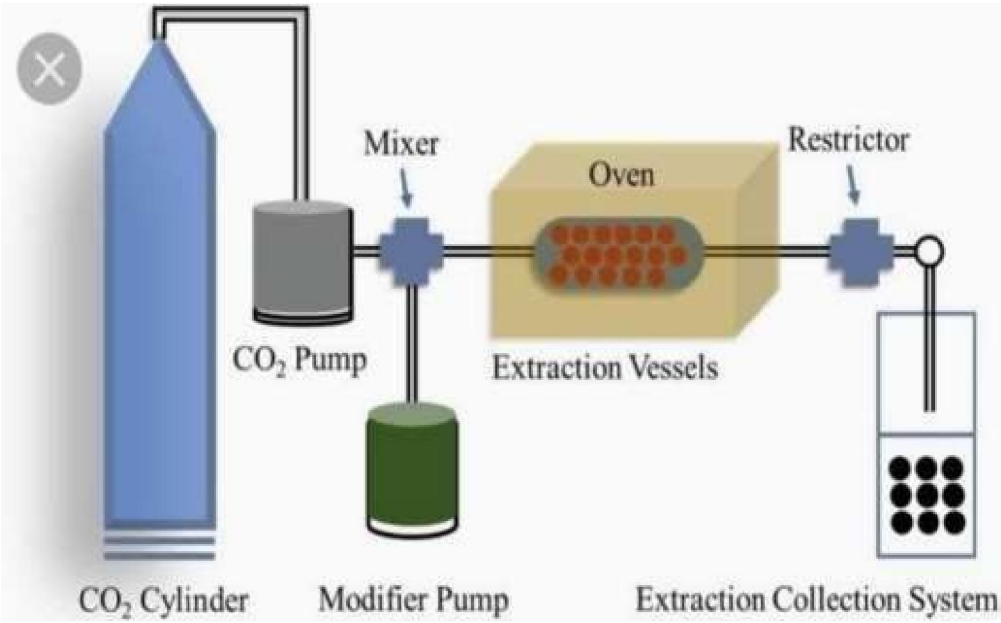

Fig No.6 Super-critical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) as a sustainable green technology has been extensively applied since the past decades. Over the critical pressure (pc) and temperature (Tc), the solvent can be transformed into the supercritical state, which shows liquid-like (solvent power, negligible surface tension) and gas-like (elevated diffusivity and low viscosity) properties. [49,50,51] Even though PLE and SFE have in common that they conduct under medium-to-high pressures, SFE operate using solvents at temperatures and pressures above their critical points, whereas PLE is based on the use of liquids at temperatures above their normal boiling points. Compared with normal liquids, supercritical fluids could enhance transport properties, which can diffuse easily through solid materials and therefore obtain faster extraction rates. SFE utilizes the outstanding physicochemical properties of supercritical fluids (SF) to extract target components from various matrices. SFE basically contains two major steps: firstly, the soluble compounds from the plant material are extracted by the supercritical solvent, then these compounds are separated from the supercritical solvent by rapidly reducing the pressure, increasing the temperature, or both. [50,51]

THERAPEUTIC USES OF ANTIOXIDANTS:

1. Anti-cancer agents in medicinal chemistry:

a. Lanthanides as anti-cancer agents:

A lot of metal-based drugs are widely used in the disease treatment of cancer. The clinical success of and colitis Aging 'cisplatin and other platinum complexes is limited by significant. Side effects are acquired or intrinsic resistance. Therefore, much attention has focused on designing new coordination compound with improved pharmacological properties and a broader range of antitumor activity. Strategies for developing new anti-cancer agents include the incorporation of carrier groups that can target tumor cells with high specificity. Also of interest is to develop complexes that bind to DNA in a fundamentally different manner than cisplatin, in an attempt to overcome the resistance pathway that has evolved to eliminate the drug. [52,53]

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b. Lycopene as a potential anti-cancer agent:

Dietary chemoprevention has emerged as a cost-effective approach to control most prevalent chronic diseases including cancer. In particular, tomato and products are recognized to confer a wide range of health benefits. Epidemiology studies have provided evidence that high consumption of tomatoes effectively lowers the risk of reactive oxygen species (ROS)-mediated diseases such cancer by improving the antioxidant carotenoid which is reported to be more stable and potent singlet oxygen quenching agent compared to other carotenoids. In addition to its antioxidants properties, lycopene shows an array of biological effects including cardio-protective, anti- inflammatory, anti-mutagenic and anti-carcinogenic activities. The cancer activities of lycopene have been demonstrated in both vitro and in vivo tumour models. [52,54]

c. Selenium derivatives as cancer preventive agents:

The role of selenium in the prevention of cancer has been recently established by laboratory experiments, clinical trials and epidemiological data , Consequently, selenium supplementation has moved from the realm of correcting nutritional deficiencies to pharmacological intervention, especially in the clinical one of domain of cancer, chemoprevention and in the control of heart failure. [52]

2. Applications of lipoic acid:

Lipoic acid protects against diseases of aging. This offer powerful antioxidant protection against three common afflictions (two of them potentially disastrous) association with the aging, stroke, heart attack and cataracts. It does it by suppressing the action of free radicals in the cells of the brain, heart and eyes. Lipid acid has an unusual relationship with four other important antioxidants: glutathione, coenzyme-Q10. vitamin C and vitamin E. Lipoic acid not only acts as a primary antioxidant in brain cells but serves to boost glutathione levels through the antioxidant network interactions. Lipoic acid in the form of gene therapy promises to be one of the most exciting and fruitful avenues of medical practice in the twenty-first century and it offer powerful antioxidant protectionagainst common afflictions including diabetes. [56]

3. Acute central nervous systeminjury:

Oxidative stress has been implicated as a potential contributor to acute central nervous system (CNS) injury by ischemic or haemorrhagic stroke or trauma. Free radicals can cause damage to cardinal cellular components such as lipids, proteins and nucleic acid e.g. DNA leading to subsequent cell death by modes of necrosis or apoptosis. The damage can become more widespread due to weakened cellular antioxidant defence systems. Moreover, acute brain injury increases the level of excitoxic amino acids (such as glutamate), which also produce ROS, thereby promoting parenchymatous destruction. Therefore, treatment with antioxidants may theoretically act as tissue damage and improve both the survival and neurological outcome. Better understanding of the pathological mechanisms of acute CNS injury would characterize the exact primary targets for drug intervention improved antioxidant design should take into consideration the relevant and specific harmful free radical. [55]

4. Neurodegenerative disease:

Oxidative stress has a mechanistic role in the development of Alzheimer's dementia. Several lines of evidence previously implied that oxidative damage to lipid membranes could disrupt normal neuronal and glial cell functioning, leading to the formation of amyloid plaques and to neuronal cell death. Hence, it is found that dietary intake of antioxidants such as vitamins E, C and beta carotene might inhibit the production of free radicals and reactive oxygen species. Antioxidants are also being investigated as possible treatments for Parkinson's disease. [57,58]

5. Uses in technology:

a. Food preservatives:

Exposure to oxygen and sunlight are the two main factors in the oxidation of food, so food is preserved by keeping in the dark and sealing it in containers or even coating it in wax, as with cucumbers. However, as oxygen is also important for plant respiration, storing plant materials in anaerobic conditions produces unpleasant flavours and unappealing

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colours. Consequently, packaging of fresh fruits and vegetables contains an approximately 8% are oxygen atmosphere. Antioxidants an especially important class of preservatives as, unlike bacterial or fungal spoilage, oxidation reactions still occur relatively rapidly in frozen or refrigerated food. These preservatives include natural antioxidants such as ascorbic acid (AA,E300) and tocopherols (E306), as well as synthetic antioxidants such as propyl gallate (PG,E310), tertiary butylhydroquinone(TBHQ), butylatedhydroxyanisole (BHA,E320) and butylatedhydroxytoluene (BHT). Since oxidized lipids are often discolored and have unpleasant tastes, it is important to avoid oxidation in fat-rich foods. Even less fatty foods such as fruits are sprayed with sulfurous antioxidants prior to air drying. Antioxidant preservatives are also added to fat-based cosmetics such as lipstick and moisturizers to prevent rancidity. [59]

b. Industrial uses :

Antioxidants are frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation and in gasolines to prevent the polymerization that leads to the formation of engine- fouling residues. They are widely used to prevent the oxidative degradation of polymers such as rubbers, plastics and adhesives that causes a loss of strength and flexibility in these materials. Polymers containing double bonds in their main chains such as natural rubber and polybutadiene are especially susceptible to oxidation and ozonolysis. They can be protected by anti-ozonants. [59]

Mechanism Of Action Of Natural Antioxidants

Natural antioxidants have been proposed to have beneficial effects on health and on different disease states, such as neurodegenerative and cardiovascular diseases, diabetes and cancer . The use of natural plant antioxidant products to handle different diseases has very ancient roots; well before the development of modern medicine with synthetic drugs and antioxidants. A lot of the biological activities of natural antioxidants have been ascribed to their ability to scavenge reactive oxygen species (ROS) that counteract oxidative stress. In the last years, a multitude of studies have suggested that their classical hydrogen- donating antioxidant activity is unlikely to be the sole explanation for their effects. First of all, natural antioxidants are subjected to an extensive metabolism in vivo that modifies their redox potentials. Moreover, the concentration of natural antioxidants and their metabolites in vivo are lower than that usually utilized in vitro. Accumulating evidence suggests that the cellular effects of natural antioxidants may also be mediated by their interactions with specific proteins central to intracellular signaling cascades, their modulation of the expression and activity of key proteins [2.10.111. their influencing of epigenetic mechanisms or their modulation of the gut microbiota. [60,61,62,63]

This special issue, concerning new mechanisms in the action of natural antioxidants in health and disease, contains nine contributions, seven research articles and two reviews, and details recent advances on this topic.

In recent years, increasing attention has been paid to natural dietetic antioxidantsand their potential effect on human health.Corsetto et al. focused on edible brown seaweeds, a rich source of natural antioxidants, extensively investigated for their ability to prevent and/or counteract different diseases . In particular, the authors studied the possible mechanisms of Fucusvesiculosus's antioxidant action and considered its bioactivity during the production of enriched rye snacks. They used a multiple-method approach, including chemical assays and cell-based bioassays, to characterize the potential mechanisms of the antioxidant action of seaweed extracts. They demonstrated that the antioxidant action of Fucusvesiculosus extracts is due to their high level of polyphenols, but also to their high Fe2+ chelating activity. Moreover, rye snacks enriched with Fucusvesiculosusshowed a higher antioxidant potential, suggesting the use of these extracts to design functional foods. [64,65,66]

Fermented foods are considered prominentconstituents of the human diet because of their content in health-promoting compounds. Fermentation is one the most ancient methods of food preparation, which increases the shelf life and improves the flavor of food matrices like soy, milk, meat, fruit and vegetables. [67]

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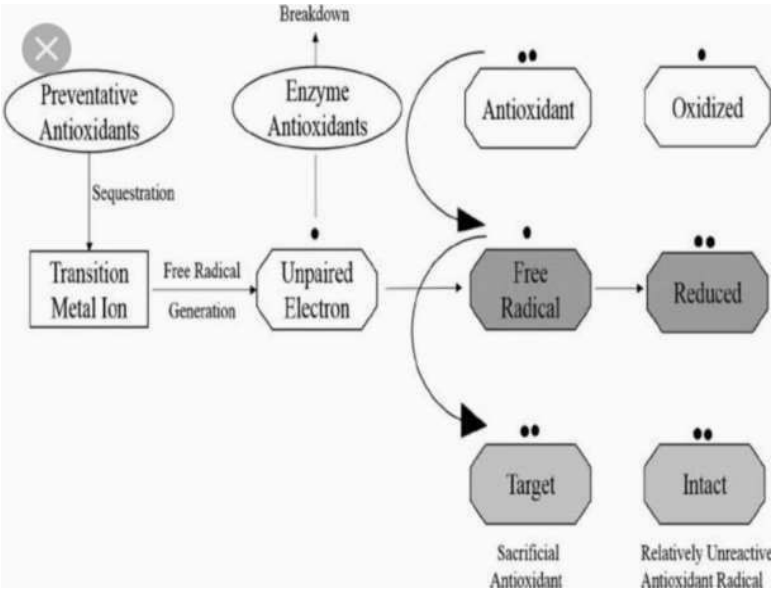

Fig No.7 Mechanism Of Action Of Natural Antioxidants

Fermented Papaya Preparation (FPP) is a product obtained from the yeast fermentation of non- genetically modified Carica Papaya Linn, and has been shown to represent a valuable approach in obtaining systemic antioxidant effects. The study of Logozzi et al. was aimed at verifying FPP's in vivo anti-aging effect, together with the modulation of the intracellular antioxidant system. Mice (C57BL/6J) were treated daily with FPP from either the 6th week or the 51st week of age. After a 10 month treatment, FPP led to an increase in telomeres length in the bone marrow and ovary, together with an increase in the plasmatic levels of telomerase activity and antioxidant levels, and a decrease of ROS. Interestingly, the treatment started at six weeks of age was more effective, suggesting a potential preventive role of FPP against molecular damages induced by age when started precociously. [68,69]

(1) Material

Freeze-dried extracts obtained from Ginkgo biloba L., Dimorphandramollis Beth, Ruta graveolens and Vitis vinifera L. leaves from local Brazilian market were usedA blend of extracts (1:1:1:1) exhibiting in vitro sun protection factor (SPF) values of 8.31 ± 0.5 from G. biloba L.7.72 ± 0.4 from mixed sample, 7.08 plus/minus 0.4 from Rgraveolens L, 5.04 plus/minus 0.2 from D. molliBenth, and 3.71 plus/minus 0.5 from V. vinifera L.was used in this workEmulsions were prepared using tribehenin and a mixture consisting of sorbitan stearate and sucrose cocoate as emulsifiers obtained from Croda (Campinas, São Paulo, Brazil)A mixture of sucrose palmitate glyceryl stearate and sucrose and glyceryl stearate citrate and xanthan gum and manna supplied by Croda (Campinas, São Paulo, Brazil) was used as thickener Caprylic/capric triglyceride hydrolyzed wheat protein/polyvinylpyrrolidone (PVP) cross-polymer and Perseagratissima (avocado) oil were also supplied by Croda (Campinas,SãoPaulo, Brazil). Phenoxyethanol, glycerin and tale were provided by PharmaSpecial (São Paulo, Brazil). Isopropanol and ethyl alcohol were provided by Synth (São Paulo, Brazil), 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol (High Performance Liquid Chromatography (HPLC) grade), formic acid and MTT 13-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide, thiazolyl blue tetrazolium bromide] by Sigma-Aldrich (São Paulo, Brazil)Phosphate buffered saline and sodium dodecyl sulphate were provided by Gibco (Waltham, MAUSA) Quercetin (93.3% of purity) and rutin (97.3% of purity) analytical standards by Acros (Itu, Brazil)

(2) Development of Emulsion and Stability Study

Nine (F1 to F9) oil-in-water (o/w) emulsions were produced according to the combinations depicted in Table 1, by heating both phases (aqueous and oily) up to 70 plus/minus 3 deg * C and then homogenized under cooling until 125 + 3 deg * C Aqueous phase, composed of phenoxiethanol, glycerin, and water, was heated at 70 deg * C in a beaker using

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a heating plate (Quimis, São Paulo, Brazil). The oil phase, composed of the remaining components listed in Table 1 (except talc), was heated at the same temperature in a beaker using a heating plate (Quimis, São Paulo, Brazil) until complete melting. The aqueous phase was then poured into the oil phase under manual stirring followed by cooling down to 135 deg * C Upon the production of the emulsion, tale was added under manual stirring. [78]

To evaluate the physical stability of emulsions, 5.0 g of each samples were subjected to three centrifugal cycles at 3000 rpm for 30 min in each cycle. This assay was performed at 27 plus/minus 2 deg * C Organoleptic characteristics (colorodor, and appearance), pH (QuimispHmeter, São PauloBrazil), densityand viscosity values were also assessedDensity was calculated based on the difference between pycnometers weight (without and with 5 mL of sample) divided by the sample volume. Viscosity assay was carried out at 1.5 rpm rotation for 30 s using spindle number 4 and rotational viscometer (BrookfieldMod LV-TSãoPauloBrazil). The result was expressed in centipoise (cP) and the assay was performed at 37 + 7 deg * C [79]

(3) Sensorial Analysis

Emulsions with better outcomes from the stability tests were subjected to sensorial analysis, usingthe sensory difference and preference tests . After signing the informed consent, fifty volunteers (37 women and 13 men) aged between 20 and 50 years old have selected the test emulsions randomly. Volunteers were given 0.1 g of the produced emulsion to be evaluated for the speed absorption, residual fatty sensorial, speed drying, stickiness, spreading, and dry touch, rating each parameter on a scale (like and dislike). The best emulsion was selected for the loading of flavonoids. [80]

(4) Production of Phytocosmetic and Stability Study

A phytocosmetic was prepared by adding 200 mg of blend of plant extract (Ginkgo biloba L., Dimorphandramollis Beth, Ruta graveolens and Vitis vinifera L-1:1:1:1) to the emulsion selected from the sensorial analysis, corresponding to 0.2% of bend extract in the final formulation. The obtained phytocosmetic was then subjected to a stability study. Stability study was conducted using the multisample analytical centrifuge Lumisizer (LUM, GmbH, Berlin, Germany). Plain emulsion (without the plant extract) and the developed phytocosmetic (emulsion with the plant extract)werediluted in distilled water(1:5;w/w) and evaluated for 2 h at 3000 rpmand at 27.5±0.5°C. [81]

The concentration of flavonoids- quercetin and rutin-was determined by High Performance Liquid Chromatography (HPLC) with diode array UV/vis detector. Emulsions submitted to stability study were dissolved in isopropanol (1:10, w/v) and filtered in 0.45 um membrane (Merck, Darmstadt,
Germany). Quercetin (50 ug/mL) and rutin (500 ug/mL) of analytical standard were analyzed by HPLC for comparison. Samples (5 µl) were injected in HPLC-DAD (Agilent, Technologies 1250 infinity, Santa Clara, United States) with flow rate of 0.3 mL/min for 10 min, at 27 ± 1 °C, using methanol grade HPLC acidified with 0.1% (v/v) of formic acid (Synth, São Paulo, Brazil) as the mobile phase. Flavonoids identification was carried out at 257 nm in a monomeric chromatographic column C (Phenomenex, Alcobendas, Spain). [82]

(5) Zeta Potential

Plain emulsion (without the plant extract) and the developed phytocosmetic (emulsion with the plant extract) were diluted in distilled water (1:500; w/w), and 1 ml of samples was subjected to Zetasizer® equipment (Malvern Instruments Ltd., Malvern, Worcestershire, UK) at 25.0±0.1 °C. Results were obtained of 10 measurements for each sample and assay was carried out in triplicate. [83]

(6) Droplet Size Distribution

For the determination of the droplet size distribution, each plain emulsion (without the plant extract) and developed phytocosmetic (emulsion with the plant extract) was diluted in distilled water prior to the analysis by laser diffraction using particle size analyzer (MasterSizer, Malvern, UK) and large volume sample dispersion units (Malvern Hydro 2000 MU, Malvern, Germany). Study was carried out at 750 rpm using obscuration range of 10-20% and water as dispersing liquid. Assays were performed in triplicate. [84]

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(7) Mechanical Analysis

The textural analysis was performed in a texturometer (Stable Micro Systems TA-XT2i, Godalming, UK), using compression mode. Penetration test was carried out in triplicate at 3 mm/s and applying 0.05 N. A cylindrical probe (SMS P/IR) was used and parameters such as rupture strength (G), adhesiveness (g.sec) and brittleness (mm) were determined. A load cell of 5 kg was used. Firmness (G) was evaluated using the extrusion cell (HDP/FE) and cylinder probe of 5 mm,measured in triplicate. [85]

For the spreadability assay, one gram of each sample was introduced in the glass plate, and otherglass plate of known weight (420 ± 1 g) was placed over the sample. After 1 min, diameter was readwith the aid of millimetric graph paper scale. This procedure was repeated successively by addingother plates of the same weight in one-minute intervals until spreading stopped. All measurementswere performed in triplicate, and results were expressed as the spreadability of samples against the applied weight.

The rheological behavior was evaluated using a Rheometer (Anton Paar, MCR 102, Ostfildern, Germany) and a cone-plate sensor (CP50-1). The data were analyzed with Rheoplus V3.61 software. Initially, the flow curve was obtained to determine the hysteresis area through an up- down test. The shear rate was applied from 0 to 100 s^{1} for the upward curve and from 100 to 05 for the downward curve. The recovery test was then performed in three intervals. In the first interval, a shear rate of 1 s^{1} was applied for 20s. Then, in the second interval, a shear rate of 100 s^{1} was applied for 60s, and finally, in the third interval, a shear rate of 1 s was applied for 100s to analyze the samples recovery. Then, the amplitude sweep was performed at a constant angular frequency of 10 rad s^{1} to define the linear viscoelasticity (LVE) range (= 0.1% for all samples). Subsequently, the frequency sweep test was performed in a frequency range from 240 to 0.1 rad s, to evaluate the viscoelastic behavior of the samples. All assays were performed in triplicate at a plate temperatureof 25 °C using a solvent trap. [86]

(8) Thermal Analysis

The samples were subjected to differential scanning calorimetry (DSC) and thermogravimetry (TG) analyses, both carried out in triplicate. Approximately 10 mg of sample were placed in an aluminum straw for DSC analysis (Mettler DSC 823e System, Mettler Toledo, Spain), at a heating rate of 15 °C/min in N2 atmosphere, from 25 to 350±1 °C temperature For TG analysis (TA- 50WSI, SHIMATZU, 50WSI, Tokyo, Japan), approximately 10 mg of sample were placed in an aluminum straw and analyzed at 10 °C/min heating rate in N_2 atmosphere with a temperature range of 25 to 500 ± 1 °C. [87]

(9) Microbiologic Control

Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testingof Chemicals. Formulations with extracts were dosed topically in a 3D Reconstructed Human Tissue model. for 42 min. After 42 h post-treatment incubation period, the tissue sample was placed in MTT 13-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue tetrazolium bromide] (Sigma-Aldrich, São Paulo, Brazil) solution at 1 mg/ml. for 3 h. The blue formazan concentration formed in viable cells was measured at 570 nm using spectrophotometer (Versamax, Molecular Devices, São Paulo, Brazil). To evaluate the reliability of the assay, SkinEthic™M RHE (1% Triton X-100) was used. Negative (phosphate buffered saline) (Gibco Waltham, MA, USA) and positive controls (5% aqueous sodium dodecyl sulphate) (Gibco Waltham, MA, USA) were used. According to the literature [47] and as described in the UN GHS (United Nations Globally Harmonized System) Category 2, a sample is considered irritant to skin if the tissue viability is less than or equal to (s) 50%, after exposure and post-treatment incubation period. [93]

(10) Statistical Analysis

All assays were carried out in three or more replicates, and statistical analysis was carried out using ANOVA test ($p < 0.05$) and Origin (v8.0) software for Windows.

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II. CONCLUSION

Antioxidants from natural sources are valuable bioactive compounds with well-demonstrated potentials for use in the food industry. Beyond their application in functional food products, attention has also been focused on their use as alternatives to their synthetic counterparts to increase product stability and avoid deterioration by oxidation during processing and storage. In the context of a circular economy, efforts are being dedicated to the use of natural antioxidants from food by products generated by the agricultural industry and from underexploited plant materials.

Each step between the extraction and the application of natural antioxidants has already been a focus of research. Regarding the extraction step, the selection of the most appropriate techniques differs according to the type of compounds targeted for recovery. More environmentally friendly techniques have been explored to avoid the large amounts of solvents used in conventional solvent extraction processes. Although replacing conventional technologies by non-conventional ones hase merged, improvements are necessary in terms of scaling up. Concerning the stabilization processes after extraction, spray drying has been the most-used process, mainly due to its simple operation and scaling up, delivering encapsulated antioxidants in the form of powder microparticles, enabling easy manipulation and dosages.

Although these compounds are derived from natural sources, their applications to food products must take into account their dosages and possible toxicological effects. Moreover, negative effects on sensory attributes, especially flavor and taste, imparted by some natural compounds, have to be addressed. This will increase the consumer propensity to purchase food products containing natural antioxidants, ultimately contributing to decreasing the prices of these products

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