Plant bioactives and biotechnological approaches to enhance their production and bioactivity

Abstract

Plants considered as an excellent source for discovering new compounds with medicinal significance in drugs/ functional foods/ nutraceutical formulations. Bioactive compounds of found in plants are those secondary metabolites exhibiting health benefit in human. It is obvious that studies related to plant bioactives engaging to develop in many aspects involving researchers from different scientific disciplines. In the explore for alternatives for producing and enhancing the desirable bioactives from plants, biotechnological approaches such as fermentation, plant tissue culture, metabolic engineering, genetic transformation, plant cell elicitation, microencapsulation (as delivery mode) are found to have potential in industrial production of bioactive plant metabolites. The present review discusses the different categories of plant bioactives along with an overview of their medicinal applications as antioxidants, anti-inflammatory agents, anti-diabetic and anti-cancer agents. The current trend on applications of recent biotechnological tools in the production of plant bioactives has also been discussed.

1. Introduction

It is well known fact that the plants with medicinal properties are the most widely used source of life saving drugs for the human beings. In indigenous medicine uses different kind of plant extracts with the bioactive constituents as medicines. Plants produce a variety of organic compounds, the great majority of which do not appear to participate directly in growth and development and these substances, traditionally referred to as secondary metabolites (Hussain et al., 2012). Bioactive compounds of plant origin are those secondary metabolites exhibiting health benefit in human (Kaur and Das 2011). Recent evidence from various epidemiological and clinical studies has proved that a diet rich in plant foods can reduce the risk of some chronic diseases, such as diabetes, obesity, cardiovascular complications, and cancer. Therefore, bioactives extracted from plants are used as pharmaceuticals, agrochemicals, cosmetics and food additives. It is obvious that works related to plant bioactives continue to develop in many aspects involving researchers from various scientific disciplines. In the search for alternatives for

production and enhancements of desirable bioactives from plants, biotechnological approaches such as fermentation, plant tissue culture, metabolic engineering, genetic transformation, plant cell elicitation, microencapsulation (as delivery mode) are found to have potential in industrial production of bioactive plant metabolites. The present review discusses the different categories of plant bioactives along with an overview of their medicinal applications as antioxidants, anti-inflammatory agents, anti-diabetic and anti-cancer agents. The current trend on applications of recent biotechnological tools in the production of plant bioactives has also been discussed.

2. Plant bioactives and their uses

With the development of analytical techniques including gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry and nuclear magnetic resonance spectrometry, the isolation, purification, and structure elucidation of bioactives in plant extracts have become available. These biologically active compounds are also known as "phytochemicals.". These phytochemicals including polyphenols, alkaloids, terpenes, saponins, etc. have been widely used in nutraceuticals as ingredients to provide a health benefit beyond basic nutrition. Table 1 shows the four most common categories of these phytochemicals such as polyphenols, alkaloids, terpenes, and saponins and their common examples and uses as described in Hussain et al (2012).

Table. 1. Plant Bioactives and their uses (Adopted from Zhao et al, 2015)

Bioactive Category		**Bioactives**	**Uses- common examples**
Polyphenols	Simple Phenolics	hydroxybenzoic acids: gallic acid, salicylaldehyde, protocatechuic acid, salicylicacid, hydroxycinnamic acids: caffeic acid, coumaric acid, sinapic acid and ferulic acid,	Hydroxybenzoic acids considered as potent antioxidants that may help to protect the human body from free radicals. Gallic acid is used as an astringent and styptic, and it also shows antimelanogenic, antineoplastic and bacteriostatic activities.

			Salicylic acid has anti-inflammatory, keratolytic, antipyretic, analgesic, antifungal, and antiseptic properties for several skin conditions such as seborrheic dermatitis and dandruff, acne, psoriasis and ichthyosis
	Coumarins	Imperatorin and xanthotoxin	Coumarins are mainly found in higher plants (e.g. Rutaceae and Umbelliferae). fruits (e.g. chicory, cloudberry and bilberry and green tea) coumarins and coumarin derivatives are having free radical scavenging activity, anti-inflammatory, anticoagulant, anticancer and Antimicrobial activities
	Lignans	Enterodiol, Enterolactone	Plant lignans show strong antioxidant activity higher than vitamin E. It may be effective in treating different other diseases such as diabetes and cardiovascular disease
	Flavonoids	Flavones : Luteolin ,Apigenin Flavonols: Myricetin ,Kaempferol, Quercetin Flavanones: Hesperetin, Naringenin, Eriodictyol Flavonols: Gallocatechin, Catechins	Apigenin possess hypotensive, antiinflammatory, antibacterial and diuretic activity while promoting smooth muscle relaxation. Quercetin is an effective antioxidant important in protecting the body against free radicals. It also shows anti-inflammatory, anticancer, antiatherosclerosis and cholesterol lowering properties, protecting eye health
	Isoflavonoids	Isoflavone, Isoflavanone, Isoflavanol, Isoflavene, Rotenoid, Daidzein, Genistein	The soy isoflavones are having structures similar as mammalian estrogens which show the capacity of working as phytoestrogens
	Anthocyanins	Cyanidin, Delphinidin, Pelargonidin, Peonidin, Malvidin, Petunidin	Antioxidant activity, antiedema, anticarcinogenic activity and anti-

			inflammatory activity, reducing capillary permeability, enhansing the regeneration of visual purple, improving vision at dusk
	Tannins	1.Condensed tannins (Proanthocyanidins) 2.hydrolysable tannins	Cinnamon can act as antitumor, antioxidant, anti-inflammatory and cholesterol-lowering agent, as a treatment for infectious diseases and preventing cardiovascular diseases.
	Quinones	Naphtodianthrone, *p*-Quinone, *o*-Quinone, Anthraquinone, Naphthoquinone,	Aloe-emodin is type of a natural anthraquinone extracting from Aloe vera L. leaves which has been reported to reveal anti-neuroectodermal tumor activity
	Stilbenes	Resveratrol	Resveratrol is a powerful antioxidant that may play a role in the chemopreventive. Inhibit carcinogenesis at the initiation, promotion, and progression stage
Alkaloids		Tropane alkaloids, indole alkaloids, quinolone alkaloids, pyrrolidine alkaloids, isoquinoline alkaloids, and izidine alkaloids	Yohimbine is an active indole alkaloid with having sexual stimulant and aphrodisiac effects which found naturally in Pausinystalia yohimbe. Caffeine - a stimulant for wakefulness and to enhance concentration and minimize the fatigue feel
Terpenes (isoprenoids)		Classify terpenes according to their molecular formula monoterpenes, C10; sesquiterpenes, C15; diterpenes, C20; sesterterpenes, C25; triterpenes, C30;	Thymol- antiworms, antibacterial Lutein -is a xanthophyll belongs to carotenoid group, which shows eye protection activity
Saponins		Chemically, saponin can be divided into two groups as triterpenoidal saponins and the steroidal saponins	*Centella asiatica* is a medicinal plant containing high amounts of triterpenoid saponins which are the primary active constituents in *C. asiatica*

		2. Ginsenosides are the primary active ingredients of ginseng and belongs to triterpene saponins

3. Biotechnological approaches to enhance production) and bioactivity

3.1 Fermentation

Fermentation is a well-established classic industrial process that has been utilized for the enhancement of shelf life, nutritional and organoleptic properties and for the removal of undesirable compounds from primary food substrates. Recently it has been explored as an economically viable cost-effective approach to improve the synthesis of secondary metabolites and their biological properties to achieve diverse functional benefits. In the concept of biotechnology, fermentation is a term that is used to describe the activity associated with the deliberate utilization of microorganisms to formulate products such as recombinant commodities, enzymes and biomass beneficial to humans. More specifically when focusing on bioactive compounds, during the process of fermentation unfavorable substrates are converted into components that are acceptable for consumption, by the action of microbial enzymes. This is in turn improves the substrate properties by enhancing the production and the bioactivity of bioactive compounds (Hussain et al., 2016).

Generally, each fermentation system is composed of three basic aspects: substrate, microorganisms and environmental conditions. Depending on the variation in these three aspects, outcomes of fermentation can vary over a wide range. The type of microorganisms used for fermentation include either bacteria or fungal groups. Thus, the fermentation is based on their action on the substrate. Depending on the microbial activity, different types of fermentation such as alcoholic, propionic, lactic and malolactic fermentation can occur. However they all possess quite similar features as they are anaerobic catabolism of organic compounds that could yield different types of bioactives. Basically, fermentation process is

classified into two systems; Solid-State Fermentation (SSF) and Submerged fermentation (SMF). SMF is based on the cultivation and growth of microorganisms on a liquid medium which contains necessary nutrients whereas SSF utilizes a solid substrate with very low moisture content that could support the growth of the microbes and facilitate product formation. However, the moisture content available in the substrate is quite sufficient for the microbial growth and metabolism (Martins et al., 2011). This part of the chapter encompasses the usage of fermentation as a biotechnological approach to enhance the contents of bioactives in different plant food groups such as vegetables, fruits, cereals and legumes. Additionally, various bioactivities of these bioactive compounds enhanced through fermentation is also discussed.

3.1.1 Enrichment of bioactive compounds in plants through fermentation

Bioactive compounds are available in different concentrations in the matrix of plants and their availability is influenced by several factors. Alteration of these factors by fermentation has been an efficient and secured method to enhance the accessibility of these bioactive compounds for beneficial purposes. Influence of fermentation on protein solubility, hydrophobic and hydrophilic domains of the components, bio-transformation and generation of novel metabolites, mobility of bioactives, glycosidic bonds and structural break down of plant cell walls have been attributed as the main causes for the improved bioactive content in post fermentation conditions (Sadh et al., 2018; Sandhu and Punia 2017; Hussain et al., 2016).

3.1.1.1 Fruits

Fruits are one of the main sources of dietary bioactives specially polyphenols which has been widely researched in recent times. They are rich sources of phenolic acids, flavonoids, proanthocyanins and hydrosable tannins which have focused the attention of researchers to enhance their value by using processing technologies including fermentation. Fermentation

process has been applied for whole fruits, fruit juices and other related products to improve their bioactive content. For example, studies conducted by Lee et al., (2016) on fermenting *Morinda citrofolia* (Noni) fruits using *Pediococcus pentosaceus* BCRC 14053 has significantly ($p<0.05$) increased the Total Phenolic Content (TPC) of the aqueous extracts of the fruits. Also the researcher has revealed that the octanoic acid content of the fruits has increased approximately by 1.5 folds compared to the non-fermented fruits. During the fermentation of *Punica granatum* (Pomegranate)fruits by *Saccharomyces cerevisiae* and juice by lactic acid bacteria (LAB) content of gallic acid and ellagic acid in fruit and juice respectively has increased due to the hydrolysis of ellagitannins and other oxidation reactions.(Gumienna et al., 2016). Fermentation of *Sambucus nigra* (Elder berry) juice with different strains of LAB increased the polyphenolic content by 60%-80% depending on the strains. This study also highlights the bacterial fermentation mechanism of producing phenyllactic acid through amino acid metabolism, modifications in hydro cinnamic acid and increase in flavonoid glycosides by deconjugation process of β-glucosidase activity (Ricci et al., 2019).

3.1.1.2 Vegetables

Consumption of vegetables either in raw or processed forms provide a numerous health benefits such as controlling degenerative diseases. Vegetables contain a number of polyphenol classes, including hydroxycinnamic acids, stilbenes, flavonols, flavan-3-ols, flavones, anthocyanins, phenolic aldehydes, tannins and proanthocyanidins (Andarwulan et al., 2012). Most commonly LAB fermentation has been employed as one of the techniques to efficiently exploit bioactives from vegetables.

Pan et al., (2015) has suggested that phenolic content in Chinese jiaotou bulbs can be increased by the fermentation of these bulbs with *Lactobacillus plantarum* ZDY 2013. Accordingly, the investigation has shown a 2.7-fold increase in the TPC and the researcher

suggests that in addition to the catalysis of glycosides and release of conjugated phenols, secretion of cellulase and tannase by the bacteria can break down the cell walls of plants and facilitate the migration of phenols and contribute to the increased TPC. The investigation of Cai et al., (2019) on enhancing the biotransformation of glucoraphanin into sulforaphane in *Brassica oleracea* (Broccoli) using strains of *Leuconostoc mesenteroides* and *Lactobacillus plantarum* has given a positive result by increasing the yield of sulforaphane in the broccoli puree. Also, the formed sulforaphane was stable throughout the storage period indicating that fermentation facilitates the production of safe stable sulforaphane in broccoli puree. Additionally, it also has increased the TPC of the puree by approximately 83%. *Cucumis sativus* (Cucumber) fermented with strains of *Lactobacillus pentosus* has been reported to show increased contents of total bioactive peptides. Also, the study has identified the synthesis of 05 bioactive peptides: IPP/ LPP, VPP, KP, and RY during the fermentation process suggesting that lactic acid fermentation of cucumber can be used to enhance its bioactive composition (Fideler et al., 2019).

3.1.1.3 Cereals

Cereal grains are considered to be an important source of dietary carbohydrates, proteins, fiber, vitamins and phytochemicals such as phenolics, saponins and phytoestrogens. Fermentation of cereals have been practiced for the production of beverages, bakery products and gruels for a long time. Recent fermentation techniques include SSF of cereals by different strains of fungi.

SSF of *Pennisetum glaucum* (Pearl millet) by *Aspergillus sojae* can be used to improve the phenolic content of finger millet. Accordingly, after 06 days of fermentation 5.3-fold of increase in phenolic content was observed. At the same time based on HPLC analysis the content of ascorbic acid, gallic acid and p-coumaric acid was higher in the fermented pearl millet compared to the unfermented pearl millet (Salar et al., 2017). Janarny and Gunathilake

(2020) have been reported the favourable effect of SSF on rice bran bioacives using *Rhizopus oryzae.* Investigations conducted by Sandhu and Punia (2017) on fermentation of different cultivars of *Hordeum vulgare* (Barley) by *Aspergillus awamorinakazawa* (MTCC-548) has revealed that SSF effectively increases the TPC and total flavonoid content (TFC) of barley with the maximum content observed at the 5^{th} day of fermentation. Fermentation of *Avena sativa* .L (Oats) by *Monas cusanka* has shown to increase the free, bound and conjugated phenolics in different fractions rising up the TPC by 18 folds. During this fermentation a significant increase in chlorogenic acid, caffeic acid, ferulic acid, catechin and rutin was observed (Bei et al., 2017). The sequences of amino acid of fermented *Chenopodium quinoa* (Quinoa) flour analyzed by chromatography showed features similar to the antioxidant peptides. This indicates the proteolytic capacity of autochthonous lactic acid bacteria that could release peptides with antioxidant activity from native quinoa proteins (Rizzello et al., 2017).

3.1.1.4 Legumes

Legumes are one of the major dietary sources of carbohydrate and protein. Fermentation of legumes has been important in improving the nutritional content by increasing the antioxidants, vitamins and also enhances the digestibility of certain other compounds. Additionally, it is important to decompose anti-nutrients present in the legumes. *Phaseolus vulgaris* (Kidney beans) subjected to SSF by *Bacillus subtilis* significantly improved ($p<0.05$) the soluble phenolic content with fermentation. This was mainly due to the hydrolysis of β-glucosidic bonds from conjugated phenolics and release of phenolic aglycones by the activity of β-glucosidase produced by the bacteria (Limón et al., 2015). Fermentation of *Glycine max* (Soybean) with *Aspergillus oryzae*, *Rhizopus oryzae*, *Bacillus subtilis* and *L. plantarum* produced an increase in the TPC of soyabean (María Landete et al., 2015). Work by Gan et al., (2017) revealed that fermentation of *Vigna unguiculate* (Mottled

cowpea) significantly increased the catechin content due to the degradation of proanthocyanidins available in the bean coat. Natural fermentation of mottled cowpea also increased the contents of bound ferulic and p-coumaric acids.

3.1.2 Enhancing bioactivity of plants through fermentation

In addition to the enrichment of bioactive compounds previous reports indicate that fermentation also enhances the therapeutical potential of plants by enhancing their bioactivities and disease preventing effects.

3.1.2.1 Antioxidant property

Bioactive substances present in plant matrix exert antioxidant activity by controlling oxidative stress through several different mechanisms including quenching of singlet oxygen, acting as reducing agents, scavenging free radicals and complexing of pro-oxidant metals. Fermentation also has had various effects on antioxidant activities.

In Oats fermented by *Monascusanka* and Quinoa flour dough fermented by LAB the DPPH and ABTS radical scavenging activities of free, bound and conjugated phenolics have significantly increased (Bei et al., 2017; Rizello et al., 2017). Considering legumes, FRAP values of naturally fermented mottled cowpea and speckled kidney beans have significantly increased indicating an increase in the reducing power of the tested grains (Gan et al., 2016). Investigations by Bhat et al., (2016) has revealed that FRAP activities of Guava fermented with *Lactobacillus planatarum* has increased and reached the highest level at the 16^{th} hour of fermentation.

3.1.2.2 Anti-inflammatory properties

Plant foods are good sources of anti-inflammatory agents due to the presence of potential bioactive compounds which exert a protective role in the inflammatory process thorough reduction of pro inflammatory enzymes and by activating prostacyclin. Enrichment of bioactives by fermentation has supported improved anti-inflammatory process through

diverse mechanisms. Studies by Puupponen-Pimiä et al., (2016) has indicated that fermentation of *Rubuscha maemorus* (Cloud berry) by *Pediococcus pentosaceus* VTT E-072742 has increased inhibitory effect on production of NO and IL6 in bacterial endotoxin LPS activated macrophages. This indicates the enhanced anti-inflammatory of cloudberries after fermentation. Fermentation of mixture of vegetables by *Lactobacillus planatarum* has shown a significant reduction in the secretion of IL 6 and TNF-α levels in RAW 264.7 cells indicating an increased anti-inflammatory property after fermentation. It was suggested that this increased in the activity corresponds to the synthesis of metabolites mainly, organic acids and glycerol (Kim et al., 2019). The results obtained in the work of Gabriele et al., (2018) indicate that sourdough fermentation is a beneficial technique to enhance the protective effect of *Triticum aestivum* (Wheat) flour against changes induced by activity of TNF-α. Fermented wheat flour (FW) pretreatments exhibited inhibitory effect on upregulation of IL-8 and COX-2 higher than unfermented wheat flour (UFW) at equivalent doses. Moreover, compared to effects of UFW, FW better regulated the proinflammatory response through modulation of NF-κB signaling pathway and upregulation of HO-1, at exposure to TNF-α and at basal conditions.

3.1.2.3 Anti-hypertensive properties

Secondary metabolites as well as functional peptides of plants have shown to exhibit anti-hypertensive properties. Various clinical trials conducted based on fermentation have revealed that peptides obtained from fermented products are capable of expressing angiotensin converting enzyme (ACE-I) inhibitory property that can applied for the treatment of hypertension. ACE plays a crucial role in controlling hypertension by involving in the rennin angiotensin pathway thus ACE inhibitory activity is used as an *in-vitro* indicator of anti-hypertensive properties of functional foods. Bioactive peptides released by the action of microbial proteases after SSF by LAB in lentils has shown to increase the ACE inhibitory

activity by 93% (Torino et al., 2013). ACE inhibitory activity in Lupin and Quinoa fermented with *Bifidobacterium breve* and *Bifidobacterium longum* has increased significantly after 24h of fermentation. This was primarily due to the changes that has occurred in proteolysis during fermentation (Ayyash et al., 2018). Investigations by Ahrén et al., (2015) have shown that in studies done using rats, administration of blueberries fermented with *Lactobacillius plantarum* were able to lower the L-NAME induced blood pressure increase within two weeks of dose administration. It has been emphasized that the novel phenolic acids such as phenyl actic acid, hydroxyphenyl lactic acid, 3,4-hydroxyphenyl-propionic acid generated during the fermentation process could have contributed to the increase in anti-hypertensive properties of blueberries.

3.1.2.4 Anticancer property

Different types of bioactive compounds from plants have been reported to combat cancer efficiently through diverse mechanisms. Plant based anticancer agents are capable of inhibiting angiogenesis, suppressing cell proliferation and mitigating chances of tumor development. Fermentation has been an efficient strategy to improve the bio efficacy of polyphenolic compounds from plants and utilize them as anticancer agents.

In the study conducted on blueberries fermented with *Lactobacillus plantarum* the fermented blueberry extract were able to inhibit the proliferation of HeLa cells, with very low cytotoxicity to normal fibroblast cells than raw blueberry extract. This suggests that fermentation of blueberries by *L. plantarum* bio-transformed the available phenolic compounds into active metabolites that are capable of inhibiting proliferation (Ryu et al., 2019). Investigation on *Brassica oleracea* (Curly kale) which is a member of *Brassicaceae* family fermented with LAB showed a statistically confirmed antitumor activity. Salicylic acid and gentisic acid produced during this fermentation process was proved to control the solid tumors in both *in vitro* and *in vivo* conditions by reducing the number of HT29 and

SW620 cells (Michalak et al., 2020). Hydrophilic extracts of *Ipomea batatas* (Sweet potato) fermented with *Lactobacillus acidophilus* exhibited an increased level of inhibition on PC-12 cell proliferation depending on the administered dose compared to the raw sweet potato extracts. The researcher suggests that this is possibly due to increased content of phenolics such as caffeic acid, p-coumaric acid and ferulic acid generated during the fermentation process (Shen et al., 2018).

3.1.2.5 Neuro protective properties

Naturally occurring bioactives are recently being investigated for the treatment of several neurodegenerative diseases. Neuro protective effects have been assessed based on attenuation of weakened acquiring and memory caused by cerebral ventricle administration of Aβ-42 peptides, mitigation of oxidative stress caused by Aβ-42 peptides in neurons and mitochondria of rat brains, control of cerebral stroke, familial amyotrophic lateral sclerosis and ischemia-reperfusion injury.

Studies conducted on a Cheonggukjang (CGK), a fermented soy bean product produced by the mixture of *Bacillus subtilis* and *Lactobacillus sakei* has shown to exert protective effects against alterations in neuronal cell death, acetylcholinesterase (Ach) activity, nerve growth factor secretion and signaling pathway in trimethyltin induced cognitive defects in mice (Go et al., 2016). Compared to unfermented ginger, administration of fermented ginger (FG) boosted recognition memory, reduced by scopolamine injection. In addition, FG was able to normalize impairment of memory in amyloid beta (Aβ) plaque-injected mice by protecting neuronal cells in their hippocampus. Also, FG restored the pre and postsynaptic protein levels decreased by a β plaque-toxicity (Huh et al., 2018).

3.2.Tissue culture technology

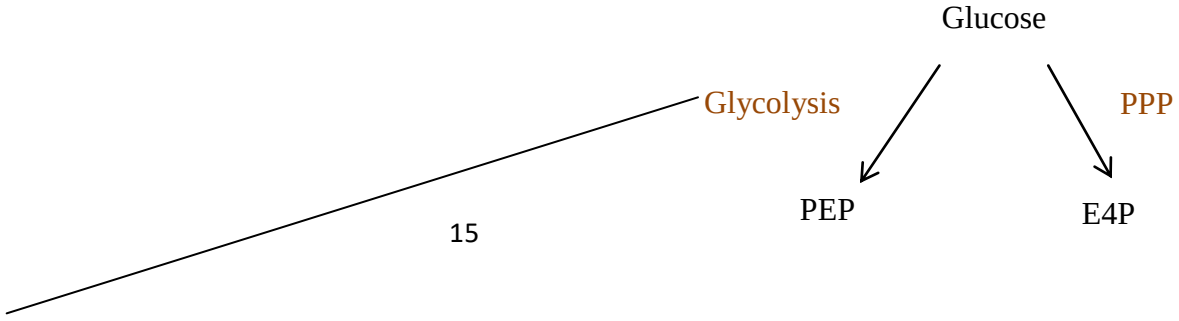
Plant cell culture and tissue culture hold great promise for controlled production of useful bioactive compounds/secondary metabolites (Hussain *et al*., 2012). Based on the recent

research studies, cell culture techniques show potential for producing specific medicinal molecules or compounds at a rate similar or higher to that of intact plants have accelerated in the last few years. In the aims of obtaining higher yields required for commercial applications, many studies have been focused on isolating the biosynthetic activities of cultured cells, achieved by optimizing the culture properties, using high-producing strains and employing precursor feeding, transformation methods, and immobilization techniques (Hussain et al., 2012).

According to Giri and Narasu (2000), transgenic hairy root cultures have transformed the role of plant tissue culture in bioactive compounds/secondary metabolite production. With regard to their genetic and biosynthetic stability, easily maintain and faster in growth, transgenic hairy root cultures are considered as more unique. Using this tissue culture technique, different types of chemical compounds has been synthesized. Advance techniques in tissue culture combine with improvement in genetic engineering aspects of nutraceuticals, pharmaceuticals, and other beneficial substances that are helpful for the synthesis of bioactive compounds. Recent developments in the fermentation technology, molecular biology, and enzymology of plant cell cultures put forward that these techniques will become a viable source of important bioactive compounds or secondary metabolites (Abdin, 2007). Genome manipulation is ensuring in relatively huge amounts of advantageous compounds synthesized by plants infected with an engineered virus, whereas transgenic plants can maintain constant levels of production of proteins without additional mediation (Abdin and Kamaluddin, 2007). Plant tissue culture in large scale is recognized to be an attractive possible perspective to conventional methods of plantation as it provides controlled supply of bioactive compounds without depending on plant availability.

3.3. Metabolic engineering and production of bioactives.

The metabolic pathways of plants are more comprehensive compared to metabolic pathways of other organisms. Through metabolic pathways, plants produce primary metabolites such as sugars, amino acids, lipids, nucleic acids and energy sources and vast range of secondary metabolites named as bioactive compounds. More than 200,000 of bioactive compounds from plant sources have been identified and studied (Aharoni and Galili, 2011). The main difference between primary and secondary metabolites is that primary metabolites participate in growth and development but secondary metabolites or bioactive compounds are not directly contribute in growth and development. However, they play an important role in plants as defense agent while people use bioactive compounds as food medicines since long time (Tiwari and Rana, 2015). Plant bioactive compounds can be divided into main three classes including phenolic compounds, alkaloids and glucosinolates (nitrogen or sulfur containing compounds) and terpenoids/isoprenoids. These groups of bioactive compounds are synthesized by different metabolic pathways such as glycolysis, Krebs cycle and pentose phosphate pathway (Aharoni and Galili, 2011).



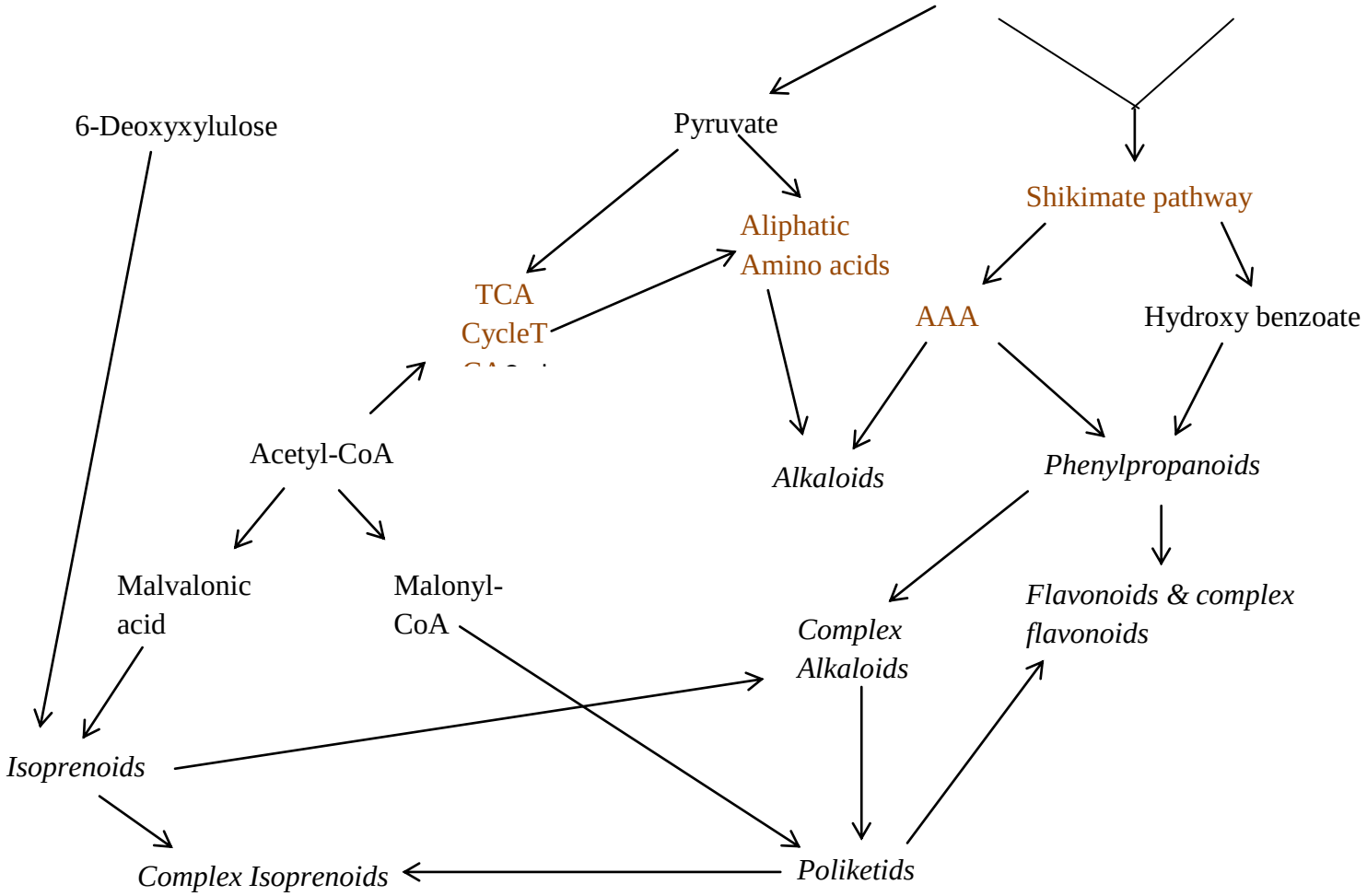


Figure 1. Production of bioactive compounds through different metabolic pathways.
Modified from Aharoni and Galili, 2011.
Note: AAA; Aromatic Amino Acids, PEP; Phosphoenol pyruvate, E4P; Erythrose-4-phosphate, PPP; Pentose phosphate pathway.

People have being using plant bioactives since ancient time for several purposes, such as color compounds, flavor enhancers, anti bacterial agents, insecticides, most importantly as therapeutic agents for different communicable and non communicable diseases. Hence, bioactive compounds have become to an interested research field in nowadays since many studies with related to human health and well being founded out that modest, long-term consumption of certain bio active compounds will have possibility of preventing the occurrence of many chronic diseases (Enfissi *et al.*, 2010). However, these bioactive

compounds are as in very limited concentrations in plants, thus extraction of large quantities of bioactive compounds from plant sources is not practical. Further, applying conventional extraction processes is not showing expectable results due to some defects such as more time consumption, cost and sometimes it may be environmentally unsafe since solvents use for isolation and purification purposes. Considering all limiting factors, metabolic engineering is an emerging and effective technique which can apply in plant biology. This technique provide different approaches such as to increase and improve productivity of plant secondary metabolites increasing the number of producing cells, increasing the carbon flux through a biosynthetic pathway by overexpression of genes, codify for rate-limiting enzymes or blocking the mechanism of feedback inhibition and competitive pathways and decrease catabolism (Hussain *et al.*, 2012). Importantly, advance techniques in microbial biotechnology have provided significant support for the expression of metabolic pathways to synthesize valuable end products from natural bio actives in microorganisms.

3.3.1. Metabolic engineering of microorganisms for the production of common plant secondary metabolites/bioactives

3.3.1.1. Flavonoids

Flavonoids can be found in all terrestrial plants. There are huge numbers of flavonoid structures synthesizing from phenyl propanoid metabolic pathway (Trantas *et al.*, 2015).

Currently, *Escherichia coli* and *S. cerevisiae* (yeast) are the most utilized microorganism species for production of flavonoids. Examples for producing certain bioactives from plats following metabolic engineering are shown in Table 2.

Table 2. Natural flavonoid products that produced during heterologous biosynthesis in various microorganisms (*Escherichia coli* or *Sacharomyces cerevisiae*)

Flavonoid target	Plant source Common name *Scientific name*	Health benefit/s	Metabolically engineered host	Externally fed precursor	Titer (mg/ L)	References
Flavonones						
Eriodictyol	Mexican oregano *Lippiagraveolens*	Anti-inflammatory and antioxidant	*E. coli*	L-tyrosine	107	Zhu *et al.*, 2014
Pinocembrin	Mexican oregano *Lippiagraveolens*	Reduce cerebral ischemia/reperfusion and blood–brain injury, anti-oxidative and anti-apoptotic effects	*E. coli*	Glucose	40.02	Wu *et al.*, 2013
Naringenin	Tomato *Solanumlycopersicum*	Antioxidant activity, anti-inflammatory, anti-lipidemic and cholesterol lowering effect	*S. cerevisiae*	Glucose	105.9	Koopman *et al.*,2012; Kanaze *et al.*, 2007
Flavones						
Hypolaetin	-	Anti-inflammatory activity	*E. coli*	Luteolin	88	Lee *et al.*, 2014; Barros *et al.*, 2016
Apigeninglucosides	Mexican oregano *Lippiagraveolens*	Antidepressant, anti-inflammatory, hepatoprotective, antithrombotic, anticancer, antioxidant and estrogenic effects	*E. coli*	Apigenin	4.67	Choi *et al.*, 2012; Tang *et al.*, 2017
Flavonols						

3-O-Xylosyl quercetin	-	Lowering of blood pressure, anti-proliferative and apoptotic effects, reduce allergy, asthma condition, cardiovascular diseases, inflammation, arthritis, diabetes and obesity	*E. coli*	Quercetin	23.78	Pandey *et al.*, 2013
Kaempferol 3-O-glucoside (Astragalin)	Bean *Phaseolus vulgaris*	Anti-oxidative, anti-allergic, anti-inflammatory, analgesic, anti-hepatotoxic properties	*E. coli*	Narigenin	109.3	Malla *et al.*, 2013
Quercetin-3-Orhamnoside	Lingonberry *Vaccinium vitis-idaea*	Anti-diabetic, anti-proliferative activity	*E. coli*	Quercetin	200	Kim *et al*.,2012

3.3.1.2. Isoprenoids

Metabolic engineering techniques related to isoprenoid compounds producing pathways are using in synthesizing many vital pharmaceutical and industrial products including terpenes and carotenoids. There are mainly two metabolic pathways to biosynthesis of isoprenoid, namely mevalonate and non-mevalonate routes. The studies have been focused on manipulating these biosynthesis pathways utilizing heterologous hosts such as *Escherichia coli* (Mijts and Dannert, 2003). The non-mevalonate pathway is common in bacteria and produce isoprenoid precursor named as isopentenyl diphosphate from glyceraldehyde-3-phosphate and pyruvate precursors following a series of catalytic paces. Overexpression of certain genes (1-deoxy-D-xylulose-5-phosphate reductoisomerase and 1-deoxy-Dxylulose-5-phosphate synthase) is used as individual or either combined way to increase the yield of carotenoid compounds when combining *E. coli* carotenoid biosynthesis pathway. Furthermore, production of lycopene in *E. coli* species can also be enhanced by changing metabolic flux to generate a balance supply of precursors in non-mevalonate pathway (Farmer and Liao, 2001) and this pathway is used to produced isoprenoid compounds such as taxadiene as well (Huang *et al.*, 2001). The mevalonate biosynthesis pathway is utilized for engineering of the fungus (*Neurosporacrassa)* by overexpressing of S. *cerevisiae* HMG-CoA reductase to increase flux to improve carotenoid compounds production (Wang and Keasling, 2002). The production level of isoprenoid through microorganism's related metabolic engineering is improved by manipulating precursor supply and by controlling downstream isoprenoid biosynthetic pathways.

3.3.1.3. Alkaloids

Plant alkaloids are very important and vital compounds which have many applications in pharmacological industry. There are more than 10,000 structurally identified compounds and

alkaloids are used as clinical drugs (Leonard *et al.*, 2009). A previous study has studied the possibility of producing cathenamine from yeast by expressing genes coding for certain enzymes from medicinal *C. roseus* plant (Geerlings *et al.*, 2001). It is very important to expand the knowledge about utilization of different microbial species in terms of synthesis of bioactive compounds and their biosynthesis pathways.

3.4. Genetic transformation towards manipulate the quality and production of natural bioactive compounds

Genetic transformation is defined as a process which the genetic material taken by an individual cell is changed by the incorporation of exogenous DNA into its genome (Zhou *et al*., 2011). That technique has been implemented to increase the quality and production of bioactive compounds. Mainly, genetic manipulation is done in hairy root culture for production of bioactive compounds which are useful as food additives, pharmaceuticals and cosmetics (Georgiev *et al*.,2007; Srivastava *et al*., 2007). The production of bioactive compounds by inoculating hairy root system with one of the soil bacterium species (*Agrobacterium rhizogenes*) is used as a popular method (Karuppusamy, 2009). In detail, bio active compounds can be synthesized by *A. rhizogenes* using hairy roots arising from infected plant material and also from intact parent roots and the yield will be similar or higher (Sevón and Caldentey, 2002). There are several studies have been conducted regarding genetic transformation on production of bioactives, for an example, glycoalkoid and saponin were produced by inducing *Solanum photeinocarpum* hairy roots and they showed rapid growth while higher yield higher than original ones (Gong *et al*., 2001). Another study conducted to produce saponin from *Phytolacca esculenta* hairy roots and result showed the total content of saponin produced from metabolic engineering was higher than in the natural roots (Shi *et al*., 2003). Further, induced hairy roots from *Taxus brevifolia* produced

bioactive compound called taxol and *Polygonum multiforum* produced chrysophanol in hairy root by genetic transformation (Wang *et al.*, 2006). Therefore, it has been proved secondary metabolites could be produces in most types of transformed hairy roots and the yield is even higher or similar as in original plant. Thus, this technique is applicable to use for industrial production of bioactive compounds.

Calli cultures and cell suspension cultures are also relate with genetic transformation engaging with the production of bioactive compounds. Secondary metabolites that are used in human medicinal purposes are frequently produced using calli cultures (Mittal and Sharma, 2017; Rady *et al*., 2018). In another study overexpression of three genes of stilbene synthase from the plant of *Picea jezoensis* Carr, *viz* in *Vitis amurensis* calli cultures showed an increase of stilbene content (Suprun *et al.*, 2019). Synthesis of t-resveratrol by transforming *Silybum marianum* (L.) cell suspension cultures with *Vitis vinifera* L stilbene synthase gene has also been studied (Hidalgo *et al.*, 2017).

3.5. RNA interference (RNAi) technology used to enhance the production of bioactive compounds

The RNAi technique is used to increase the accumulation of secondary metabolites by down regulating the expression of genes in metabolic pathways. RNAi technology is always applicable whenever other approaches such as co-suppression and antisense RNA become failed to block the enzyme activity which is coded by multi genes. RNAi is associated with silencing of double-strand RNA mediated gene

RNAi is a process of double-strand RNA (dsRNA)-mediated gene silencing when only such situation of mRNA associated with dsRNA is degraded. A precursor of isoquinoline alkaloids referred as reticuline accumulation is approached by RNAi-mediated suppressing of berberine

bridge-forming enzyme in *Eschscholzi acalifornica.* Further, studies have been conducted following RNAi technology to increase β-carotene and lutein contents of potato. Mechanism of above application is down regulating the expression of β-carotene hydroxylase which can convert β-carotene to zeaxanthin, reducing β-carotene content in potato (Van Eck *et al.*, 2007; Amar *et al.*, 2016). Table 3 shows application of RNAi-mediated gene silencing for bioactive products in medicinal plants.

Table 3. RNAi-Mediated Gene Silencing in Medicinal Plants for synthesizing bioactive compounds

Plant	Therapeutic effect of the bioactive compound	Product	Enzyme	Reference
1. *Salvia miltiorrhiza*	Antiviral, antimicrobial, and anti-inflammatory activities	Rosmarinic acid	Phenylalanine ammonia-lyase	Song and Wang, 2011
2. *Withanias omnifera*	Improving immune system, antioxidant, antihyperglycemic, anti-inflammatory, and anti-cancer activities	Withanalides	Cycloartenol synthase	Mishra *et al.*, 2016
3. *Papavers omniferum*		Codeine and morphine	Codeinonereductase (COR)	Allen *et al.*, 2004
		Morphinan alkaloids	Salutaridinol	Allen *et al.*, 2008
			7-O-acetyltransferase	Kempe *et al.*, 2009
		Morphine, codeine, sanguinarine	Berberine bridge enzyme and N-methyl coclaurine 3′-hydroxylase	Frick *et al.*, 2004
4. *Artemisia annua*		Salicylic acid and artemisinin	Cinnamate-4-hydroxylase	Kumar *et al.*, 2016

3.6. Plant Cell Elicitation

Plant secondary metabolites, which are of pharmaceutical importance include phenolic compounds, alkaloids and their glycoside derivatives, volatile oils, resins etc. They are commonly known as phyto-pharmaceuticals and mostly isolated from wild and cultivated plants. The yield of these compounds from plants is often low and largely depends on plant physiology. The chemical synthesis of most compounds is not successful since it is extremely difficult and infeasible to generate the same efficacy and pharmacological specificity. Although application of biotechnological techniques to enhance the recovery and synthesis of secondary metabolites in plant cell or organ cultures appeared to be an effective approach, due to lack of knowledge about the synthesizing pathway of these metabolites, it had only limited success (Namdeo, 2007). Since compounds can be produced without affected by soil conditions and climatic changes, free of microbes and insects, with reduced labor cost and using automated control of cell growth to yield specific metabolites, exploring synthesis of secondary metabolites using biotechnological techniques from plant cell and organ cultures would be worth experimenting further (Varma, 2010).

Elicitation is one of the biotechnological strategies experimented for enhancing the secondary metabolite production. It is a process that stimulates or enhances the synthesis of secondary metabolites by the plant cells in-vitro, as they respond physiologically and morphologically to physical, chemical or microbial factors which are known as ‘elicitors’ (Mejía-Tenienteet al., 2010).Secondary metabolites may be the products of chemical adaptions to environmental stress by the plant cells, which act as defensive or offensive chemicals against any harmful agents (Namdeo, 2007). Also secondary metabolite synthesis in plants can be induced by factors like UV-irradiation, osmotic shock, fatty acids, and heavy metal ions which create stressful

conditions in plant cells. Therefore, term ‘elicitor’ can be defined as a substance which initiates or enhances the biosynthesis of compounds, when introduced in small concentrations to a living cell system, (Namdeo, 2007).

Classification of elicitors

Elicitors can be categorized either based on their ‘nature’ or based on their ‘origin’.

When classified according to their ‘nature’ two types of elicitors can be identified as abiotic elicitors and biotic elicitors. Substances which are of non-biological origin, mostly physical factors such as metal ions and high pH are called abiotic elicitors. Substances with biological origin such as plant-based polysaccharides (pectin or cellulose), micro-organisms (fungi, bacteria or herbivores, plant cell wall fragments) and glycoproteins or intracellular proteins which works by activating or inactivating either enzymes or ion channels are known as biotic elicitors (Namdeo, 2007).

When classified according to their ‘origin’ two types of elicitors can be identified as endogenous elicitors and exogenous elicitors. Substances whose origin is inside the cells (galacturonide or hepta-β-glucosides) are called endogenous elicitors whereas substances originated outside the cell, such as polysaccharides, polyamines and fatty acids are termed as exogenous elicitors. (Namdeo, 2007).

Mechanisms of elicitation

Treating plants with elicitors causes a series of reactions that results in the accretion of defensive secondary metabolites in plants. However, the exact mechanism of elicitation through biotic and abiotic elicitors on secondary metabolite synthesis needs further investigation. Some of the mechanisms proposed in this regard are messenger Ca^{2+}, inhibition/ activation of intracellular

pathways, factors influencing cell membrane integrity, and changes in osmotic stress (Namdeo, 2007).

Namdeo (2007) reported the common mechanism by which plant cells generate biochemical responses when an elicitor is introduced into it. The elicitor initially binds to the plasma membrane receptor and this leads to the influx of Ca^{2+} ions into the cytoplasm from the extracellular environment as well as intracellular Ca^{2+} reservoirs. Elicitation also leads to rapid changes in protein phosphorylation patterns and protein kinase activation. During this process, cytoplasm acidification is caused by H^{+}-ATPase inactivation as an result of stimulation of mitogen-activated protein kinase and G-protein activation. Also studies have reported the decrease in membrane polarization and extracellular increase of pH in elicitor treated plant tissues. Bioactive fatty acid derivatives and reactive oxygen species like superoxide anion and H_2O_2which are having direct antimicrobial effect, are produced. H_2O_2 also acts as a secondary messenger and is involved in the transcriptional activation of defence genes.

Another hypothesis relates about the accumulation of proteins associated with defensive action and pathogenesis (chitinases and glucanases) that contribute to the release of oligomers glycoproteins, and protease inhibitors (Namdeo, 2007).

However, reporting the exact mechanism of elicitation and their interconnection is highly complex and requires in depth studies. All elicitors do not follow the same series of actions but varies depending on their origin, specificity, environment, stage of their growth cycle and nutritional uptake (Namdeo, 2007).

Characteristics of Elicitors

Characteristics of elicitor includes concentration and selectivity, age of culture, cell line, nutrient composition, quality of cell wall materials and enhancement of product accumulation etc. If the

elicitor concentration is high, higher amount of bioactive compounds will be produced. Also increasing dosage of elicitor has been identified to induce hypersensitive response causing cell death, whereas, induction requires an optimum concentration (Namdeo, 2007). With high exposure time, higher production was observed, but when extending the exposure time further it lowered the production. Age of subculture and nutrient composition of selected medium plays an important role in process of elicitation mediated synthesis of bioactive compounds. Apart from these characteristics, the efficiency of elicitation also varies depending on elicitor specificity, clones of microbial elicitor used, availability of growth regulators and the environmental conditions.

Applications of Plant cell elicitation

The exact molecular structures of elicitors like polysaccharides, oligosaccharides, proteins, glycoproteins, and fatty acids have been elucidated in recent studies (Angelova et al., 2006). Many carbohydrate elicitors have been used on different cultures to produce secondary metabolites. Chitosan has been used on N. tabacum, E. californica cultures to produces metabolites like phytoalexins.β-1,6-1,3-Glucans have been used upon Glycine maxcultures to produce isoflavonoids. Chitin, alginate, pectin, guar gum rhamsan and xanthan have been used as elicitors to produce Anthraquinones from Morinda citrifolia cultures (Radman et al., 2003, Angelova et al., 2006).

3.7. Microencapsulation for the improved delivery of bioactive compounds into foods/medicine

Phytochemicals or plant bioactive compounds are recognized as beneficial products for chronic diseases in human. Many studies, focused on developing functional food products and

nutraceuticals using these phytochemicals encounter difficulties in maintaining their stability until delivery to target site. Since they are more prone to degradation during processing and storage, there must be a way to increase their stability within food matrix and external environment to protect against degradation. Many natural bioactive compounds are unstable and readily oxidized with exposure to light, heat and pH (Corrêa-Filho et al., 2019). Food industries mostly look for functional products having more shelf life and designed to release at the target site during desired time and increased bioavailability after release. Microencapsulation is a process that can enhance stability of bioactive compounds which are sensitive to adverse environmental conditions by entrapping the compounds within a polymeric material that acts a protective coating. This application is generally used to either preserve or stabilize functionally active ingredients in pharmaceuticals and food-based applications.

Microencapsulation is a physiochemical or mechanical process that in which liquid droplets or small particles are wrapped in a shell or coated with a continuous film of polymeric material to produce small particles whose size ranges are within nanometers to millimeters. These so formed wrapped particles are called microcapsules or microspheres, for protection and/or later release (Corrêa-Filho et al., 2019).Microcapsules have been widely used in the pharmaceutical sector and currently it is highly recommended to the food industries to enhance their product quality with high thermal stability, controlled release of compounds, texture/sense improvement and sheltered from being volatile (Yang et al., 2020).

Microcapsules are different from microspheres due to their structural and morphological variations. Microcapsules are hollow and possess an internal spatial system while microspheres have a dense matrix (Corrêa-Filho et al., 2019). Microencapsulation process protects the active ingredient from harmful environmental conditions by the coating material, which is termed as the

encapsulating agent or wall material. Active ingredients are microencapsulated in monolayer or multilayer's of wall materials with a variety of molecular interactions such as electrostatic attraction, van der Waals forces, and hydrogen bonding (Yang et al., 2020). The wall material protects the core material from adverse conditions until it passes through the human gastro-intestinal tract where the wall material of microcapsules is dissolved in lower pH of the gastric acid. This facilitates the core substances to be released and readily absorbed in the small intestine. Microcapsule preparation is a low-cost activity which requires a smaller production cost and simple equipment. Also the wall and core materials should be in line with the food safety and regulatory standards (Yang et al., 2020) and made out of food grade edible polymers like maltodextrin, inulin and arabic gum. There are many studies which have documented the microencapsulation of natural bioactive compounds such as phenolic compounds, carotenoids, other organic compounds and essential oils (Corrêa-Filho et al., 2019).

Microencapsulation methods

Microencapsulation can be carried out using many techniques such as pan coating, polymerization, salting out, air suspension coating, (Tarun and Murthy, 2011), freeze drying spray drying, coacervation, electrospraying, ionic gelation, and fluidized bed coating(Corrêa-Filho et al., 2019), emulsification coacervation, layer-by-layer, extrusion, supercritical, solvent evaporation, and nanocapsule preparation (Yang et al., 2020).

Spray drying microencapsulation is done by atomizing the emulsion of wall material and core material in the dry and high-temperature environment. During this step moisture is evaporated by the heat exchange between droplets and drying medium. This leads to the formation of solidified droplet shells which coats the core material. This is widely used for mass production of materials

like arabic gum and modified starch which have good water solubility, low viscosity, and good fluidity (Corrêa-Filho et al., 2019, Yang et al., 2020).

Emulsification is a microencapsulation technique where chemical embedding and cross-linking aids in encapsulation. In this method, the mixture of core material and wall material is added into a large amount of continuous phase, containing the emulsifier to form a stable emulsion. Then microencapsulation is facilitated by the cross-linking agent. The production cost of this method is usually very high and therefore, this is not feasible (Yang et al., 2020).

Freeze-drying is a method where quick frozen ice is sublimated into vapor using high vacuum conditions. Due to sublimation of ice, the process is kept cool and safe for biological samples. The economic feasibility of this method is low due to high energy cost and long processing time (Corrêa-Filho et al., 2019, Yang et al., 2020).

In Coacervation technique, an emulsion of core material and wall material is formed. Then another substance or solvent is added to reduce the solubility of the wall material. The core material evenly aggregates the added substance to form microcapsules. This is a commonly used technique as it does not need any special equipment, has mild processing conditions and does not detoriate the core material severely. (Yang et al., 2020).

In layer-by-layer assembly technique, attach to each other spontaneously to form stable molecular aggregates. This technique is used to produce multilayer microcapsules. However, product quality for some products may be poor due to weak interactions between layers (Yang et al., 2020).

Extrusion technique is a physical technique to form microcapsules by squeezing core material and colloid mixture into the hardening bath to form liquid drops through the needle tube under pressure. Though it is not an economical approach it is commonly used for entrapping all kinds

of volatiles, pigments and other thermolabile (Yang et al., 2020, Bamidele and Emmambux, 2020).

Supercritical technique is used for nonvolatile substances which dissolve in the supercritical fluid. At this technique small particles are formed within a short time period, by decompressing through the pore capillary which leads to oversaturation and particle formation. Hollow microcapsules are separated and precipitated under controlled conditions. Selection of supercritical CO_2 is primarily to due to its low critical temperature, low viscosity, high mass transfer, and nontoxicity (Yang et al., 2020).

Electrospray technique makes use of high voltage electric field to decompose the polymer fluid transported by the conductive capillary pump into the fine droplets. The formed polymers are collected to obtain microcapsules after evaporating the solvent. This technique can be widely applied to encapsulate bioactive substances, volatile compounds and can be used for sustained-release of preservatives in food based applications. (Yang et al., 2020).

Fluidized Bed Coating is a tool used to produce microparticles, using powder coating in a batch processor or a continuous setup. The particle to be coated should be spherical and should posess a narrow particle size distribution. Initially, the particles are air suspended at a predefined temperature and then sprayed with coating material. (Đorđević et al., 2014).

Nano capsulation is a technique that can be used to produce nano sized capsules which can penetrate through capillaries, tissues and easily absorbed by cells. Thus this facilitates more precise targeting of the core materials. However, additional high energy based treatments such as are high pressure or intense mechanical agitation are required to prepare (Gómez et al., 2018).

Polymers used in microencapsulation

According to Yang et al (2020), there are many natural food grade materials that are used for microencapsulation process with several different properties in each. Cellulose and its derivatives such as methyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose and hydroxypropyl methyl cellulose (HPMC) possess improved solubility and thermoplasticity. Chitosan is another material that has many food specific properties such as biodegradability and osmotic enhancement effects which is used in fresh fruit and vegetable-based applications. Alginate is famous as a coating material due to its properties such as flocculability, film-forming ability and biocompatibility. Alsoit is easy to handle as it requires mild reaction conditions and is nontoxic and harmless. Starch and its hydrolysates are also used as wall materials owing to their properties like easiness of retrogradation and dehydration. Pectin is another compound widely used in the production of ice cream, jam and fruit juices due to its gelatinization and emulsification stability. Protein holds several beneficial processing properties such as solubility and emulsification, as well as the physiological ability to resist oxidation as well as biocompatibility and biodegradability. In addition, the susceptibility of protein to pH changes facilitates their application in strict pH-controlled release conditions (Yang et al., 2020). Many above discussed materials are added together in compatible ratios to prepare composite wall materials with enhanced properties.

Application of microencapsulation in food/medicine for improved delivery of bioactive compounds

Many studies have been conducted to find the real scenario behind the microencapsulated bioactive compound after being incorporated into different food matrices. A study reported microencapsulation of carotenoids from sea buckthorn extract using whey protein isolate and arabic gum as wall materials and incorporating them into muffins increased the stability of the

bioactive compounds after 21 days' storage at 25°C by 55 % (Ursache et al., 2018). Polyphenol rich green tea extract was microencapsulated in maltodextrin by spray-drying and freeze-drying and then incorporated to bread (Pasrijaet al., 2015). The study evaluated the stability of polyphenols and quality of the baked bread upon incorporation of microcapsules. The amount of polyphenols present in the baked bread decreased by 33% after baking (Corrêa-Filho et al., 2019). However, through optimization of the above discussed microencapsulation techniques thus formed microparticles containing phytochemicals can be incorporated into food matrices to enhance the delivery of therapeutic effects to humans.

Conclusions

It is well known that various plants are recognized as vital sources for the extraction and synthesis of novel bioactives with therapeutical potential. These can be used in the formulation of functional foods, drug, nutraceuticals. Numerous research have shown that plant based bioactive molecules are the secondary metabolites which could deliver health benefits to human including control and prevention of many chronic diseases. It is obvious that studies related to plant bioactives involving diverse aspects and various scientific disciplines are emerging recently. Based on various research conducted in searching alternatives approaches for the production and enhancements of beneficial plant bioactive molecules, several biotechnological approaches such as fermentation and plant tissue culture are found to have potential to be developed into industrial scale production of plant bioactives. Different categories of plant bioactives and their therapeutical applications as antioxidants, anti-inflammatory agents, anti-diabetic and anti-cancer agents have been studied by scientists. Also, applications of various

biotechnological approaches for the production of plant bioactives has also been reviewed and reported.

COMPETING INTERESTS

Authors have declared that no competing interests exist

References

Abdin MZ. Enhancing bioactive molecules in medicinal plants. In: Y Zhu, B Tan, B Bay, C Liu, editors. Natural Products-Essential resources for human. Singapore: World Scientific Publishing Co. Pvt. Ltd; 2007. p. 45-57.

Abdin MZ, Kamaluddin A. Improving quality of medicinal herbs through physico-chemical and molecular approaches. In: MZ Abdin, YP Abrol, Narosa, editors. Traditional systems of medicine. India: Publishing House Pvt. Ltd; 2006. p. 30-9.

Ahrén, I. L., Xu, J., Önning, G., Olsson, C., Ahrné, S., & Molin, G. (2015). Antihypertensive activity of blueberries fermented by Lactobacillus plantarum DSM 15313 and effects on the gut microbiota in healthy rats. *Clinical Nutrition*, *34*(4), 719–726. https://doi.org/10.1016/j.clnu.2014.08.009

Aharoni, A. and Galili, G. (2011) 'Metabolic engineering of the plant primary – secondary metabolism interface Asaph Aharoni and Gad Galili', *Current Opinion in Biotechnology*. Elsevier Ltd, 22(2), pp. 239–244. doi: 10.1016/j.copbio.2010.11.004.

Abou-Shoer, M.I., Shaala, L.A., Youssef, D.T., Badr, J.M. and Habib, A.A.M., 2008. Bioactive brominated metabolites from the Red Sea sponge Subereamollis. *Journal of natural products*, *71*(8), pp.1464-1467.

Ali, M., Kim, I.-D., Bilal, S., Shahzad, R., Saeed, M., Adhikari, B., Shin, D.-H. (2017). Effects of Bacterial Fermentation on the Biochemical Constituents and Antioxidant Potential of Fermented and Unfermented Soybeans Using Probiotic Bacillus subtilis (KCTC 13241). *Molecules, 22(12), 2200.* doi:10.3390/molecules22122200

Allen, R. S. *et al.* (2004) 'RNAi-mediated replacement of morphine with the nonnarcotic alkaloid reticuline in opium poppy', *Nature Biotechnology*, 22(12), pp. 1559–1566. doi: 10.1038/nbt1033.

Allen, R. S. *et al.* (2008) 'Metabolic engineering of morphinan alkaloids by over-expression and RNAi suppression of salutaridinol 7-O-acetyltransferase in opium poppy', *Plant Biotechnology Journal*, 6(1), pp. 22–30. doi: 10.1111/j.1467-7652.2007.00293.x.

Angelova, Z., Georgiev, S. and Roos, W., 2006. Elicitation of plants. *Biotechnology & Biotechnological Equipment*, *20*(2), pp.72-83.

Andarwulan, N., Kurniasih, D., Apriady, R. A., Rahmat, H., Roto, A. V., & Bolling, B. W. (2012). Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. *Journal of Functional Foods*, *4*(1), 339–347. https://doi.org/10.1016/j.jff.2012.01.003

Ankolekar, C., Pinto, M., Greene, D., & Shetty, K. (2012). In vitro bioassay based screening of antihyperglycemia and antihypertensive activities of Lactobacillus acidophilus fermented pear juice. *Innovative Food Science & Emerging Technologies, 13, 221–230.* doi:10.1016/j.ifset.2011.10.008

Ayyash, M., Johnson, S. K., Liu, S. Q., Al-Mheiri, A., &Abushelaibi, A. (2018). Cytotoxicity, antihypertensive, antidiabetic and antioxidant activities of solid-state fermented lupin, quinoa and wheat by Bifidobacterium species: In-vitro investigations. *LWT, 95,* 295–302. https://doi.org/10.1016/j.lwt.2018.04.099

Ben-Amar, A. *et al.* (2016) 'BENTHAM SCIENCE Impact Factor: 2.43 Reverse Genetics and High Throughput Sequencing Methodologies for Plant Functional Genomics', *Current Genomics*, 17(6), pp. 460–475. doi: 10.2174/1389202917666616052010.

Barros, H. R. de M. *et al.* (2016) 'Evaluation of the distribution and metabolism of polyphenols derived from cupuassu (Theobroma grandiflorum) in mice gastrointestinal tract by UPLC-ESI-QTOF', *Journal of Functional Foods*. Elsevier Ltd, 22, pp. 477–489. doi: 10.1016/j.jff.2016.02.009.

Bamidele, O.P. and Emmambux, M.N., 2020. Encapsulation of bioactive compounds by "extrusion" technologies: a review. *Critical Reviews in Food Science and Nutrition*, pp.1-19.

Bei, Q., Liu, Y., Wang, L., Chen, G., & Wu, Z. (2017). Improving free, conjugated, and bound phenolic fractions in fermented oats (Avena sativa L.) with Monascusanka and their antioxidant activity. *Journal of Functional Foods*, *32*, 185–194. https://doi.org/10.1016/j.jff.2017.02.028

Bhat, R., Suryanarayana, L. C., Chandrashekara, K. A., Krishnan, P., Kush, A., & Ravikumar, P. (2015). Lactobacillus plantarum mediated fermentation of Psidium guajava L. fruit extract. *Journal of Bioscience and Bioengineering*, *119*(4), 430–432. https://doi.org/10.1016/j.jbiosc.2014.09.007

Cai, Y. X., Wang, J. H., McAuley, C., Augustin, M. A., &Terefe, N. S. (2019). Fermentation for enhancing the bioconversion of glucoraphanin into sulforaphane and improve the functional attributes of broccoli puree. *Journal of Functional Foods*, *61*. https://doi.org/10.1016/j.jff.2019.103461

Choi, S. H. *et al.* (2012) 'Glycosylation of various flavonoids by recombinant oleandomycin glycosyltransferase from Streptomyces antibioticus in batch and repeated batch modes', *Biotechnology Letters*, 34(3), pp. 499–505. doi: 10.1007/s10529-011-0789-z.

Corrêa-Filho, L.C., Moldão-Martins, M. and Alves, V.D., 2019. Advances in the application of microcapsules as carriers of functional compounds for food products. *Applied Sciences*, *9*(3), p.571.

D'Auria, M.V., Zampella, A., Paloma, L.G., Minale, L., Debitus, C., Roussakis, C. and Le Bert, V., 1996. Callipeltins B and C; bioactive peptides from a marine Lithistida sponge Callipelta sp. *Tetrahedron*, *52*(28), pp.9589-9596.

Đorđević, V., Balanč, B., Belščak-Cvitanović, A., Lević, S., Trifković, K., Kalušević, A., Kostić, I., Komes, D., Bugarski, B. and Nedović, V., 2015. Trends in encapsulation technologies for delivery of food bioactive compounds. *Food Engineering Reviews*, *7*(4), pp.452-490.

Enfissi, E. M. A. *et al.* (2010) 'Integrative transcript and metabolite analysis of nutritionally enhanced DE-ETIOLATED1 downregulated tomato fruit', *Plant Cell*, 22(4), pp. 1190–1215. doi: 10.1105/tpc.110.073866.

Farmer, W. R. and Liao, J. C. (2001) '<Farmer-2001-Precursor-balancing-for-lycopene-production.pdf>', pp. 57–61.

Fideler, J., Johanningsmeier, S. D., Ekelöf, M., &Muddiman, D. C. (2019). Discovery and quantification of bioactive peptides in fermented cucumber by direct analysis IR-MALDESI mass spectrometry and LC-QQQ-MS. *Food Chemistry*, *271*, 715–723. https://doi.org/10.1016/j.foodchem.2018.07.187

Frick, S. *et al.* (2004) 'Transformation of opium poppy (Papaver somniferum L.) with antisense berberine bridge enzyme gene (anti-bbe) via somatic embryogenesis results in an altered ratio of alkaloids in latex but not in roots', *Transgenic Research*, 13(6), pp. 607–613. doi: 10.1007/s11248-004-2892-6.

Gabriele, M., Pucci, L., Árvay, J., & Longo, V. (2018). Anti-inflammatory and antioxidant effect of fermented whole wheat on TNFα-stimulated HT-29 and NF-κB signaling pathway activation. *Journal of Functional Foods*, *45*, 392-400.https://doi.org/10.1016/j.jff.2018.04.029

Gan, R. Y., Li, H. B., Gunaratne, A., Sui, Z. Q., &Corke, H. (2017, May 1). Effects of Fermented Edible Seeds and Their Products on Human Health: Bioactive Components and Bioactivities. *Comprehensive Reviews in Food Science and Food Safety*. Blackwell Publishing Inc. https://doi.org/10.1111/1541-4337.12257

Gan, R. Y., Shah, N. P., Wang, M. F., Lui, W. Y., &Corke, H. (2016). Fermentation alters antioxidant capacity and polyphenol distribution in selected edible legumes. *International Journal of Food Science and Technology*, *51*(4), 875–884. https://doi.org/10.1111/ijfs.13062

Go, J., Kim, J. E., Kwak, M. H., Koh, E. K., Song, S. H., Sung, J. E., … Hwang, D. Y. (2016). Neuroprotective effects of fermented soybean products (Cheonggukjang) manufactured by mixed culture of Bacillus subtilis MC31 and Lactobacillus sakei 383 on trimethyltininduced

cognitive defects mice. *Nutritional Neuroscience*, *19*(6), 247–259. https://doi.org/10.1179/1476830515Y.0000000025

Georgiev, M. I., Pavlov, A. I. and Bley, T. (2007) 'Hairy root type plant in vitro systems as sources of bioactive substances', pp. 1175–1185. doi: 10.1007/s00253-007-0856-5.

Gómez, B., Barba, F.J., Domínguez, R., Putnik, P., Kovačević, D.B., Pateiro, M., Toldrá, F. and Lorenzo, J.M., 2018. Microencapsulation of antioxidant compounds through innovative technologies and its specific application in meat processing. *Trends in food science & technology*, *82*, pp.135-147.

Gong Yulian, Shi Heping, Li Ling, Pan Ruichi. Induction of Solanumphoteinocarpum hairy roots and production of secondary metabolites Journal of Tropical and Subtropical Botany. 2002 ;10(1):58-62.

Geerlings, A. *et al.* (2001) 'Biotransformation of tryptamine and secologanin into plant terpenoid indole alkaloids by transgenic yeast', *Applied Microbiology and Biotechnology*, 56(3–4), pp. 420–424. doi: 10.1007/s002530100663.

Giri A, Narasu ML. Transgenic hairy roots. recent trends and applications. Biotechnol Adv 2000;18:1-22.

Gumienna, M., Szwengiel, A., &Górna, B. (2016, May 1). Bioactive components of pomegranate fruit and their transformation by fermentation processes. *European Food Research and Technology*. Springer Verlag. https://doi.org/10.1007/s00217-015-2582-z

Hashemi, S. M. B., Mousavi Khaneghah, A., Barba, F. J., Nemati, Z., SohrabiShokofti, S., & Alizadeh, F. (2017). Fermented sweet lemon juice (*Citrus limetta*) using Lactobacillus plantarum LS5: Chemical composition, antioxidant and antibacterial activities. *Journal of Functional Foods*, *38*, 409–414. https://doi.org/10.1016/j.jff.2017.09.040

Hashemi, S. M. B., Mousavi Khaneghah, A., Barba, F. J., Nemati, Z., SohrabiShokofti, S., & Alizadeh, F. (2017). Fermented sweet lemon juice (*Citrus limetta*) using Lactobacillus plantarum LS5: Chemical composition, antioxidant and antibacterial activities. *Journal of Functional Foods, 38, 409–414.* doi:10.1016/j.jff.2017.09.040

Hidalgo, D. *et al.* (2017) 'Silybum marianum cell cultures stably transformed with Vitis vinifera stilbene synthase accumulate t-resveratrol in the extracellular medium after elicitation with methyl jasmonate or methylated β-cyclodextrins', *Engineering in Life Sciences*, 17(6), pp. 686–694. doi: 10.1002/elsc.201600241.

Huang, Q. *et al.* (2001) 'Engineering Escherichia coli for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol', *Bioorganic and Medicinal Chemistry*, 9(9), pp. 2237–2242. doi: 10.1016/S0968-0896(01)00072-4.

Hashimoto, T., Yun, D. J. and Yamada, Y. (1993) 'Production of tropane alkaloids in genetically

engineered root cultures', *Phytochemistry*, 32(3), pp. 713–718. doi: 10.1016/S0031-9422(00)95159-8.

Hidalgo, D. *et al.* (2017) 'Silybummarianum cell cultures stably transformed with Vitisviniferastilbene synthase accumulate t-resveratrol in the extracellular medium after elicitation with methyl jasmonate or methylated β-cyclodextrins', *Engineering in Life Sciences*, 17(6), pp. 686–694. doi: 10.1002/elsc.201600241.

Hoornstra, D., Vesterlin, J., Parnanen, P., Al-samadi, A., Zlotogorski-hurtvitz, A., Vered, M., & Salo, T. (2018). Fermented Lingonberry Juice Inhibits Oral Tongue Squamous Cell Carcinoma Invasion In Vitro Similarly to Curcumin. *In Vivo, 32(5), 1089–1095.* doi:10.21873/invivo.11350

Huh, E., Lim, S., Kim, H. G., Ha, S. K., Park, H. Y., Huh, Y., & Oh, M. S. (2018). Ginger fermented with: Schizosaccharomyces pombe alleviates memory impairment via protecting hippocampal neuronal cells in amyloid beta1-42 plaque injected mice. In *Food and Function* (Vol. 9, pp. 171–178). Royal Society of Chemistry. https://doi.org/10.1039/c7fo01149k

Hussain, A., Bose, S., Wang, J. H., Yadav, M. K., Mahajan, G. B., & Kim, H. (2016). Fermentation, a feasible strategy for enhancing bioactivity of herbal medicines. *Food Research International*. Elsevier Ltd. https://doi.org/10.1016/j.foodres.2015.12.026

Hussain, M.S., Fareed, S., Saba Ansari, M., Rahman, A., Ahmad, I.Z. and Saeed, M., (2012). Current approaches toward production of secondary plant metabolites. *Journal of pharmacy & bioallied sciences*, *4*(1), p.10.

Janarny, G. and Gunathilake, K.D.P.P., (2020). Changes in rice bran bioactives, their bioactivity, bioaccessibility and bioavailability with solid-state fermentation by Rhizopus oryzae. *Biocatalysis and Agricultural Biotechnology*, *23*, p.101510. https://doi.org/10.1016/j.bcab.2020.101510

Juan, M.-Y., & Chou, C.-C. (2010). Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715. *Food Microbiology, 27(5), 586–591.* doi:10.1016/j.fm.2009.11.002

Kempe, K. *et al.* (2009) 'RNAi suppression of the morphine biosynthetic gene salAT and evidence of association of pathway enzymes', *Phytochemistry*. Elsevier Ltd, 70(5), pp. 579–589. doi: 10.1016/j.phytochem.2009.03.002.

Kumar, R. *et al.* (2016) 'RNAi down-regulation of cinnamate-4-hydroxylase increases artemisinin biosynthesis in Artemisia annua', *Scientific Reports*. Nature Publishing Group, 6(1), pp. 1–12. doi: 10.1038/srep26458.

Kim J, Choi K-B, Park JH, Kim KH (2019) Metabolite profile changes and increased antioxidative and antiinflammatory activities of mixed vegetables after fermentation by *Lactobacillus plantarum*. PLoS ONE 14(5):0217180. https://doi.org/10.1371/journal.pone.0217180

Kanaze, F. I. *et al.* (2007) 'Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects', *European Journal of Clinical Nutrition*, 61(4), pp. 472–477. doi: 10.1038/sj.ejcn.1602543.

Karuppusamy, S. (2009) 'A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures', *Journal of Medicinal Plants Research*, 3(13), pp. 1222–1239.

Kaur S, Das M (2011) Functional foods: an overview. Food Sci Biotechnol 20(4):861–875

Kim, B. G., Kim, H. J. and Ahn, J. H. (2012) 'Production of bioactive flavonol rhamnosides by expression of plant genes in Escherichia coli', *Journal of Agricultural and Food Chemistry*, 60(44), pp. 11143–11148. doi: 10.1021/jf302123c.

Koopman, F. *et al.* (2012) 'De novo production of the flavonoid naringenin in engineered Saccharomyces cerevisiae', *Microbial Cell Factories*, 11, pp. 1–15. doi: 10.1186/1475-2859-11-155.

Lee, H., Kim, B. G. and Ahn, J. H. (2014) 'Production of bioactive hydroxyflavones by using monooxygenase from Saccharothrix espanaensis', *Journal of Biotechnology*. Elsevier B.V., 176(1), pp. 11–17. doi: 10.1016/j.jbiotec.2014.02.002.

Le, B., Anh, P. T. N., & Yang, S. H. (2020). Enhancement of the Anti-Inflammatory Effect of Mustard Kimchi on RAW 264.7 Macrophages by the Lactobacillus plantarum Fermentation-Mediated Generation of Phenolic Compound Derivatives. *Foods, 9(2), 181*. doi:10.3390/foods9020181

Lee, H. H., Yin, L. J., Tai, H. M., & Jiang, S. T. (2016). Facilitating the release of bionutrients from Morindacitrifolia (noni) by cellulase hydrolysis and lactic acid bacteria fermentation and their effects on α-amylase and α-glucosidase activities. *Journal of Marine Science and Technology (Taiwan), 24*(3), 648–655. https://doi.org/10.6119/JMST-015-1124-1

Lee, H., Song, B., Kim, H., Kim, J., Yun, W., Park, J., Hwang, D. (2017). Butanol extracts of Asparagus cochinchinensis fermented with Weissellacibaria inhibit iNOS-mediated COX-2 induction pathway and inflammatory cytokines in LPS-stimulated RAW264.7 macrophage cells. *Experimental and Therapeutic Medicine*. doi:10.3892/etm.2017.5200

Limón, R. I., Peñas, E., Torino, M. I., Martínez-Villaluenga, C., Dueñas, M., & Frias, J. (2015). Fermentation enhances the content of bioactive compounds in kidney bean extracts. *Food Chemistry, 172*, 343–352. https://doi.org/10.1016/j.foodchem.2014.09.084

Liu, F., Chen, Z., Shao, J., Wang, C., & Zhan, C. (2017). Effect of fermentation on the peptide content, phenolics and antioxidant activity of defatted wheat germ. *Food Bioscience, 20, 141–148*. doi:10.1016/j.fbio.2017.10.002

Leonard, E. *et al.* (2009) 'Opportunities in metabolic engineering to facilitate scalable alkaloid production', *Nature Chemical Biology*, 5(5), pp. 292–300. doi: 10.1038/nchembio.160.

Malla, S. *et al.* (2013) 'Regiospecific modifications of naringenin for astragalin production in Escherichia coli', *Biotechnology and Bioengineering*, 110(9), pp. 2525–2535. doi: 10.1002/bit.24919.

Mittal, J. and Sharma, M. M. (2017) 'Enhanced production of berberine in In vitro regenerated cell of Tinospora cordifolia and its analysis through LCMS QToF', *3 Biotech*. Springer Berlin Heidelberg, 7(1), pp. 1–12. doi: 10.1007/s13205-016-0592-6.

Mijts, B. N. and Ã, C. S. (no date) 'Engineering of secondary metabolite pathways', pp. 597–602. doi: 10.1016/j.copbio.2003.09.009.

María Landete, J., Hernández, T., Robredo, S., Dueñas, M., De Las Rivas, B., Estrella, I., & Muñoz, R. (2015). Effect of soaking and fermentation on content of phenolic compounds of soybean (Glycine max cv. Merit) and mung beans (Vigna radiata [L] Wilczek). *International Journal of Food Sciences and Nutrition*, *66*(2), 203–209. https://doi.org/10.3109/09637486.2014.986068

Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz, J., Aguilar, C. N., & Teixeira, J. A. (2011, May). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnology Advances*. https://doi.org/10.1016/j.biotechadv.2011.01.008

Mishra, S. *et al.* (2016) 'RNAi and homologous over-expression based functional approaches reveal triterpenoid synthase gene-cycloartenol synthase is involved in downstream withanolide biosynthesis in withania somnifera', *PLoS ONE*, 11(2). doi: 10.1371/journal.pone.0149691.

Michalak, M., Szwajgier, D., Paduch, R., Kukula-Koch, W., Waśko, A., &Polak-Berecka, M. (2020). Fermented curly kale as a new source of gentisic and salicylic acids with antitumor potential. *Journal of Functional Foods*, *67*. https://doi.org/10.1016/j.jff.2020.103866

Mejía-Teniente, L., Torres-Pacheco, I., González-Chavira, M.M., Ocampo-Velazquez, R.V., Herrera-Ruiz, G., Chapa-Oliver, A.M. and Guevara-González, R.G., 2010. Use of elicitors as an approach for sustainable agriculture. *African Journal of Biotechnology*, *9*(54), pp.9155-9162.

Namdeo, A.G., 2007. Plant cell elicitation for production of secondary metabolites: a review. *Pharmacogn Rev*, *1*(1), pp.69-79.

Pan, M., Wu, Q., Tao, X., Wan, C., Shah, N. P., & Wei, H. (2015). Fermentation of Allium chinense Bulbs With Lactobacillus plantarum ZDY 2013 Shows Enhanced Biofunctionalities, and Nutritional and Chemical Properties. *Journal of Food Science*, *80*(10), M2272–M2278. https://doi.org/10.1111/1750-3841.12994

Pandey, R. P. *et al.* (2013) 'Production of 3-O-xylosyl quercetin in Escherichia coli', *Applied*

Microbiology and Biotechnology, 97(5), pp. 1889–1901. doi: 10.1007/s00253-012-4438-9.

Park, E. hye, Bae, W. young, Kim, J. yeon, Kim, K. tae, & Paik, H. dong. (2017). Antimelanogenic effects of Inulabritannica flower petal extract fermented by Lactobacillus plantarum KCCM 11613P. *Journal of Zhejiang University: Science B*, *18*(9), 816–824. https://doi.org/10.1631/jzus.B1600234

Pasrija, D., Ezhilarasi, P.N., Indrani, D. and Anandharamakrishnan, C., 2015. Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT-Food Science and Technology*, *64*(1), pp.289-296.

Puupponen-Pimiä, R., Nohynek, L., Juvonen, R., Kössö, T., Truchado, P., Westerlund-Wikström, B., … Oksman-Caldentey, K. M. (2016). Fermentation and dry fractionation increase bioactivity of cloudberry (Rubuschamaemorus). *Food Chemistry*, *197*, 950–958. https://doi.org/10.1016/j.foodchem.2015.11.061

Radman, R., Saez, T., Bucke, C. and Keshavarz, T., 2003. Elicitation of plants and microbial cell systems. *Biotechnology and applied biochemistry*, *37*(1), pp.91-102.

Radman, R., Saez, T., Bucke, C. and Keshavarz, T., 2003. Elicitation of plants and microbial cell systems. *Biotechnology and applied biochemistry*, *37*(1), pp.91-102.

Rady, M. R., Aboul-Enein, A. M. and Ibrahim, M. M. (2018) 'Active compounds and biological activity of in vitro cultures of some Echinacea purpurea varieties', *Bulletin of the National Research Centre*. Bulletin of the National Research Centre, 42(1), pp. 1–12. doi: 10.1186/s42269-018-0018-1.

Rajasekaran, A., Kalaivani, M. Protective effect of *Monascus* fermented rice against STZ-induced diabetic oxidative stress in kidney of rats. *J Food Sci Technol* **52,** 1434–1443 (2015). https://doi.org/10.1007/s13197-013-1191-8

Ricci, A., Cirlini, M., Calani, L., Bernini, V., Neviani, E., Del Rio, D., … Lazzi, C. (2019). In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria. *Food Chemistry*, *276*, 692–699. https://doi.org/10.1016/j.foodchem.2018.10.046

Rizzello, C. G., Lorusso, A., Russo, V., Pinto, D., Marzani, B., &Gobbetti, M. (2017). Improving the antioxidant properties of quinoa flour through fermentation with selected autochthonous lactic acid bacteria. *International Journal of Food Microbiology*, *241*, 252–261. https://doi.org/10.1016/j.ijfoodmicro.2016.10.035

Ryu, J. Y., Kang, H. R., & Cho, S. K. (2019). Changes Over the Fermentation Period in Phenolic Compounds and Antioxidant and Anticancer Activities of Blueberries Fermented by Lactobacillus plantarum. *Journal of Food Science*, *84*(8), 2347–2356. https://doi.org/10.1111/1750-3841.14731

Sadh, P. K., Kumar, S., Chawla, P., &Duhan, J. S. (2018, October 8). Fermentation: A boon for production of bioactive compounds by processing of food industries wastes (By-Products). *Molecules*. MDPI AG. https://doi.org/10.3390/molecules23102560

Salar, R. K., Purewal, S. S., & Sandhu, K. S. (2017). Fermented pearl millet (Pennisetumglaucum) with in vitro DNA damage protection activity, bioactive compounds and antioxidant potential. *Food Research International*, *100*, 204–210. https://doi.org/10.1016/j.foodres.2017.08.045

Sandhu, K. S., &Punia, S. (2017). Enhancement of bioactive compounds in barley cultivars by solid substrate fermentation. *Journal of Food Measurement and Characterization*, *11*(3), 1355–1361. https://doi.org/10.1007/s11694-017-9513-6

Shen, Y., Sun, H., Zeng, H., Prinyawiwatukul, W., Xu, W., & Xu, Z. (2018). Increases in Phenolic, Fatty Acid, and Phytosterol Contents and Anticancer Activities of Sweet Potato after Fermentation by Lactobacillus acidophilus. *Journal of Agricultural and Food Chemistry*, *66*(11), 2735–2741. https://doi.org/10.1021/acs.jafc.7b05414

Sevón, N. and Oksman-Caldentey, K. M. (2002) 'Agrobacterium rhizogenes-mediated transformation: Root cultures as a source of alkaloids', *Planta Medica*, 68(10), pp. 859–868. doi: 10.1055/s-2002-34924.

Shi HP, Liang P, Quan H. [Induction and culture of hairy boots in Phytolaccaesculenta and its saponin production]. Sheng wu Gong Cheng xuebao = Chinese Journal of Biotechnology. 2003 Jan;19(1):46-49.

Song, J. and Wang, Z. (2011) 'RNAi-mediated suppression of the phenylalanine ammonia-lyase gene in Salvia miltiorrhiza causes abnormal phenotypes and a reduction in rosmarinic acid biosynthesis', *Journal of Plant Research*, 124(1), pp. 183–192. doi: 10.1007/s10265-010-0350-5.

Srivastava, S. and Srivastava, A. K. (2007) 'Hairy Root Culture for Mass-Production of High-Value Secondary Metabolites', pp. 29–43. doi: 10.1080/07388550601173918.

Suprun, A. R. *et al.* (2019) 'Effect of spruce PjSTS1a, PjSTS2, or PjSTS3 gene overexpression on stilbene biosynthesis in callus cultures of Vitis amurensis Rupr', *Biotechnology and Applied Biochemistry*. doi: 10.1002/bab.1839.

Torino, M. I., Limón, R. I., Martínez-Villaluenga, C., Mäkinen, S., Pihlanto, A., Vidal-Valverde, C., & Frias, J. (2013). Antioxidant and antihypertensive properties of liquid and solid state fermented lentils. *Food chemistry*, *136*(2), 1030-1037.

Tang, D. *et al.* (2017) 'Pharmacokinetic properties and drug interactions of apigenin, a natural flavone', *Expert Opinion on Drug Metabolism and Toxicology*. Taylor & Francis, 13(3), pp. 323–330. doi: 10.1080/17425255.2017.1251903.

Tarun, G. and Murthy, R.S.R., 2011. Patented microencapsulation techniques and its application. *Journal of Pharmacy Research*, *4*(7), pp.2097-2102.

Tiwari, R. and Rana, C. S. (2015) 'Plant secondary metabolites : a review Plant secondary metabolites : a review', (October).

Trantas, E. A. *et al.* (2015) 'When plants produce not enough or at all : metabolic engineering of flavonoids in microbial hosts', 6(January), pp. 1–17. doi: 10.3389/fpls.2015.00007.

Ursache, F.M., Andronoiu, D.G., Ghinea, I.O., Barbu, V., Ioniţă, E., Cotârleţ, M., Dumitraşcu, L., Botez, E., Râpeanu, G. and Stănciuc, N., 2018. Valorizations of carotenoids from sea buckthorn extract by microencapsulation and formulation of value-added food products. *Journal of Food Engineering*, *219*, pp.16-24.

Varma, V., 2010. Advancements in the production of secondary metabolites. *Journal of Natural Products*, *3*.

Van Eck, J. *et al.* (2007) 'Enhancing beta-carotene content in potato by RNAi-mediated silencing of the beta-carotene hydroxylase gene', *American Journal of Potato Research*, 84(4), pp. 331–342. doi: 10.1007/BF02986245.

Xiao, Y., Xing, G., Rui, X., Li, W., Chen, X., Jiang, M., & Dong, M. (2014). Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with Cordyceps militaris SN-18. *Journal of Functional Foods, 10, 210–222.* doi:10.1016/j.jff.2014.06.008

Yang, M., Liang, Z., Wang, L., Qi, M., Luo, Z. and Li, L., 2020. Microencapsulation Delivery System in Food Industry—Challenge and the Way Forward. *Advances in Polymer Technology*, *2020*.

Yueliang Zhao, YiZhen Wu, and Mingfu Wang. (2015). Bioactive Substances of Plant Origin, Hand Book of Food Chemistry, DOI 10.1007/978-3-642-36605-5_13967

Wang, G. Y. and Keasling, J. D. (2002) 'Amplification of HMG-CoA reductase production enhances carotenoid accumulation in Neurospora crassa', *Metabolic Engineering*, 4(3), pp. 193–201. doi: 10.1006/mben.2002.0225.

Wang, B. *et al.* (2006) 'Genetic transformation of Echinacea purpurea with Agrobacterium rhizogenes and bioactive ingredient analysis in transformed cultures', *Colloids and Surfaces B: Biointerfaces*, 53(1), pp. 101–104. doi: 10.1016/j.colsurfb.2006.08.003.

Wu, J. *et al.* (2013) 'Metabolic engineering of Escherichia coli for (2S)-pinocembrin production from glucose by a modular metabolic strategy', *Metabolic Engineering*. Elsevier, 16(1), pp. 48–55. doi: 10.1016/j.ymben.2012.11.009.

Zhu, S. *et al.* (2014) 'Efficient synthesis of eriodictyol from l-tyrosine in escherichia coli', *Applied and Environmental Microbiology*, 80(10), pp. 3072–3080. doi: 10.1128/AEM.03986-13.

Zhou, M., Zhu, X. and Shao, J. (2011) ‘Production and metabolic engineering of bioactive substances in plant hairy root culture’, pp. 1229–1239. doi: 10.1007/s00253-011-3228-0.