PHENOLIC AND ANTIOXIDANT ACTIVITY IN ASIAN VEGETABLES

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Contents

- 1. Introduction
- 2. Classification of phenolic compounds
- 3. Antioxidant activity
- 4. Phenolics in Asian vegetables
- 4.1. Beet Root
- 4.2. Carrot
- 4.3. Radish
- 4.4. Sweet Potato
- 4.5. Turmeric
- 4.6. Spinach
- 4.7. Fenugreek
- 4.8. Amaranthus
- 4.9. Chenopodium
- 4.10. Coriander
- 4.11. Asparagus
- 4.12. Lettuce
- 4.13. Fennel
- 4.14. Mustard
- 4.15. Cruciferous Vegetables
- 4.16. Cowpea
- 4.17. French Bean
- 4.18. Broad Bean
- 4.19. Brinjal
- 4.20. Tomato
- 4.21. Capsicum
- 4.22. Onion and Shallot

- 4.23. Garlic
- 4.24. Cucurbitaceous Vegetables
- 4.25. Bamboo shoots
- 4.26. Jackfruit
- 4.27. Moringa
- 4.28. Asian Tuber Crops
- 4.29. Malabar spinach
- 5. Correlation between Phenolic Compounds and Antioxidant Activity
- 6. Effect of Processing and Storage on Phenolics in Vegetables
- 7. Bioavailability
- 8. Ways to Promote Vegetable Consumption in Children
- 9. Conclusions
- Acknowledgement Glossary Bibliography

Biographical Sketches

Summary

Vegetables are rich source of dietary fiber, vitamins, phenolic compounds and minerals known to improve immunity and lower incidence of type-2 diabetes, cancer, and neurodegenerative disease. The present chapter aims to provide a comprehensive overview of the different classes of phenolics, their mechanism of action, bioavailability and associated antioxidant activity (AOX) present in foods. Detailed information on different phenolics present in different Asian vegetables is presented. The chapter also covers and summarizes the effect of various processing methods in improving the total phenolic content (TPC) and AOX of vegetables. Role of anthocyanin (ACN) rich vegetables and fermented vegetables in diet has also been highlighted. The information provided in the chapter will be of immense value to researchers, nutritionists, and students; for improving their diet by incorporating more healthy vegetables in diet.

1. Introduction

Fruits and vegetables (F&V) are an integral part of human nutrition as they are a source of vitamins, minerals and antioxidant essential for fulfilling our basic physiological needs. The World Health Organization (WHO) recommends consumption of at least 400 g of F&V each day or 5-a-day, to prevent non-communicable diseases such as heart disease, stroke and some types of cancer (Chawner & Hetherington, 2021). Overwhelming epidemiological evidence has demonstrated that consumption of F&V helps to counteract oxidative stress which is responsible for the pathogenesis of various lifestyle diseases such as cardiovascular, diabetes, Alzheimer's, arthritis, Parkinson's and cancer. The sedentary lifestyle, consumption of processed food, exposure to pollution and lack of exercise plays an important role in inducing oxidative stress in our body (Sharifi-Rad et al., 2020).

Oxidation is a normal necessary process that regulates the normal functioning of our body and generates free radicals. Free radicals are reactive oxygen species (ROS) that help fight pathogens and antigens; however, when they are produced in excessive amounts, there is imbalance between ROS and defense related antioxidants. This situation leads to oxidative stress subsequently diseases. The most common ROS includes superoxide radical (O^{-2}), singlet oxygen (${}^{1}O_{2}$), hydrogen peroxide ($H_{2}O_{2}$), and hydroxyl radical (OH). ROS damage lipid, protein, and DNA; break down the structural and cellular proteins. They also break lipid membrane and increase membrane fluidity and permeability. Protein damage involves site-specific amino acid modification, peptide chain fragmentation, cross-linked reaction product aggregation, enzymatic inactivation, and proteolysis. In DNA damage, they oxidize deoxyribose, breaks strand, modify bases and crosslink DNA-protein. High amounts of free radicals damage cells and cause apoptosis, leading to the pathogenesis of cancer, diabetes, and cardiovascular disease. Oxidative stress also stimulates the immune response and causes allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis, or food allergies.

Antioxidants are compounds that are capable of quenching free radicals and break radical chain reactions, maintain haemostasis and prevent oxidative damage. A more biologically relevant definition of antioxidant is "a synthetic or natural substance added to products to prevent or delay their deterioration by the action of oxygen in the air (oxidation). In biochemical terms, antioxidants are enzymes or organic substances that are capable of counteracting oxidative stress in animal tissues. On the nutritional perspective, antioxidants can be classified into endogenous and exogenous antioxidants. Endogenous antioxidants are synthesized in the cells and include all enzymatic antioxidants along with some non-enzymatic ones (i.e., thiols antioxidants and coenzyme Q10). Whereas, exogenous antioxidants have to be supplemented through diet since their synthetic pathways are usually present only in microbial or plant cells. Common exogenous antioxidants include ascorbic acid, phenolics, vitamin E, Bcarotene, organosulfur compounds, glucosinolates and selenium. Vegetables are surplus in antioxidants and have been conferred the status of 'functional foods' as they provide health-promoting effects besides fulfilling basic physiological needs of the body (Kaur & Kapoor, 2001). Vegetables like, beetroot, tomato, broccoli, and garlic are good examples of functional foods.

The term "Asian Vegetables" encompasses a diverse array of plant species that are either indigenous to Asia or have become deeply integrated into Asian diets over centuries due to historical events like colonization, migration, and trade. Understanding the nativity of these vegetables is complex, as many now-ubiquitous vegetables, such as cruciferous varieties, and potatoes were introduced to Asia from other parts of the world. Conversely, vegetables like bamboo shoots, moringa, yam, taro, luffa, jackfruit, amaranth, Malabar spinach and several other vegetables have roots in Asian soil and remain staples in traditional culinary practices across the continent. These vegetables not only contribute to the nutritional and medicinal value of Asian diets but also reflect the rich agricultural heritage of the region. While modern cultivation practices often follow market demands and global health trends, many of these traditional vegetables considered in this chapter are given in subsections 4.1. to 4.29.

The human body utilizes natural, endogenous, and synthetic antioxidants as a defense against reactive species. Convincing evidence from the dietary patterns such as 'Mediterranean diet' (MD) and 'Japanese diet' (JD) supports the beneficial health

effects of consuming diet rich in fruits, vegetables, whole grain legumes, olive oil, polyphenols and vitamins (Siervo et al., 2021). The MD has been recognized as the healthiest lifestyle by UNESCO and the Council of the EU; and also recommended by the US Department of Health (Maggi et al., 2021). Data from multiple randomized clinical trials have demonstrated the powerful effect of MD against oxidative stress, neurodegenerative diseases, inflammation, cardiovascular disease and type 2 diabetes. Japanese population also has been known for having the longest life expectancy in the world, with food being the key factor associated with the longevity of the population. Balanced consumption of energy, grains, vegetables, fruits, meat, fish, eggs, soy products, dairy products, and alcoholic beverages have positively contributed to longevity and lower risk of cerebrovascular disease in Japan. Lower mortality rates associated with the Japanese diet have been documented by 'Dietary Intake Reference Guide' (Htun et al., 2017). Overwhelming scientific evidence supports the role of vegetables-based diet and adherence to a JD and MD in improving cognitive decline, dementia and Alzheimer's disease.

There are specific benefits of consuming vegetables compared to fruits and selective promotion of vegetables may improve health and reduce premature mortality. They are low in fat, high in mineral and dietary fiber content which improves gut health. In addition, bioactive phenolics, protect health through various antioxidant mechanisms; inhibit the oxidation of low density cholesterol (LDL), platelet aggregation, and prevent carcinogen formation (Wallace et al., 2020).

2. Classification of Phenolic Compounds (PCs)

Phenolic compounds (PCs) are organic compounds characterized by a hydroxyl group (-OH) attached to a carbon atom of an aromatic ring. In simpler terms, phenol is a monohydroxy benzene (C_6H_5OH) and is also known as benzaldehyde, similar to alcohol but has a strong hydrogen bond (Figure 1). Based on the origin, PCs can be classified into natural and synthetic PCs.



Figure 1. Basic structure of phenol

Natural PCs are the most abundant secondary metabolites and more than 8000 polyphenols have been identified in higher plants. These are widely found in plantbased foods (fruits, vegetables, cereals, olive, legumes, chocolate, etc.) and beverages (tea, coffee, beer, wine, etc.). PCs are responsible for sour, bitter and astringent taste of foods, for example, the bitter taste of spinach, lettuce, fenugreek and other vegetables. Generally, they are produced naturally or in response to environmental stress, UV radiation and against insect pest attack. They are synthesized through two pathways; i) shikimic acid where phenylpropanoids are formed and ii) acetic acid pathway in which simple phenols are formed. In plants, PCs range from simple phenolic acids to highly polymerized compounds such as tannins. Common example of PCs includes ferulic acid, gallic acid, biphenols (resveratrol), and polyphenols (tannic acid). In plant cells, PCs occur in two forms namely, free or bound. The bound or insoluble form, attached to other molecules frequently to sugars (glycosyl residue) and proteins in cell walls of plant materials. Free and soluble PCs referred to as aglycones, that are less common, as they are toxic in the free state (Shahidi & Yeo, 2016). Most bound-insoluble phenolics are bound to cell wall substances such as fiber, pectin, cellulose and structural protein through covalent bonds and that constitute a relatively large amount (20-60%) in vegetables and legumes. Bound phenolics can be released by basic, acidic and enzymatic hydrolysis methods and show strong biological activities.



Figure 2. Classification of phenolic compound

Natural phenolics have been classified into six categories, *viz.* phenolic acid, flavonoids, stilbenes, coumarins, lignans and tannins. The major class of PCs is summarized in Figure 2. Substituted derivatives of hydroxybenzoic and hydroxycinnamic acids are the predominant phenolic acids in plants, later being the more common ones. Hydroxycinnamic acids include caffeic, p-coumaric and ferulic acids, which frequently occur in bound form as simple esters with quinic acid or glucose. A well-known bound hydroxycinnamic acid is chlorogenic acid, which is a combined caffeic and quinic acids. Hydroxybenzoic acid derivatives usually occur in the form of glucosides; p-hydroxybenzoic, vanillic and protocatechuic acids represent the most common forms (Shahidi & Ambigaipalan, 2015).



Figure 3a. Asian vegetables (Page1/3)

Flavonoids are the biggest subgroup, which is further divided on the basis of their structure into various subgroups like flavanols, flavanones, isoflavones, flavones and ACN. ACN are the major flavonoids responsible for blue, red, and purple coloration in

flowers and fruit. They have been referred to as 'chameleon pigments' as they change color depending upon the pH of cytoplasm. They appear as red under acidic conditions and blue under alkaline conditions. ACN occurs in the form of a glycoside of anthocyanidin (aglycone), common types of anthocyanidins present in vegetables are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin. Vegetables such as black carrot, red cabbage, red radish, red onions and purple potato are rich in ACN and possess high AOX (Figures 3a.b.c). Due to the broad spectrum of pharmacological properties of ACN, they are used for nutraceuticals in supplements. Tannins are high molecular (>500 Da) PCs generally referred to as anti-nutritional factors, as they have adverse effects on health. Tannins decrease protein digestibility and reduce the availability of essential amino acids. They are known to reduce the transport of thiamine and folic acid thereby decreasing their bioavailability. Polyphenols can also chelate iron and have inhibitory effects on the absorption of iron resulting in the deficiency of iron in the body.

Synthetic PCs are commonly used as preservatives in foods. These compounds are added to food to prevent or delay the onset of lipid oxidation during the processing and storage of foods. Currently permitted phenolics for food use include butylated hydroxyanisole (BHA, E-320), butylated hydroxytoluene (BHT, E-321), propyl gallate (PG, E-310) and tertiary-butylhydroquinone (TBHQ, E-319). Excessive addition of these synthetic phenolics has been reported to cause carcinogenicity, cytotoxicity, and oxidative stress. They can form molecular complexes with nucleic acid structure and cause damage to the double-helical structure of DNA. BHA is reported as the main cause of apoptosis in the human body. Toxic effects associated with their use, have prompted the food industry to look towards the natural phenolics in foods as preservative agents. As a result, a large number of phenolic rich plant extracts and by-products from the F&V processing industry are being explored for their efficacy as preservatives in lieu of synthetic ones.

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Dr. Charanjit Kaur is a fellow of National Academy of Sciences (NASC), India and Professor in the Division of Food Science and Postharvest Technology. Her research interest is in the area of "Developing high value functional foods and nutraceutical ingredients from horticultural crops". Her significant work envisages use of un-conventional processing through enzymes, and microwave and ultrasound assisted techniques for enhancing recovery of bio-active phyto-chemicals from waste. She has guided more than 20 MSc and PhD students and currently has 8 PhD students under her supervision. She has published nearly 130 research articles in high impact factor journal with total citations of 8013, h-index of 34 and i-10 index of 72. Her students have many acclaimed awards including 'IARI Merit Gold Medal', 'Jawaharlal Nehru Award', 'Prime-Minister Fellowship' and 'DuPont innovation award' to their credit.

Dr. Manoj Kumar is a Scientist (Plant Biochemistry) at ICAR–Central Institute for Research on Cotton Technology, Mumbai, India. He is a recipient of prestigious 'Prime Minister Fellowship award' and 'IARI Gold medal' for his outstanding research on natural colorants from black carrot. His research interest is in the field of extraction and characterization of proteins and other bioactive compounds from plant-based matrices. Currently he is on deputation to East Carolina University, Greenville, North Carolina, USA for undertaking training under CRISPR/Cas Technology funded by SERB, DST, India. He has published more than 150 research and review articles in peer-reviewed international journals.