CHAPTER 1

INTRODUCTION

Epidemiological studies have reported that regular consumption of fresh fruits and vegetables provides 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.[1-4] Fruits and vegetables are considered very rich in phytochemicals. Phytochemicals are defined as bioactive non nutrient secondary plant metabolites with health promoting properties and helps in prevention of major chronic diseases. [5] The phytochemicals include polyphenols or phenolic compounds; dietary fibre, ascorbic acid, carotenoids, micronutrients etc. [6] The polyphenols present in fruits and vegetables act as antioxidants and have free radical destroying properties. They act as inhibitors of lipid peroxidation, prevent DNA oxidative damage and prevent inhibition of cell communications, all of which are precursors to degenerative diseases. [7] A polyphenol compound even at a very low concentration is considered having antioxidant property if it can delay, retard or prevent the oxidation or free radical induced oxidation and moreover, the resulting compounds formed after scavenging of the free radicals must be stable. [8] The phenolic acids free radical destroying property of phenolic acids could be due to their redox properties. They act as reducing agents, hydrogen donors and singlet oxygen scavengers. [9]

The polyphenols consists of an aromatic ring carrying one or more hydroxyl moieties. The classification of polyphenols includes two major group viz. non flavonoid and flavonoid group (Fig. 1.1). They are produced through number of biosynthetic pathways giving rise to secondary metabolism (Fig 1.2.).The non flavonoid group consists of phenolic acids, tannins, acetophenones, phenylacetic acid, xanthones, stibenes, chalcone, lignans and secoiridoids while the flavonoids are divided into subclasses flavonone, flavones, dihydroflavanols, flavonol, flavan-3-ols, anthocyanidins, isoflavones and proanthocyanidins. [10]

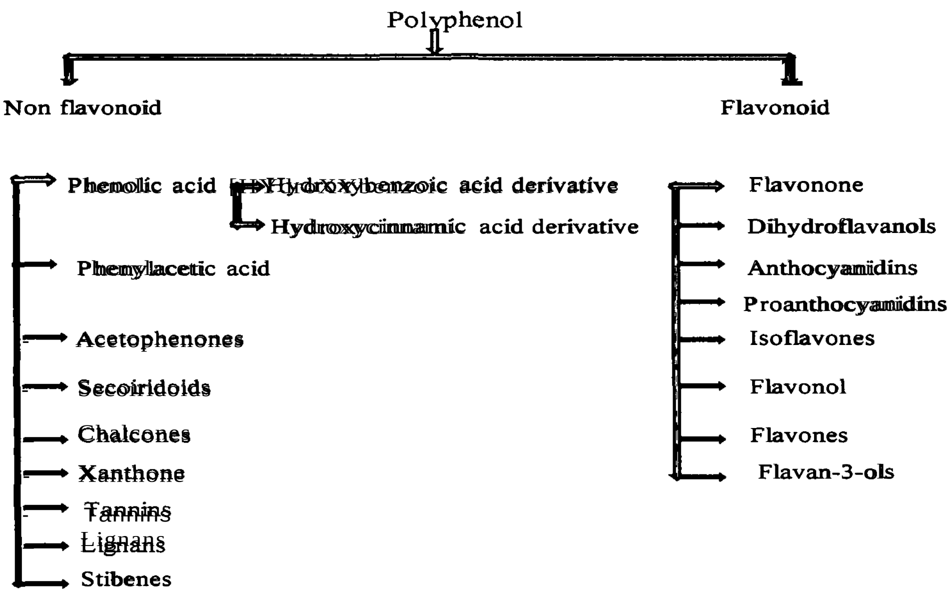

Fig. 1.1. Flowchart of polyphenol classification

Free radicals play an important role, both in health and disease. But, free radicals are useful only when they are produced in the right amount at the right place at the right time. Alterations to any of these parameters leading to free radical imbalance can lead to lipid peroxidation, cell death and genetic damage as a result of the extremely reactive nature of free radicals. [8] A variety of defense mechanisms do exist to quench potentially damaging free radicals, including enzymes like superoxide dismutase and glutathione peroxidase, micronutrients and excision and repair processes that remove free radical- induced damage. These defensive measures function as part of a complex system with significant interdependence and additive or synergistic effects. As antioxidant defenses are produced within the body and/or derived from the diet, efficient functioning of the antioxidant defense system is very much dependent on the optimal functioning of the body's metabolism and nutrition. Antioxidants terminate the free radical initiated chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants often act as reducing agents. Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation.

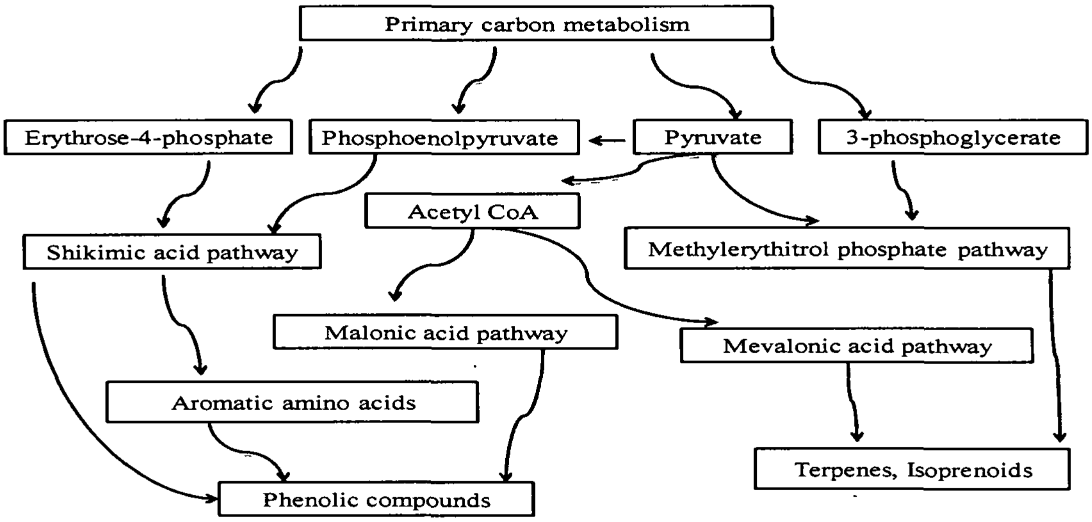

Fig. 1.2. Major biosynthetic pathways for secondary metabolites (adapted from Saltveit, 2009) [11]

The antioxidants serve as physical barriers to prevent reactive oxygen species (ROS) generation or ROS access to important biological sites inside the body. They act as chemical traps that absorb energy and electrons and thus quench ROS (e.g. carotenoids, anthocyanidins). Some antioxidants act as metal chelators to prevent ROS generation (e.g. catechins) while, some are chain-breaking antioxidants which scavenge and destroy ROS (e.g. flavonoids, vitamin C, and vitamin E).

1.1. Polyphenols in fruits and vegetables

Fruits are naturally very rich in polyphenols. In most of the apple varieties, hydoxycinnamic acid derivatives, flavan-3-ols, flavanols and their conjugates, dihydrochalcones and proanthocyanidins are the major polyphenols present. [12] Up to 87% of the total amount of hydroxycinnamic acid in apple fruit is chlorogenic acid. [13] Similarly, apricot and peach are good source of polyphenols. [14] Flavonoids like flavanol-3-ol catechin, malvidin-3-glucoside, are present in grapes and wine. [15] Tannins and resveratrol are also abundant in grapes. They are believed to have anticarcinogenic, anti-inflammatory, antibacterial, antiallergic and antioxidant properties. [16, 17] The citrus fruits are also very rich in flavonoids. It contains limonoids which can prevent cancers and atherosclerosis. [18] Other flavonoids abundant in citrus fruits are naringin, narirutin, and hesperidin. Apart from them, hydroxycinnamic acids like coumaric acid, caffeic acid, ferulic acid are also predominant phenolic acids in citrus fruits. [9] The hydrocinnamic acids help in inhibition of LDL oxidation *in vitro*. [16] Flavonols in their glycosylated forms are mainly found in the outer tissues of fruits and vegetables. [19-20] Quercetin, a flavonoid is abundant in capers, onions, asparagus, lettuce etc. [21] Vegetables are rich source of kaemferol compared to friuits. [21] Potatoes are excellent sources of ascorbic acid, α-tocopherol and polyphenolic compounds. [22] Spinach, brussel sprouts; broccoli, beets, red bell pepper, onion, eggplant and cauliflower are rich in polyphenols and have high antioxidant properties. [23] Ferulic acid and *p*-coumaric acid are found in generous amount in eggplant, broccoli and aspharagus. [24]

1.2. Effect of processing on polyphenols and antioxidant properties

Fruits are commonly consumed raw; however, vegetables usually require to be processed before consuming. With the advent of industrialization and globalization, a rapid rise of processed food products market has occurred. Fruits and vegetables are increasingly being processed to make them available throughout the year. Processing methods can increase or decrease the health promoting properties of the fruits and vegetables.

A significant reduction in phytochemical occurs upon processing of fruits and vegetables. Processing conditions can be divided into domestic, conventional or industrial which are further categorized into thermal and non thermal processing. Thermal processing includes blanching, pasteurisation, canning, microwaving, steaming, etc., while non thermal processing consists of high pressure processing, pulse electric field, UV light, ozonation and sonication. [25] Garci-Viguera et al. [26] reported upto 70% reduction in anthocyanin content in strawberry when processed into jam. But in some cases, heat treatment had a positive effect on the polyphenol content by inactivating the polyphenol oxidase and stabilizing the polyphenols as well as releasing the bound phenolic acids that enhances their bioavailability and antioxidant activities.

Literature has cited that domestic cooking methods can result in significant changes in the composition and bioavailability of antioxidant compounds. [27] These changes could be both beneficial and detrimental depending on the extent and type of treatment conditions. Variety of effects like destruction, release and structural transformation of the phytochemicals take place during the cooking process. Cooking treatments like boiling, microwaving [27], baking, frying and griddling lead to changes in texture and nutritional properties of the vegetables. Studies have reported that cooking softens the cell walls which lead to increase in the extraction of carotenoids. [28] However, other studies have reported that cooking can also lead to loss in essential vitamins and antioxidants, mostly water soluble and heat labile compounds. The extent of loss is dependent on the type of cooking treatment [29] and the phytochemical composition of the cooked vegetables. The adverse effect of thermal processing in fruits, mainly during pasteurisation and spray drying has been reported for mulberry juice, durian juice and cashew apple juice on their bioactive components [30, 31]. Overall, phytochemicals and their antioxidant properties in fruits and vegetables are dependent on a number of factors which includes variation among cultivars, environmental conditions, locations and agronomic factors, maturity stages and types of processing techniques. [32]

1.3. Utilisation of fruit by-products

Residues from the processing of fruits, considered to create an environmental problem, are being increasingly recognized as sources for recovery of valuable products. Efforts towards identifying alternative natural and safe sources of food antioxidants, especially of plant origin, have notably increased in recent years. [33, 34] In this regard, the recovery of phenolic compounds from industrial wastes is gaining considerable attention, especially due to the antioxidant properties that these compounds exert. Studies have shown that the waste residues from fruits and vegetables after processing and the non-edible portions from various fruits (mainly seeds and peels) can be good sources of dietary fibres and antioxidants. [35]

Peels from various fruits have remarkably good polyphenol concentration. [36] Ajila et al. [37] 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. Peel and pomace from lemon and apple contain good amount of polyphenolic compounds. [38] Studies have reported that citrus fruit peels and pomace exhibit good antioxidative, antihypertensive, antiobesity and antihyperglycemic properties. [39, 40] Similarly, in avocado seeds, high phenolic content was reported. [41] Extracts of white and red grape pomace showed the presence of glycosylated flavonols such as quercetin and kaempferol. [42]

Saura-Calixto et al. [43] described the association of polyphenolic compounds and carotenoids with the matrix of the dietary fibre. Perez-Jimenez et al. [44] reported polyphenols with a concentration of 19740 mg/100 g dry weight in grape dietary fibre. The dietary fibre component has been proven to assist in regulation of blood glucose and lowering of serum cholesterol [45] and exert a number of protective effects on cardiovascular diseases, colorectal cancer and obesity. [46] In order to take advantage of the dietary and functional properties of fibre, some high dietary fibre formulated foods are currently being developed. [47, 48]

Dietary fibre consist polysaccharide and lignin that escapes digestion in the small intestine by the digestive enzymes. The recommended level of intake is 30-35g/day. Dietary fibre is divided into soluble and insoluble fractions. The soluble fibres consists of mainly pectin, gum, mucilages and some hemicelluloses and accounts for 30-50% of the total dietary fibre intake to improve dietary benefits. The insoluble fibres include cellulose, lignans and hemicelluloses. [49] The fibre acts as a bulking agent helps in lowering blood cholesterol and intestinal absorption of glucose. The colonic bacteria ferment the dietary fibre and during the process numerous metabolites are produced as by-products and are readily absorbed by the body. Prominent among these by-products are short chain fatty acids which are believed to play a positive role in prevention of colonic cancer and maintained of overall colon health. The other functional properties of fibre such as water-and oil- holding capacity, swelling capacity and viscosity could be exploited to develop newer functional formulations. Therefore, the recovery of polyphenols and dietary fibre from waste residues of fruits has wide scope as an alternate source of phytochemicals for the food and pharmaceutical industries.

1.4. Fruits and vegetables of Assam, India

A variety of fruits and vegetables are cultivated in Assam. The phytochemical content and antioxidant properties of the raw fruits and vegetables and the effects of processing on these properties have not been systematically studied. In addition to the common fruits that are available easily in the market of Assam, a few seasonal fruits like poniol (*Flacourtia jangomas* (Lour.) Raeusch), hogplum (*Spondias pinnata L. Kurz*), carambola (*Averrhoa carambola L.)*, leteku (*Baccurea sapida* Muell Arg.), and different jamun (*Syzygium* sp.) varieties are traditionally believed to have some therapeutic properties and are used in many traditional medicines. Similarly, vegetables like banana blossom of (*Musa balbisiana* Colla.), roselle leaves (*Hibiscus acetosella* Welw.) and teasel gourd (*Momordica dioica* Roxb.) are believed to have health promoting properties. Therefore, study on their phytochemical properties is required to harness their goodness into the diet of the people. A detail study of the fruits and vegetables of this region for their phytochemical and health promoting properties as well as extent of impact of different processing technique are still lacking. Further, the recovery of phenolics from processing waste of fruits of Assam and their composition have not been reported in literature.

Pomace of *Averrhoa carambola* (also known as carambola or 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 proanthocyanindins and has insoluble fibre-rich fraction (FRF) as the major fraction. [50] The phytochemicals have wide scope to be used in development of number of functional products after their extraction and recovery from the pomace. The fruit due to its good phytochemical content is used traditionally to cure many diseases. The carambola fruit is available in Assam and has immense potential for commercialization. Previous studies have reported that its fibre has desirable functional properties, *in vitro* hypoglycemic effects [51, 52], and *in vivo* hypolipidemic and hypocholesterolemic effects. [53] This insoluble FRF could be a promising source of food fibre or low calorie bulk ingredient in functional food applications. However, the efficacy and functions of the fibre from carambola upon incorporation into a fruit beverage and subsequent *in vivo* study have not been reported.

The work reported in this thesis attempted to study the phytochemical and antioxidant content of fresh fruits and vegetables available in Assam, India and the effect of different processing methods on their phytochemical and antioxidant content. The pomace of carambola was used to extract polyphenol compounds and dietary fibre. The fibre rich fraction from the pomace was incorporated in a mixture of fruit juices to develop a functional beverage powder and the quality and functional properties of the developed beverage powder were studied for health promoting properties *in vivo* using an animal model.

1.5. Objectives

Following are the objectives that were followed for the present study

1. To determine the phytochemical content and antioxidant capacities in selected fresh fruits and cooked vegetables of Assam.
2. To study the effect of pasteurisation and spray drying on the phytochemical content of selected fruits.
3. To optimize the phenolic extraction from *Averrhoa carambola* pomace and microencapsulate the phenolics by spray and freeze drying methods.
4. To study the *in vitro* physicochemical, phytochemical and functional properties of fibre rich fractions derived from by-products of selected fruits.
5. To develop a functional beverage powder fortified with carambola pomace fibre.
6. To study the effect of the functional beverage powder on the serum cholesterol and glucose levels and on the functions of liver enzymes.

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