

REVIEW OF LITERATURE

Awareness about the relation between diet and health among the consumers has stimulated scientists to conduct research on the health promoting aspects of the chemical compounds derived from natural sources. These health promoting compounds are generally referred to as phytochemicals or bioactive compounds that are mainly derived from plant sources and plays important functional roles in disease prevention. The phytochemicals or bioactive compounds are defined as bioactive non nutrient secondary plant metabolites with health promoting properties and help in prevention of major chronic diseases. ^[1] Some of the major examples of phytochemicals include polyphenols, vitamins, carotenoids, dietary fibre, micronutrients, etc. ^[2] Polyphenols which includes phenolic acids and flavonoids act as natural free radical scavengers and thus have antioxidant properties. ^[3] They usually inhibit lipid peroxidation, prevent DNA oxidative damage and prevent inhibition of cell communications all of which are precursors to degenerative diseases. ^[4] This chapter reviews the reported literature on polyphenols in fruits and vegetables, their biosynthesis and functional roles; on effect of processing on polyphenols; and on fruit juice processing for enhanced functionality.

2.1. Polyphenols as important phytochemicals in plants

The polyphenols are secondary metabolites consisting of an aromatic ring carrying one or more hydroxyl moieties. Currently, more than 8,000 polyphenolic structures are known. ^[5] They are classified into non flavonoid and flavonoid groups according to the number of carbon atoms of the basic skeleton. The non flavonoid group consists of phenolic acids, tannins, acetophenones, phenylacetic acid, xanthenes, stilbenes, chalcone, lignans and secoiridoids while the flavonoids are divided into subclasses flavonone, flavones, dihydroflavanols, flavanol, flavan-3-ols, anthocyanidins, isoflavones and proanthocyanidins. ^[6] The phenolic acids and flavonoids are the major polyphenols which are being studied extensively for their bioactive roles.

2.1.1. Phenolic acids

The phenolic acids are further grouped into hydroxybenzoic acid and hydroxycinnamic acid derivatives. The major phenolic acids commonly found in plants are the hydroxybenzoic acid derivatives mainly ellagic acid, gallic acid, gentisic acid, salicylic acid, vanillic acid, whereas common hydroxycinnamic acid derivatives are coumaric acid, ferulic acid, caffeic acid, chlorogenic acid, sinapic acid, etc. The phenolic acids are mostly present in bound form in plant foods. Hydroxybenzoic acid derivatives are present in foods in the form of glucosides but in some cases occur as glucose esters also. Similarly, hydroxycinnamic acids occur frequently in foods as simple esters with quinic acid or glucose [7-9]

Hydroxybenzoic acids consist of C₆-C₁ structure derived directly from benzoic acid (Fig. 2.1a). Through the processes of hydroxylation and methylations of the aromatic ring, different structures of individual hydroxybenzoic acid are derived. [10]

Hydroxycinnamic acids consist of C₆-C₃ structures (Fig. 2.1b) and are found in conjugated forms as esters of hydroxyacids such as quinic, shikimic and tartaric acid and their sugar derivatives. [11]

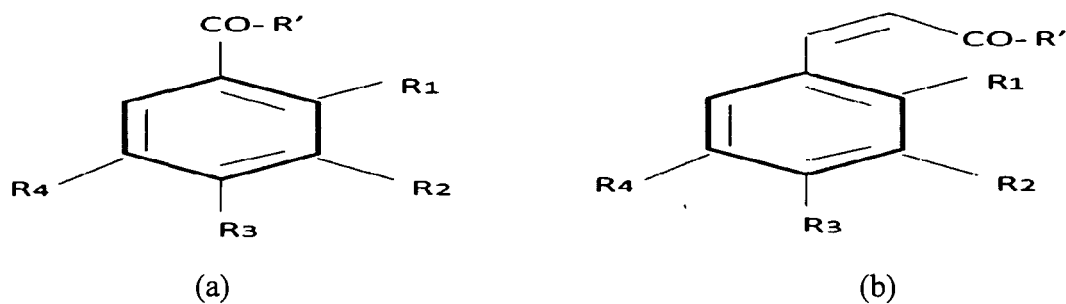


Fig.2.1. Basic structure of hydroxybenzoic and hydroxycinnamic acid derivatives

2.1.2. Flavonoids

Flavonoids are derivatives of diphenylpropanes (C₆-C₃-C₆) and two benzene rings linked through three carbons that usually form an oxygenated heterocycle (Fig. 2.2). [12] The flavonoids are further classified into subclasses based on their structural variations within the rings. They are divided into flavonone, flavones, dihydroflavanols, flavonol, flavan-3-ols, anthocyanidins, isoflavones and proanthocyanidins. [6]

The flavonoids often occur as glycosides, glycosylation renders the molecule more water-soluble and less reactive toward free radicals. Glucose is the common sugar that takes part in glycoside formation with flavonoids although glycosylation with other sugars like galactose, rhamnose, xylose and arabinose as well as disaccharides such as rutinose have been reported. ^[13] The flavonoid variants are all related by a common biosynthetic pathway, incorporating precursors from both the shikimate and the acetate-malonate pathways. ^[14] The variation in flavonoid also take place due to further modifications that occur at various stages of their synthesis pathway, resulting in an alteration in the extent of hydroxylation, methylation, isoprenylation, dimerization and glycosylation (producing O- or C-glycosides).

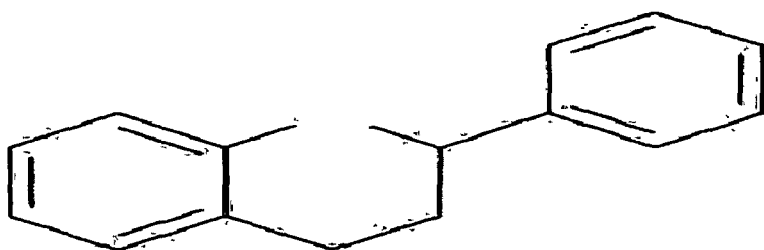


Fig.2.2. Basic structure of a flavonoid

2.1.3. Biosynthesis of polyphenol

The biosynthesis and accumulation of secondary compounds can occur either during developmental differentiation ^[15] or it can be induced by exogenous factors such as light, temperature and wounding. ^[16, 17] The common precursor of phenolic compounds synthesis is phenylalanine, a metabolite obtained through the shikimic acid pathway as presented in Figure 2.3. ^[10, 15] The shikimic acid pathway converts simple carbohydrate precursors into the amino acids phenylalanine and tyrosine. Many secondary phenolic compounds are derived from phenylalanine and tyrosine. Compounds derived from phenylalanine and to some lesser extent from tyrosine are referred to as phenylpropanoids. The phenylpropanoids include trans-cinnamic acid, coumaric acid and their derivatives; benzoic acid derivatives; and coumarins called as phenylpropanoids lactones.

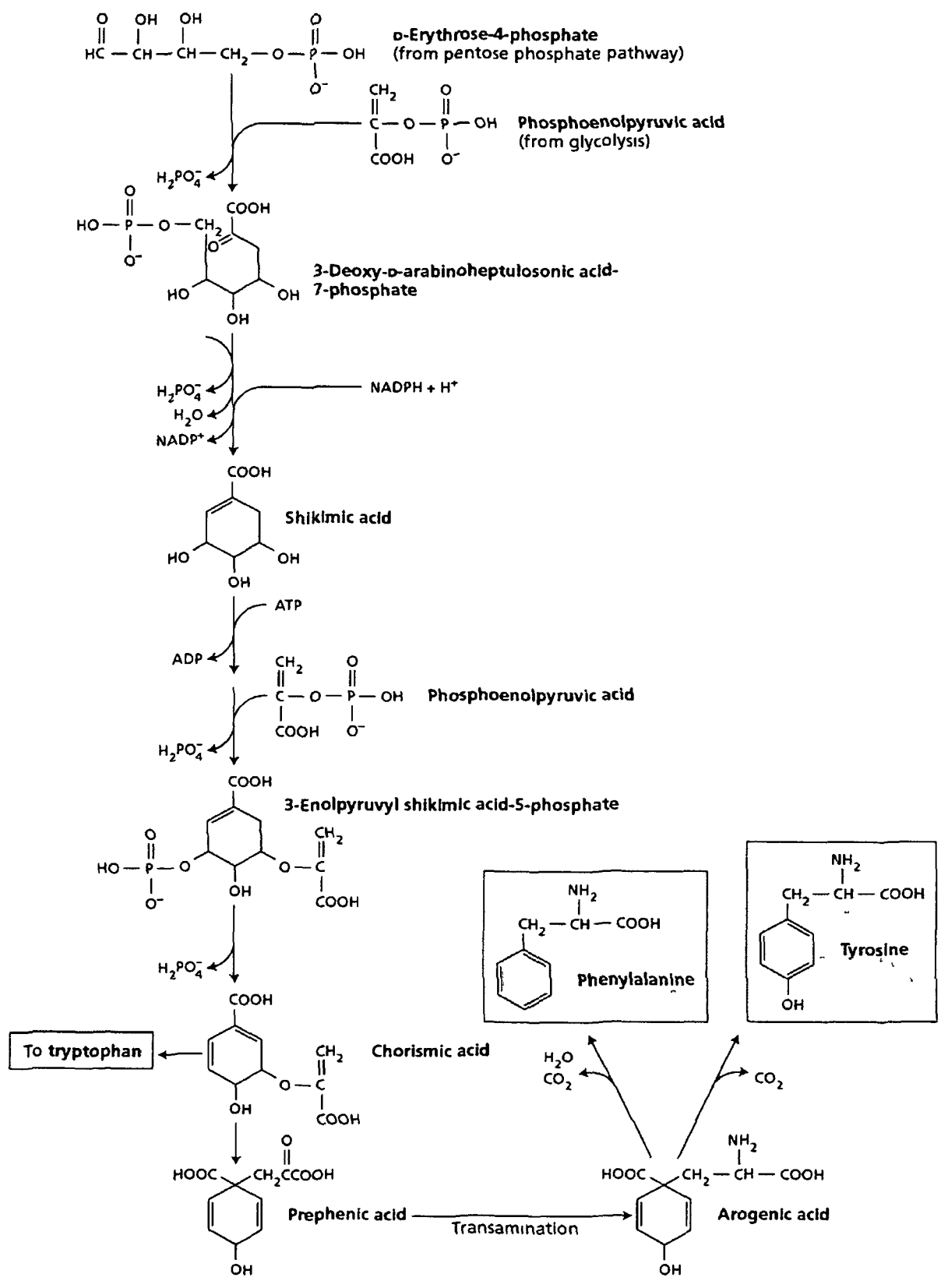


Fig.2.3. Shikimic acid pathway leading to synthesis of phenylalanine

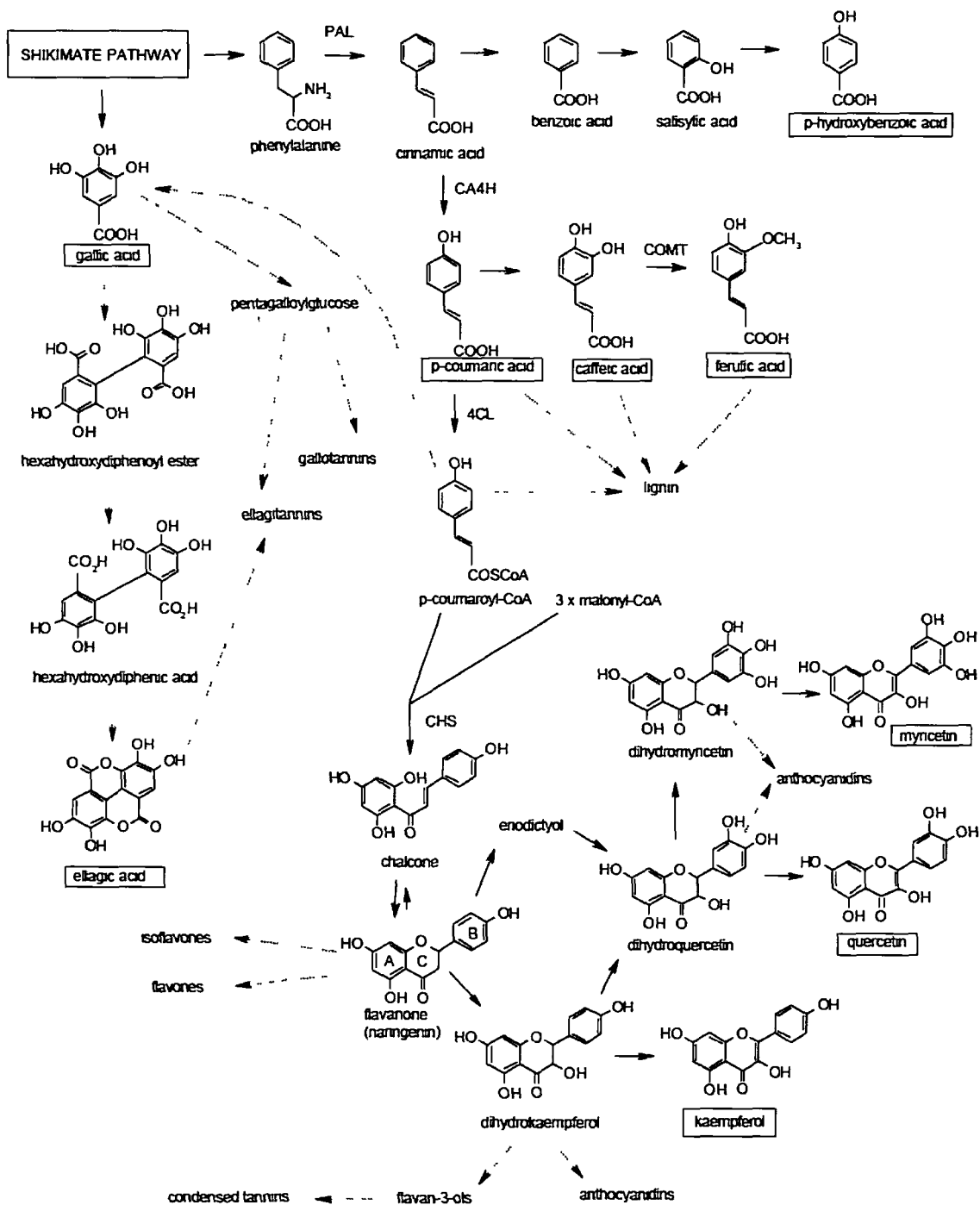


Figure 2.4. Biosynthesis of hydroxycinnamic acids, hydroxybenzoic acids and flavonoids. [15-21] Arrows indicates reaction steps catalysed by the specific enzyme. Major Enzymes: CA4H, cinnamic acid 4-hydroxylase; CHS, chalcone synthase; 4CL, 4-coumarate:coenzyme A ligase; PAL, phenylalanine ammonialyase.

The key enzyme involved in the catalysis of deamination of L- phenylalanine into trans-cinnamic acid with the release of NH₃ is phenylalanine ammonia lyase (PAL). This deamination initiates the main phenylpropanoids pathway (Fig. 2.4).

In some cases, the shikimic acid pathway precursors directly lead to formation of gallic acid which in turn gets converted into ellagic acid. The PAL activity is under the influence of both intrinsic and extrinsic environmental factors. Pathogenic attack or mechanical injury and stress increases PAL activity. Similarly, PAL activity also increases during the production of lignin for strengthening the secondary xylem cells and production of pigments in flowers. The initiation of the phenolic acid synthesis pathway by the PAL with the introduction of a hydroxyl group at the 4-position of the ring of the cinnamic acid gives *p*-coumaric acid (i.e. 4-hydroxycinnamic acid). Addition of a second hydroxyl group at 3-position to *p*-coumaric acid gives caffeic acid while O-methylation of this hydroxyl group yields ferulic acid. ^[22]

Flavonoids are derived from *p*-coumaric acid when condensed with malonic acid to form chalcones catalysed by the enzyme chalcone synthetase (CHS). The chalcone thus formed is then transformed by isomerisation into naringenin catalysed by the chalcone isomerase enzyme. The activity of chalcone isomerase like PAL is controlled by both intrinsic and extrinsic factors. The naringenin thus obtained is the precursor or progenitor of basically all the subsequent flavonoid structures. ^[22]

2.1.4. Functional role of polyphenols (phenolic acids and flavonoids)

The polyphenols play an important role in the biological medium because of their antioxidant properties having wide range of biological effects. The free radical destroying capacity in polyphenol is due to its property to accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components. However, the efficiency of polyphenols as antioxidant compounds greatly depends on their chemical structure. ^[23] Phenol itself is inactive as an antioxidant, but *ortho*- and *para*-diphenolics have antioxidant capacity, which increases with the substitution of hydrogen atoms by ethyl or *n*-butyl groups. Among the polyphenols, flavonoids are the most potent plant antioxidants because they possess one or more of the following structural elements involved in the antiradical activity: (i) an *o*-diphenolic group (in ring B), (ii) a 2–3 double bond conjugated with the 4-oxo function, and (iii) hydroxyl groups in positions 3.

Studies have shown that polyphenols protect LDL oxidation *in vivo* with significant positive effect in atherosclerosis.^[24] They also protect DNA from oxidative damage.^[25] In addition, polyphenols can inhibit platelet aggregation^[26], improve endothelial dysfunction^[27], lower risk of myocardial infarction.^[28] They can prevent neurodegenerative diseases^[29], protect against neurotoxic drugs^[30] and has anticarcinogenic effect.^[31] The effectiveness of polyphenols in treatment of diabetes and osteoporosis had also been reported.^[32] The flavonoids have antithrombotic and anti-inflammatory properties.^[33, 34] The antimicrobial property of polyphenolic compounds has been well documented.^[35]

Bravo^[36] opined that the anticarcinogenic property of polyphenols might be due to their inhibiting role in several of the steps that lead to the development of malignant tumors, inactivating carcinogens, inhibiting the expression of mutant genes and the activity of enzymes that are involved in the activation of procarcinogens and activating enzymatic systems involved in the detoxification and degradation of xenobiotics.

Various epidemiological studies have shown that the consumption of polyphenols or polyphenol rich diet is inversely proportional to the risk of cardiovascular diseases. Arts and Hollman^[37] studied the correlation between the intake of flavonols, flavones, catechins and lignans and the risk of coronary heart diseases and strokes. Studies have established that an increasing notion that reduction of cellular expression and decreased activity of antioxidant proteins and consequent augmentation of oxidative stress are fundamental causes for aging processes and neurodegenerative diseases.^[38, 39] The free-radical theory of aging states that organisms age because cells accumulate free radical damage over time.^[40] This generally paves the way for the onset of many degenerative diseases like heart failure^[41], myocardial infarction^[42], Alzheimer's disease^[43] and chronic fatigue syndrome.^[44] Therefore, many researchers like Calabrese et al.^[45] advocated the activation of antioxidant pathways particularly for tissue with relatively weak endogenous antioxidant defenses such as the brain. Endogenous defense mechanisms can be inadequate for the complete prevention of oxidative damage with increasing age and different sources of dietary antioxidants can be helpful in destroying the free radicals.^[46] Studies had shown that diet is a key environmental factor and a potential tool for the control of chronic diseases.^[47] Fruits and vegetables have been shown to exert a protective effect.^[48, 49] The high content of vitamins A, C, and E and polyphenol antioxidants in fruits and vegetables may be the main factor responsible for these

effects.^[50-52] Rats when supplemented in their diet with aqueous extracts of spinach, strawberry, or blueberry rich in polyphenols improved their cognitive functions and neuronal signal transduction.^[53] Grape polyphenols were found to reduce the neurodegenerative changes induced by chronic ethanol consumption in rats, and improved the synaptic function measured on isolated synaptosomes.^[54] Similarly, intravenous administration of caffeic acid and isoferulic acid to rats reduces the fasting glycemia and attenuates the increase of plasma glucose during intravenous glucose tolerance test.^[55, 56]

2.2. Polyphenols in fruits and vegetables

Fruits and vegetables are naturally very rich in these phytochemicals.^[57, 58] Studies had established that fruits and vegetables when consumed on daily basis in the diet have many beneficial effects. Phenolic compounds in addition to being antioxidants were reported to also exhibit anti-inflammatory, anti-carcinogenic, anti-arthritic activities *in vitro*.^[59, 60] Epidemiological studies have reported that regular consumption of fresh fruits and vegetables has many health promoting properties and helps in prevention of many degenerative diseases like cardiovascular heart disorders, atherosclerosis, cancer, Alzheimer's and other age related disorders.^[61-63] Scalbert and Williamson^[64] has reported dietary intake of about 1 g/day of polyphenols which represents approximately two-thirds of the total daily phenolic intake, with approximately one third of it's as phenolic acids. The most abundant types of polyphenols found in diet are the flavonoids, most often in their glycoside conjugate forms. More than 4000 chemically unique flavonoids have been identified in plants, particularly fruits, vegetables, nuts, seeds, and flowers. The polyphenols in wine include phenolic acids, anthocyanins, tannins, and other flavonoids.^[65]

Phenolic compounds content in immature fruits is much higher than in mature fruits.^[66] Distribution of phenolics in plants at the tissue, cellular and subcellular levels is not uniform.^[67] Phenolic acids present in grape berries and their skins contain caftaric acid (*trans*-caffeoyltartaric acid), coumaric acid and *trans*-ferulic acid (ester of ferulic acid bound to tartaric acid).^[68, 69] They also consist of flavonols such as quercetin 3-glucuronide, quercetin 3- glucoside, myricetin 3-glucuronide and myricetin 3-glucuronide^[69] and flavanonols, such as astilbin (dihydroquercetin 3-rhamnoside) and engeletin (dihydrokaempferol 3-rhamnoside). In addition to that they also contain a number of stilbenes for example *trans*- and *cis*- resveratrols.^[70] Grape seeds and skins are also good

source of proanthocyanidins, flavonols and flavan-3-ols.^[69] Procyanidins are the predominant proanthocyanidins in grape seeds, while prodelfinidins along with procyanidins are dominant in grape skins and stems.^[71, 72]

In apples, hydroxycinnamic acid (HCA) derivatives such as chlorogenic acid, flavan-3-ols (monomeric and oligomeric), flavonols and their conjugates, dihydrochalcones and procyanidins are present.^[73, 74] Number of flavonol glycosides has been identified in apple fruits, namely rutin, hyperin, isoquercitrin, reynoutrin, avicularin and quercitrin.^[75-78] The vacuole in the epidermal and subepidermal cells of apple skin contains anthocyanins.^[79] The procyanidins in apple fruit are mixture of oligomers and polymers made of (-) -epicatechin and (+)-catechin as monomeric units.^[80]

Similarly, citrus fruits consist of cinnamic acid derivatives, coumarins and flavonoids (flavonones, flavones and flavonols) as their major groups of phenolic compounds.^[81, 82] Generally the phenolic compounds in the form of esters, amides and glycosides are embedded in the flavedo of citrus fruits.^[81, 83, 84] Among citrus fruits which exhibited intermediate antioxidant capacity, oranges are the most effective followed by grapefruit. This is in agreement with the higher concentrations of phenolic compounds and vitamin C present in orange with respect to grapefruit.^[85] Depending on the species and cultivar the distribution of phenolic compounds differs. For example, the major flavanone glycosides found in grapefruit are naringin; naringenin 7-neohesperidoside and narirutin, naringenin 7-rutinoside, while in sour oranges naringin, neohesperidin and hesperetin 7-neohesperidoside and narirutin and hesperedin, hesperetin 7-rutinoside in sweet oranges are predominant.^[86] On the other hand, hesperedin, narirutin and didymin (isosakuranetin 7-rutinoside) are the predominant flavanone glycosides in navel^[87] and blood oranges.^[88]

The different fruits belonging to the berry family are also rich sources of polyphenols. Sariburun et al.^[89] reported that high antioxidant capacity of berries is likely due to the high content of phenolic acids and flavonoids such as anthocyanins. For example, blueberries contains rich amount of phenolic acids, catechins, flavonols, anthocyanins and proanthocyanidins.^[90-92] Other phenolic acids identified in blueberry fruits are gallic, caffeic, *p*-coumaric, ferulic and ellagic acids.^[92] Blueberries are also rich in anthocyanins like 3-galactosides and 3-arabinosides of cyanidin, delphinidin, peonidin, petunidin and malvidin and 3-glucosides of cyanidin, delphinidin, peonidin, petunidin and malvidin.^[93, 94] Apart

from that, catechin, myricetin, quercetin and kaempferol were also detected in blueberries. Another berry fruit known as cranberries contains good amount of anthocyanin, proanthocyanidins and phenolic acids. ^[95, 96]

Among vegetables, spinach, peppers, and asparagus had the highest antioxidant activity. In tomatoes, the flavonols are located in the skin. ^[97] Compared to the large sized tomatoes, cherry tomatoes have higher flavonols level. ^[98] The major flavonols are a mixture of rutin, quercetin 3-rhamnosyldiglucoside, kaempferol 3-rhamnosylglucoside and kaempferol 3-rhamnosyldiglucoside. ^[99] Major phenolic acids in carrots include *p*-hydroxybenzoic acid, syringic acid and neochlorogenic acid, chlorogenic acid. ^[100, 101] The presence of coumarins in the periderm tissue of carrot tissue was also reported in carrot tissues. ^[102, 103] Lettuce is another good source of flavonoids such as quercetin conjugates, namely quercetin 3-(6-malonylglucoside), quercetin 3-glucoside, quercetin 3-glucuronide, quercetin 3-rhamnoside, quercetin 3-galactoside, and quercetin 3-(6-malonylglucoside)-7-glucoside. ^[104, 105] Spinach leaves consists of flavonoids such as patuletin, jaceidin and spinacetin ^[106] Onions contain quercetin, isorhamnetin, myricetin, kaempferol mostly distributed and concentrated in the skin. ^[107] Approximately 50% of flavonols were detected in the top quarter part of the scales. The red onion skin and the outer fleshy layer ^[108] are the major areas containing anthocyanins, namely peonidin 3-glucoside, cyanidin 3-glucoside and cyanidin 3-arabioside and their malonylated derivatives, cyanidin 3-laminariobioside and delphinidin and petunidin derivatives. ^[109] In peppers, capsaicinoids and flavonoid conjugates like quercetin and luteolin are the major phenolic compounds found. ^[11-113] The distribution of the compounds responsible for the pungency in peppers viz. capsaicinoids, acid amides of vanillylamine and C8 and C13 branched fatty acids ^[114], are accumulated predominantly in the epidermal tissue of the placenta. ^[115, 116]

2.3. Polyphenol bioavailability and metabolism

Polyphenols are absorbed from the gut in their native or modified form. However, the mechanism involving the absorption, biodistribution and metabolism of polyphenols is known only partially. There exists great variability in the bioavailability of the different polyphenols. ^[117] The bioavailability is lower for complex flavonoid conjugates with several sugars and acylated with hydroxycinnamic acids complex and condensed tannins. The amount of large molecule-complexed phenolics is generally higher than that of simpler

phenolics. However, studies have showed that the microbial population in the gut helps in breakdown of these complex phenolics into simpler metabolites with better absorption and potent bioactive properties than their parent compound. ^[118] The absorption pattern and behavior of some polyphenols were tried to be explained using experiments on animals by many researchers. It was found that they are subsequently metabolized into products which are eventually detected in plasma that retain at least part of the antioxidant capacity and are then excreted. ^[119, 120] Studies on human model had reported the detection of quercetin after the consumption of onions, tea, and apple juice. ^[121-123] Manach et al. ^[123] reported the retention of antioxidant activity of the plasma metabolites derived from some polyphenols. Similarly, consumption of red wine leads to the accumulation of o-methylcatechin, a catechin metabolic product, in plasma. ^[124] Pietta et al. ^[125] detected green tea flavanols in plasma and some monohydroxy and dihydroxybenzoic acids in urine, accounting for approximately 15% of the polyphenols administered. It was assumed that these phenolic acids are formed as a result of bacterial metabolization of catechin and quercetin in the gut. Another possibility in polyphenol metabolism is the methylation of one or more phenolic hydroxyls which was sometimes observed in catechin, epicatechin and flavonoids metabolism. ^[126, 127] The methylation is apparently mediated by catechol-O-methyl transferase, an enzyme present in liver and kidney. As reported by Morand et al. ^[120], appearance of glucuronic acid and sulfate conjugate of isorhamnetin which is a 3' methylation product of quercetin as metabolite in rats administered with 0.2% quercetin suggest the occurrence of methylation. Likewise, daidzin is transformed into equol, by the intestinal microflora which has got higher antioxidant activity. ^[128] Similarly, Piskula and Terao ^[126] reported the presence of epicatechin, methylated and conjugated with glucuronic acid and sulfate, as the plasma metabolite with the longer half life, after a single dose of epicatechin to rats. Another mode of polyphenol metabolism is glucuronidation which occurs in the intestine and in the liver ^[120, 126] and sulfation which apparently occurs only in the liver. ^[126, 129]

2.4. Effect of processing of fruits and vegetables on the polyphenol content

Fruits are often consumed in their native raw forms and vegetables are cooked to make them soft and more palatable. But with the technological development in the food industries, a number of processing methods have developed which help in increasing the

shelf life and sometime the acceptability of the fruits and vegetable. In addition to that, processing of fruits and vegetables ensures their availability in off season and in places where these are generally not grown or available. Processing also minimizes the loss of quality to some extent during transportation. Intact fruits and vegetables obviously are more prone to deleterious changes induced by respiratory, metabolic and enzymatic activities, as well as by transpiration, pest and microbial spoilage and temperature-induced injury during long distance transportation and hence spoilage loss is very high. But processing of a plant product has both positive and negative features. Types and extent of processing could impact on their phytochemical and antioxidant status. Most such changes may impact adversely on the antioxidant status of these products. ^[130] Fruits and vegetables undergo several types of oxidative reactions during the processing. These may involve removal of electrons from atoms leading to formation of an oxidised form. These reactions may cause browning reactions, loss or changes to flavour or odour, changes in texture and loss of nutritional value from destruction of vitamins and essential fatty acids.

2.5. Processing effects on the polyphenol content of fruits and vegetables

Thermal processing of fruits and vegetables has significant effect on their physical and chemical properties. Generally, they are recognized as one of the major factors on the destruction or changes of natural phytochemicals, which may affect the antioxidant capacity in foods. ^[131] Steaming, canning, microwave heating, boiling, blanching are common processing treatments given to the vegetables. Likewise, thermal pasteurisation, microwave pasteurisation, spray drying, canning, etc are some of the common processing treatments given to fruits. Cooking can influence the composition, concentration and bioavailability of bioactive compounds in vegetables. Both positive and negative effects have been reported depending upon differences in process conditions and morphological and nutritional characteristics of vegetable species. In general, phenolic compounds are present in fruits and vegetables in free and bound forms. On application of some kind of processing, tissue disruption could release these bound phenolics and could increase the polyphenols yield during extraction that may either enhance or reduce the antioxidant activity. For example, both hydrophilic and lipophilic- ORAC values in cooked tomatoes are significantly higher than in their raw forms. ^[132] In peppers, cooking by boiling in water decreases the radical

scavenging activity, whereas microwave heating in absence of water increase their activity. [133]

In some cases, application of heat inactivates the polyphenol oxidase enzyme and thus lowers the rate of oxidation of the phenolic compounds. But the efficiency of the thermal treatment in retention of the phenolic compounds and their antioxidant activity is dependent on the individual phenolic acid present and their susceptibility to heat and overall property of the food matrix in which they are embedded. [134] Also, reduction in antioxidant compounds in thermally treated fruits and vegetables may also be attributed to consumption of ascorbic acid and polyphenols as reactants in the maillard reaction.

Rossi et al. [135] reported, high recovery of phenolic compounds and strong radical-scavenging activity to DPPH and hydroxyl radicals in blueberry juice after steam blanching for 3 min than the non-blanched blueberry fruits. Antioxidant activities in processed tomatoes [136, 137] and coffee [138] were retained or were higher compared to their fresh equivalents.

The increase or retention of antioxidant activities in processed foods could be due to development of new compounds with potential antioxidant capacity although the content of naturally occurring antioxidants has significantly decreased due to the heat processing. [131, 138, 139] Microwave heating treatments either retains or decreases a small quantity of total phenolic content in cauliflower, peas, spinach and Swiss chard, whereas significant reduction had been reported after boiling for 6-13 min. [140] The same investigators observed significant increase or retention of total antioxidant capacity in cauliflower, peas, spinach, Swiss chard, potatoes, tomatoes and carrots. In another study, researchers investigated the effect of microwave cooking on different radical scavenging capacity (lipoperoxyl, hydroxyl and ABTS radicals) of vegetables. [141] The researchers observed 30-50% loss of lipoperoxyl radical scavenging capacity in most of the vegetables; artichoke, asparagus, garlic, onion, and spinach retained scavenging capacity; and eggplant, maize, pepper, and Swiss chard increased their antioxidant activity after microwave cooking. However, microwave cooking of broccoli severely degrades phenolic content and antioxidant capacity in florets and stems and potatoes. [142, 143] But Turkmen et al. [144] reported studies on green vegetables and herbs and found phenolic content and antioxidant capacity increased or remained unchanged after microwave irradiation.

Tiwari et al. ^[145] reported a decrease in anthocyanin content of blackberry juice during sonication treatment. The destruction of anthocyanins during sonication could be due to oxidation reaction that is promoted by the interaction with free radicals formed during sonication ^[146] or sonochemical reactions including generation of free radicals, enhancement of polymerization/depolymerisation reactions and other reactions. ^[147]

Another thermal processing method in fruits is pasteurisation. Pasteurisation of juice is based on the principle of 5 log reduction of the most resistant micro-organisms of public health significance. ^[148] The degree of heat and duration of exposure applied during pasteurisation generally has detrimental effect on the juice phenolic compounds. For example, greater phenolic degradation (48%) was found in apple juices pasteurised at 94°C compared with those pasteurised at 72°C. ^[149]

2.6. Fruit by-products, their phytochemical content and utilization

By-products produced in the fruit industries pose serious environmental and health risk. Due to increased industrial growth in the food sector, after processing of the edible part, large quantities of fruit wastes such as citrus fruit skins, pineapple residues, sugarcane bagasse and other fruit residues (principally peels and seeds) are being generated ^[150] that pollute the environment. However, from the past some years, utilization of these wastes as sources for extraction of secondary metabolites like bioactive compounds or phytochemicals has been proved to be highly cost-effective and also it has helped to minimize the environmental hazards. ^[151] The phytochemicals present in the fruit waste generally consist of polyphenols or phenolic compounds, carotenoids, dietary fibre, sugars, minerals, organic acids.

Apple pomace is a rich source of polyphenols, minerals and dietary fibre. ^[152, 153] Pectin consists of 10–15% of apple pomace, on a dry weight basis and is recovered by acid extraction and precipitation. The total phenolics, total flavonoids, total flavan-3-ols, and some individual phenolic compounds such as epicatechin, its dimer (procyanidin B2), trimer, tetramer and oligomer, quercetin glycosides, chlorogenic acid, phloridzin and 3- -hydroxy-phloridzin contributed significantly to the antiradical activities of apple pomace. ^[154]

Pomace represents approximately 20% of the weight of processed grapes. Its composition varies considerably, depending on grape variety and technology of wine

making. Grape pomace contains high content of organic substances such as sugars, tannins, polyphenols, polyalcohols, pectins and lipids which poses detrimental effects on the flora and fauna of discharged zones. ^[155, 156] From the grape seeds, grape seed oil is mainly produced in Italy, France and Spain. The seed oil has high content of polyunsaturated fatty acids and also phenolic compounds such as gallic acid, catechin and epicatechin, and a wide variety of procyanidins. Extracts of white and red grape pomace showed the presence of glycosylated flavonols such as quercetin and kaempferol. ^[157] In addition to that ethanol, tartrates, citric acid, grape seed oil, hydrocolloids, and dietary fibre are recovered from grape pomace. ^[158, 159] The antioxidant activity of the natural phenolic extracts obtained from grape waste increases their prospect of commercialization as natural powerful antioxidants in foods in order to increase the shelf life of food by preventing lipid peroxidation and protecting from oxidative damage. Further, their hypoglycaemic and lipid lowering properties makes them a good candidate for incorporation as ingredient in development of functional food products.

Ajila et al. ^[160] studied the bioactive compounds and antioxidant potential in mango peel extract and reported that mango peel contains a number of valuable compounds such as polyphenols, carotenoids, enzymes and dietary fibre. Citrus wastes are good sources of phytochemicals. The total phenolic and flavonoid content in peels of lemons, oranges, and grapefruits were higher than those in the peeled fruits. ^[161] The flavonoid classes present in citrus peel are flavones, flavanones, flavonols, isoflavones, anthocyanidins and flavanols. ^[162] They were assumed to have antioxidative, anti-cancer, anti-viral, and anti-inflammatory activities as well as inhibit human platelet aggregation. ^[163] Similarly, consumption of citrus peel showed significant reduction of both total and HDL-cholesterol levels of the serum ^[164] and also exhibited a protective role against diet-induced atherosclerosis and thyroid dysfunction in a rat model. ^[165]

Pomace of *Averrhoa carambola* (also known as carambola/star fruit) is a by-product obtained in large quantity after juice extraction. It is a good source of phytochemical compounds like phenolic acids and proanthocyanidins and has insoluble fibre-rich fraction (FRF) as the major fraction. ^[166]

The phytochemicals from the fruit waste are extracted using different methods among which solvent extraction technique for polyphenol extraction is the most common

method applied. The extracted polyphenols from different fruits and their by-products are generally, incorporated to develop functional food products. The phytochemicals are added as such directly into the food to develop a product in most of the cases. But some kind of processing or delivering system is required in some cases to ensure the effectiveness of the phytochemical for their targeted functions. This is because phytochemicals as such are prone to destruction and oxidation from environmental factors like light and oxygen. Moreover, phytochemicals mainly polyphenols have an inherent astringent taste which could hamper or mask the overall sensory desirability of the food product to which they are added. ^[167] Microencapsulation technique is widely studied to deliver the phytochemicals to the consumer without destruction. It protects the core material by encapsulating it with a wall or coating material that prevents exposure to adverse environmental conditions and also promotes controlled release of the encapsulate. ^[168] Desai and Park ^[169] summarized the reason for the use of microencapsulation technique.

- Protection to the core material from degradation due to its interaction to the outside environment
- Reduction of the evaporation or transfer rate of the core material to the outside environment
- Modification of the physical characteristics of the original material to allow easier handling
- Smooth release or delivery of the core material slowly over time, or at a particular time
- To mask unwanted flavor or taste of the core material
- Dilution of the core material when only small amounts are required, while achieving uniform dispersion in the host material
- To help separate the components of the mixture that would otherwise react with one another.

Presently, spray drying, freeze drying, spray cooling, extrusion, coacervation, liposome entrapment, co-crystallisation, emulsion, etc. ^[169, 170] are some of the widely used encapsulation techniques used in the food industries. Widely used coating or wall materials for encapsulation are maltodextrin, starch, modified starch and protein conjugates, gums, pectin, etc. The encapsulation for polyphenol compounds overcomes the hurdles of

unpleasant tastes or flavors, improves the bioavailability and half-life of the compound *in vivo* and *in vitro* as well as increases their stability. ^[171]

Dietary fibre as defined by American Association of Cereal Chemists (AACC) ^[172] is the remnant of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fibres are classified as soluble or insoluble based on their solubility in water. Soluble dietary fibres include pectic substances, gums, mucilage, and some hemicelluloses, whereas cellulose, other types of hemicelluloses and lignin are included in the insoluble fraction. ^[173] Soluble fibres increase the viscosity and help to reduce glycaemic response and plasma cholesterol. ^[174] Insoluble fibres are more porous and help to increase faecal bulk and decrease intestinal transit. ^[175, 176] Previous studies on carambola pomace have reported that its fibre has desirable functional properties, *in vitro* hypoglycemic effects, and *in vivo* hypolipidemic and hypocholesterolemic effects. ^[177-179]

Dietary fibre consists of carboxyl and hydroxyl side chain groups in their structure and act as cation exchange resin. ^[180] Studies have shown that many cations can be exchanged by dietary fibre, especially some toxic cations. Then, such absorbed toxic ions can be excreted with the faeces. ^[181-184] The fibre also plays an important role in prevention of cancer, high blood pressure ^[185], heart disease ^[186] and cardiovascular disease. ^[187] Moreover, it has glucose absorption property ^[188], prevents constipation ^[189], lowers the level of total cholesterol and triglycerides ^[190], inhibits the synthesis of hepatic fatty acid and maintains stable blood sugar levels after meals and thus reduces postprandial blood glucose (reduces insulin responses) and reduces pre-prandial cholesterol levels. In addition, dietary fibres increase the faecal bulk and stimulate colonic fermentation that helps in maintenance of the colon health. ^[191] The colonic bacteria takes part in the fermentation of the dietary fibre and secondary metabolites having bioactive role are produced as end products. The short chain fatty acids such as butyrate, propionate, acetates and many other metabolites produced during microbial fermentation of the dietary fibre helps in prevention of colon cancer and maintenance of overall colon health. ^[192] They possess anti-proliferative, anti-inflammatory and apoptotic properties. ^[193]

Saura-Calixto et al. ^[194] described the association of polyphenolic compounds and carotenoids with the matrix of the dietary fibre. This suggests that antioxidant dietary fibre could be incorporated in fatty foodstuffs for improving their oxidative stability and prolonging their shelf life. ^[195]

In new product development field, fibre imparts functional properties such as increase in water holding capacity and oil holding capacity, enhanced emulsification and/or gel formation ability which could be exploited to develop newer products with improved quality and value additions. Indeed, dietary fibre incorporated into food products (bakery products, dairy, jams, meats, soups) can modify textural properties, avoid syneresis, stabilise high fat food and emulsions, and improve shelf-life. ^[196, 197] There is therefore wide scope for utilization of dietary fibre from fruit wastes in development of functional foods.

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