CHAPTER 2

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A diet having high quantities of fruits and vegetables has been widely recommended (mainly during the past twenty years) due to their health-promoting benefits (Rodriguez-Casado, 2016). These contain ample amounts of minerals (usually electrolytes), vitamins and phytochemicals. In addition, these food sources are also a good source of dietary fiber.

2.1. COMMONLY CONSUMED FRUITS AND VEGETABLES IN INDIA

The commonly consumed fruits throughout India include pomegranate, banana, mango, sapodilla, jambolan, kinnow and grapes. Pomegranate fruit is a berry (having seeds in the range from 200 to 1400 in number) having a reddish colored skin and is around 5 to 12 cm in diameter (Morton, 1987). Its seeds are placed in a spongy white membrane, and every single seed is surrounded by a watery pulp. Banana fruit is a parthenocarpic berry that is constituted by a peel and an edible pulp which is nutritionally rich (Singh et al., 2016). Each fruit grows in a cluster that is hanging from top of the plant. In addition, it varies in firmness, color as well as in the size and is curved and elongated in shape. The mango fruit is a juicy drupe that is usually sweet in taste, having pulpy soft to fibrous texture. Sapodilla fruit is a berry that has a diameter ranging from 4 to 8 cm and contains high levels (approximately 85%) of sugar and starch (Fernandes et al., 2011). The pulp of this fruit is very sweet (malty in flavor), grainy in texture, containing 1 to 6 seeds and varies in color from pale yellow to brown. Jambolan fruit is an ovoid and oblong structure having a length of around 1.5 to 3.5 cm (Ayyanar and Subash-Babu, 2012). It is green in color when unripe and its color changes from pink to crimson and finally black upon maturity. Moreover, the taste of this fruit is a mixture of sweet and slightly sour. Kinnow fruit is orange to reddish colored citrus fruit having high
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amounts of juice and can be peeled with ease. Grapes are the fruits growing in clusters (varying from 15 to 300) with colors varying from green to blue tones.

The commonly consumed vegetables in India include carrot, beetroot, bitter gourd, brinjal, spinach and mentha. Carrot is a root vegetable that is usually orange colored, although black, purple, yellow and red varieties are also present. The outer cortex is pulpy, while the inner core is non-pulpy. Carrots of high quality carrots have more percentage of the cortex than core. Carrots are commonly consumed as a salad or as vegetables along dhal dishes in India. Beetroot is a vegetable which is either eaten raw or added with any other salad vegetable. Moreover, in some places it is also boiled and added into pickles. In India, it is usually chopped, then cooked as well as spiced and served as a side dish. In addition, beetroot is nowadays added to soups, ice cream, confectionery items, sherbet and yogurt due to its attractive color value (Bazaria and Kumar, 2016). Bitter gourd is a vegetable having an oblong shape and a warty exterior. The cross-section of this vegetable is hollow, with a thin layer of flesh that surrounds a central cavity filled with seeds. It is usually often eaten green or as it just begins to turn yellow in color. Brinjal is a vegetable having purple skin and white flesh (having a meaty texture). Although it is bitter to taste when raw, yet it becomes soft as well as tender after cooking and develops a rich flavor. Spinach is a green leafy vegetable containing a wide variety of nutrients including essential minerals, vitamins, and phytochemicals (Zhang et al., 2009). Mentha is a herbaceous perennial plant that has leaves in opposite pairs. It is commonly consumed as a vegetable in India and is useful in gastric troubles (Khalsa and Tierra, 2008).

2.2. FRUITS AND VEGETABLES AS VALUABLE SOURCES OF MINERALS

Fruits and vegetables are rich sources of minerals. More than twenty minerals are essential for humans in the diet, which differ in their daily requirements. The overall concentration of minerals in fruits and vegetables is dependent on plenty of factors which include the degree of maturity, genetic constitution, characteristics of the soil, climate and harvest time (Martinez-Ballesta et al., 2010). Minerals play important roles in the human body. Potassium is important for maintaining the physical fluid system balance as
well as helps to assist functions related to the nervous system. Among fruits, bananas are a rich source of potassium. On the other hand, calcium participates in the essential functioning of tissues, while sodium is important for maintaining balance of fluids related to physiological functions in the body. Usually, spinach contains around 600 mg/100 g of calcium (Bender, 2013). Fruit juices and raw vegetables have been reported to have sodium in the amounts ranging from 0.05 to 277 mg/100 g and from 2.3 to 94.1 mg/100 g, respectively (Szefer and Grembecka, 2006). Other important minerals required in lesser amounts are iron, copper, manganese, and zinc. Iron is mainly required for the synthesis of myoglobin and hemoglobin, while copper and manganese (as a co-factor) are required for enzyme-related functions (Huskisson et al., 2007). In addition, zinc is also important for the activity as well as structure of various enzymes. Martinez-Ballesta et al. (2010) reported iron and copper amounts ranging from 0.1 to 3 mg/100 g and 0.01 to 0.2 mg/100 g, respectively in various fruits and vegetables. Szefer and Grembecka (2006) reported manganese and zinc contents ranging from 0.01 to 0.67 mg/100 g and 0.02 to 0.6 mg/100 g, respectively in different fruits and vegetables.

2.3. HEALTH BENEFITS OF CONSUMING FRUITS AND VEGETABLES IN A DIET

Fruits and vegetables confer several human health benefits. The health promoting effects of fruits and vegetables have been widely screened, as it has been reported that their consumption leads to reduction in the incidence of different types of cancers (Larsson et al., 2006). Gandini et al. (2000) reported a reduction in breast cancer with enhancing intake of fruits as well as vegetables after studying a meta-analysis of twenty-six studies. Additionally, some studies also suggest reduction in prostate cancer risk with fruit and vegetable intake (Giovannucci et al., 2003; Stram et al., 2006). Apart from cancer, Bazzano et al. (2001) observed a lower incidence of heart diseases in people who regularly consumed fruits and green leafy vegetables. Bone status also has been suggested to improve with increased fruit and vegetable intake as plenty of fruits are potassium rich that generate metabolites which reduce dissolution of bones (Prynne et al., 2006). Radhika et al. (2008) studied the relationship between intake of fruits as well as
vegetable with the cardiovascular disease risk factors in some south Indians (living in urban areas). They reported that consuming high amounts of fruits and vegetables had approximately half the percentage of the total protective effect against this disease. In spite of these health promoting effects, Kimmons et al. (2008) concluded that intake of fruit and vegetables was pretty low in many countries and a lot of effort was needed for its increase.

2.4. BIOACTIVE COMPOUNDS (POLYPHENOLIC COMPOUNDS AND DIETARY FIBER) PRESENT IN FRUITS AND VEGETABLES

Many bioactive components are present in different fruits and vegetables. Morales-Soto et al. (2014) reported that even micronutrient supplements may not provide the similar health benefits as those provided by the bioactive compounds present in fruits and vegetables (as these work synergistically with one another). Some of the bioactive compounds (polyphenolic compounds and dietary fiber) present in fruits and vegetables are discussed in detail in the following sub sections:

2.4.1. Polyphenolic compounds

Polyphenolic compounds are a large group of secondary metabolites present in plants having many hydroxyl groups attached on at least one or more aromatic groups (Stevenson and Hurst, 2007; Haminiuk et al., 2012). These compounds affect the sensory properties of foods (mainly flavor and color), contributing to aroma as well as taste (Landete et al., 2012). Moreover, these also contribute to the astringency of foods. The main classes of polyphenolic compounds are phenolic acids, flavonoids, stilbenes and lignans. The compounds having one aromatic ring (such as phenolic acids) are not polyphenolic compounds in true sense, but many characteristics and properties are shared with them (Weichselbaum and Buttriss, 2010). Therefore, these are included in the group of polyphenolic compounds. The structures of polyphenolic compounds range from one C6 aromatic ring (hydroxybenzoic acids); with C6-C3 structure (hydroxycinnamic acids), C6-C3-C6 structure (flavonoids), C6-C2-C6 structure (stilbenes) and C6-C4-C6 structure (lignans). A large number of these compounds (around 8000) have been identified
including flavonoids (around 4000), and still their number is increasing (Ignat et al., 2011). About 1 g/day of polyphenolic compounds (with one-third for phenolic acids) have been recommended (Scalbert and Williamson, 2000). These compounds are synthesized during normal plant development and also in response to stress conditions (Naczk and Shahidi, 2004). Some of these compounds are soluble in water (such as phenolic acids and flavonoids), while some are insoluble (such as hydroxycinnamnic acids and tannins). Fruits and vegetables are the two most abundant sources of polyphenolic compounds in the human diet (Martinez-Avila et al., 2014).

Phenolic acids constitute the compounds that are derived from hydroxybenzoic acid (gallic acid, ellagic acid and vanillic acid) and hydroxycinnamic acid (ferulic acid, caffeic acid and coumaric acid). The content of hydroxybenzoic acid in plants is usually low, except in some red fruit, onions and black carrots that have concentrations of more than 10 mg/kg on fresh weight basis [FW] (Shahidi and Naczk, 1995). Caffeic acid is a well known phenolic acid, representing more than three-fourths of total content of hydroxycinnamic acids in many fruits (Manach et al., 2004). Moreover, phenolic acids not only affect the color of the foods but also their taste (McSweeney and Seetharaman, 2015). Flavonoids are polyphenolic compounds that have a low molecular weight, containing around 15 carbon atoms that are organized in a C6–C3–C6 configuration. Structurally, these contain two aromatic rings (A and B) that are joined by a three-carbon bridge in the form of a heterocyclic ring (C). Ring A is derived from the malonate pathway, while ring B is derived (through shikimate pathway) from the amino acid phenylalanine (Merken and Beecher, 2000). Flavonoids are usually present as glycosides in foods rather than as free compounds (Robbins, 2003). Flavonoids can be subdivided into various classes (flavonols, flavones, flavanols, flavanones, isoflavones and anthocyanidins) that result from the varying substitution patterns in the ring C. The most abundant flavonoids in foods are the flavonols. These compounds mainly include quercetin, myricetin and kaempferol. The examples of flavanols are catechin and epicatechin, flavones are apigenin and luteolin, flavanones are hesperitin and naringenin, isoflavones are daidzein and genistein, while those of anthocyanidins are cyanidin and
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delphinidin. Manach *et al.* (2004) reported that flavones were less abundant than flavonols in fruits and vegetables. Stilbenes are another class of polyphenolic compounds that have the presence of a 1,2 diphenylethylene nucleus with substituted hydroxyl groups attached to the aromatic rings. Resveratrol is a well known stilbene characterized by a trihydroxystilbene skeleton. Other polyphenolic compounds include tannins (water soluble bitter and astringent compounds having molecular weights ranging from 500 to 3,000) and lignans (mainly composed of two phenylpropanoid moieties attached using their side chain).

2.4.1.1. Extraction of polyphenolic compounds

The extraction of polyphenolic compounds is an essential step for their isolation as well as identification and finally their use. There are plenty of available methods for their extraction and no single method can be classified as a standard method. Usually the extraction of polyphenolic compounds is affected by several parameters and most important step is to select the optimum extraction conditions. The preparation of samples is a crucial step in the quantification of these compounds, as it not only influences the accuracy but also the repeatability during the analysis part (Zhao *et al.*, 2011). The testing of many parameters including the solute to solvent ratio, particle size of the samples, mass transfer efficiency, agitation and temperature is recommended for achieving the maximum extraction (Yang *et al.*, 2011; Haminiuk *et al.*, 2012). The use of finely powdered samples considerably improves the extraction of polyphenolic compounds, due to the increase in the surface area as well as disruption of cell walls in the plant material. Various organic solvents (methanol, ethanol, ethyl acetate and acetone) as well as their combination with water are used for the extraction of polyphenolic compounds depending upon their selectivity, recovery and price.
Figure 1: Various classes of polyphenolic compounds along with their examples
2.4.1.2. Identification and quantification of polyphenolic compounds

Freeze drying is the main preservation method that is used for identification as well as quantification of polyphenolic compounds in fruits and vegetables. It is very widely used technique because it preserves the phenolic contents of foods and also might inactivate the enzymes during the application of cold temperatures (McSweeney and Seetharaman, 2015). Moreover, fruits and vegetables have a limited shelf life owing to their high water contents. Therefore, dehydration using freeze drying is desirable as it retards the growth of microorganisms that deteriorate fresh food substances. Other methods such as hot drying might be used in some instances, but it has a negative effect on the nutrients present in the foods (Haminiuk et al., 2012). Asami et al. (2003) reported a decrease in the total phenolic content in berries with hot drying technique which might be due to the thermo-labile nature of anthocyanins. Garau et al. (2007) reported that longer drying times as well as high temperatures of drying decreased the total phenolic content (TPC) of orange peel and pulp. Many studies have reported that freeze drying is a better dehydration technique than other drying methods. Some of these studies include as those reported for blueberries (Mejia-Meza et al., 2010), grape skins (De Torres et al., 2010) and strawberries (Mogol et al., 2010). However, freeze drying is mostly suitable for food products of high value (owing to its higher relative cost than other drying methods) such fruits and vegetables, few herbs and coffee (McSweeney and Seetharaman, 2015).

Folin-Ciocalteu reagent colorimetric method and aluminium chloride colorimetric assay have been widely reported in the literature for the estimation of TPC and total flavonoid content (TFC), respectively (Granato et al., 2010). These assays use spectrophotometric analysis (in the visible region of the spectrum) for quantifying the polyphenolic compounds. The benefits of using this technique are low cost and simplicity. However, these assays cannot be used for quantifying individual polyphenolic compounds. Liquid chromatography is an important technique that physically separates (rapidly as well as easily) a mixture of compounds in present in a liquid phase. RP-HPLC is the main technique that is used for separating polyphenolic compounds, where the mobile phase is more polar than the stationary phase. Hydrophobic alkyl chains make up the stationary phase, wherein silica bonded C18 column is widely used for the separation
of polyphenolic compounds. Other lesser used column lengths include the C4 and C8. The retention times of polyphenolic compounds are lower for polar molecules (such as gallic acid and protocatechuic acid) that are eluted easily, while these are higher for molecules that are less polar (such as quercetin and kaempferol). Polyphenolic compounds are identified and afterwards quantified using a gradient elution in the HPLC method instead of isocratic mode, because of their similarity as well as their chemical complexity (Merken and Beecher, 2000). The mobile phase usually consists of high quality ultrapure acidified (using acetic or formic acid) water as a polar solvent, while methanol and acetonitrile form the less polar solvents. Acidification is done to lower the ionization of polyphenolic compounds and their carboxylic groups, so as to improve the resolution and retention time (Haminiuk et al., 2012). The maximum absorbance of polyphenolic compounds lies in either the visible or the ultraviolet regions of the spectra. Therefore, the optimum absorbance for each individual compounds is important for accurate analysis. Diode array detectors (DAD) are mostly used in analysis of polyphenolic compounds as large number of results can be obtained in a single run which increases the throughput of the HPLC system.

2.4.1.3. Antioxidant and antimicrobial activities of polyphenolic compounds

Oxidation reaction (involves the transfer of electrons between electron rich species/molecules) can give rise to free radicals such as reactive oxygen species. These free radicals are highly reactive, often do not last long in a given form and contain unpaired valence electron. The resulting oxidative stress might damage some cellular components such as DNA, RNA, proteins and lipids and may cause degenerative illnesses such as multiple sclerosis, Parkinson’s disease and cancer in humans (Thériault et al., 2006).

Antioxidants (species or molecules that prevent or slow the oxidation of another molecule) donate an electron to the free radical, thereby converting it into an innocuous molecule. These compounds can be categorized as primary antioxidants or secondary antioxidants. Primary antioxidants actively inhibit oxidation reactions, while secondary ones inhibit oxidation indirectly, using other mechanisms such as binding pro-oxidants or oxygen scavenging (Craft et al., 2012). Most of the primary antioxidant reactions can be
grouped into the categories of hydrogen-atom transfer (where an antioxidant quenches free radical species by donation of hydrogen atoms) and single-electron transfer (where an antioxidant transfers a single electron to support in the reduction of a target compound). At present, antioxidants are mainly included in various food products as preservatives for extending their shelf-life as well as to maintain their quality. Fruits and vegetables have been considered rich sources of a wide array of phytochemicals that have strong antioxidant activities (Yahia, 2010).

Polyphenolic compounds are one of the most well studied groups of antioxidants. These compounds inhibit the pro-oxidative action of metals by chelation process, where these bind with the metal ions, forming complexes that are not capable of promoting oxidation. Therefore, polyphenolic compounds function as secondary antioxidants (Rice-Evans et al., 1997). At low concentrations, these compounds protect food from oxidative rancidity when added to food products (Karakaya, 2004). Over the past two decades, owing to the growing popularity of polyphenolic compounds, many new scientific methods have been developed for measuring their content or their antioxidant capacity of polyphenolics present in foods (Deng et al., 2011; Craft et al., 2012). The methods involving the quantification of total antioxidant capacity find a commonplace in today’s medical and scientific laboratories because of the presence of many different types of antioxidants in biological systems. Antioxidant potential of polyphenolic compounds not only depends on the number but also on the arrangement of hydroxyl groups in the individual molecules (Sang et al., 2002). Hoelz et al. (2010) reported that increases in either ionization potential or hydroxyl bond homolytic dissociation enthalpy negatively affect the antioxidant activity of a polyphenolic compound. The in vitro antioxidant activity measurements can produce valuable data (when chosen and properly performed) which involves the potential capability of antioxidants in vivo (only in the case of screening). In addition, TPC can have a strong relation with the observed antioxidant activity within a system. However, this is not always true in some occasions. It is observed that in sources where a strong correlation is observed, it typically concluded that polyphenolic compounds were mainly responsible for the antioxidant activities. On the contrary, in sources where strong correlations were not observed, it was concluded that there were mainly antioxidants other than polyphenolic compounds present in the
system or a proper quantification was not done. In vivo, the mode of action of various polyphenolic compounds differs, even when these are not present in high levels for eliciting their antioxidant effects. The mechanisms might include changes in the metabolism of hormones as well their regulation or signaling pathways (Spencer et al., 2009).

Apart from the antioxidant activity, the antimicrobial activity of polyphenolic compounds present in fruits and vegetables as well as medicinal plants has been widely investigated against wide ranges of microorganisms. Among these, flavonoids and tannins have received the most attention owing to their higher antimicrobial activity compared with other polyphenolic compounds and wide spectrum (Daglia 2012). These are able to suppress some virulence factors of microorganisms such as biofilm inhibition and also might show synergism with some antibiotics. These beneficial effects of polyphenolic compounds has led to the development of new food preservatives (in order to avoid synthetic preservatives used in food) and to treat some infections caused by microorganisms, considering the increase in microbial resistance against antibiotics currently available in the market (Jayaraman et al., 2010).

Cushnie et al. (2007) reported that polyphenolic compounds caused the aggregation of the bacterial cells which prevented infection. Moreover, Lin et al. (2008) reported that quercetin as well as kaempferol inhibited the catalytic activity of various bacterial topoisomerases which might explain the synergism between ciprofloxacin and these polyphenolic compounds. Furthermore, Saavedra et al. (2010) reported that some phenolic acids (such as caffeic acid, ferulic acid and gallic acid) showed antibacterial activity against some Gram-positive and Gram-negative bacteria and these compounds were found to be even more efficient against these bacteria than conventional antibiotics (such as streptomycin and gentamicin).

2.4.1.4. Polyphenolic compounds present in selected fruits and vegetables

Many polyphenolic compounds have been characterized by several authors in fruits and vegetables. In pomegranate fruit, anthocyanins, lignans, flavonoids (that are non-colored), gallotannins and ellagitannins have been mainly identified by some authors (Fischer et al., 2011; Mena et al., 2012). Campillo et al. (2015) characterized gallic acid,
ellagic acid, punicalagins, corilagin and punicalin in fresh pomegranate fruits using HPLC technique. In case of bananas, Russell et al. (2009) identified sinapic acid, ferulic acid, gallic acid, vanillic acid, p-hydroxybenzoic acid, p-coumaric acid and gentisic acid as the major phenolic acids. In addition, catechin, gallocatechin, epicatechin and condensed tannins have been characterized in banana fruit pulps (Bennett et al., 2010). Kim et al. (2007) identified gallic acid (main polyphenolic compound), ferulic acid, coumaric acid and hydrolysable tannins in mango fruits. Ribeiro et al. (2008) characterized mangiferin, isomangiferin, kaempferol and quercetin as well as its derivatives in some mango varieties grown in Brazil. In sapodilla fruits, gallic acid, chlorogenic acid, leucocyanidin and catechin have been previously identified (Mathew and Lakshminarayana, 1969). Moreover, Lakshminarayana et al. (1969) reported that simple polyphenolic compounds (such as phenolic acids) decreased, while leucoanthocyanidins increased with maturation in sapodilla fruits. Jambolan fruit pulp has been shown to mainly contain petunidin, malvidin and delphinidin (Veigas et al., 2007; Sharma et al., 2008). de Carvalho Tavares et al. (2016) also identified petunidin, malvidin and delphinidin in jambolan fruits. Additionally, they identified gallic acid, ellagic acid, laricitrin, myricetin, dihydromyricetin, gallocatechin, ellagitanin and proanthocyanidins in various concentrations in jambolan pulp. Hayat et al. (2010) detected catechin, rutin, naringenin, isorhamnetin, kaempferol and hesperidin as the major polyphenolic compounds in the peels of kinnow fruit. In grapes, Yang et al. (2009) reported the presence of hydroxybenzoic acids (such as gallic acid), hydroxycinnamic acids and stilbenes (such as resveratrol). Antonioli et al. (2015) identified catechin, epicatechin and malvidin-3-glucoside as the main polyphenolic compounds present in grapes.

Alasalvar et al. (2001) reported chlorogenic acid as the main phenolic acid in orange, black and yellow carrots. They also reported that highest content of this phenolic acid was present in black carrots. Kammerer et al. (2004) reported the presence of ferulic acid, caffeic acid, coumaric acid and quercetin in carrots. Vulić et al. (2012) identified as well as quantified ferulic acid, vanillic acid, hydroxybenzoic acid, caffeic acid, protocatechuic acid, betanin, isobetanin and vulgaxanthin in the pomaces of some beetroot varieties. Horax et al. (2005) identified catechin, epicatechin, chlorogenic acid,
gallic acid and gentisic acid as the major polyphenolic compounds present in bitter gourd. Kubola and Siriamornpun (2008) reported the presence of gallic acid (as the major polyphenolic compound), tannic acid, p-coumaric acid, benzoic acid, ferulic acid, caffeic acid and catechin in bitter gourd. Singh et al. (2009) identified the presence of 5-caffeoylquinic acid, quercetin and myricetin derivatives in brinjal. Chu et al. (2000) reported quercetin and myricetin as the main polyphenolic compounds present in spinach. Moreover, apart from quercetin and myricetin, some other polyphenolic compounds namely kaempferol, apigenin and luteolin have also been reported in spinach leaves (Dehkharghanian et al., 2010). Taamalli et al. (2015) reported gallocatechin, quercetin and dihydrokaempferide as the main polyphenolic compounds in mentha.

2.4.2. Dietary fiber
The dietary pattern of human beings has shifted towards a diet predominantly containing refined grains, added sugars as well as fats but lower amounts of fruits and vegetables. This change along with the shift to a sedentary lifestyle is the possible reason responsible for the increased prevalence of heart diseases, obesity, cancer and chronic diseases such as diabetes. Over the past few decades, dietary fiber has received quite a lot of positive attention owing to its ability to reduce blood cholesterol levels, ease constipation and lower the incidence of diabetes and coronary heart disease (Telrandhe et al., 2012). At present, there has been a great increase in the knowledge about dietary fiber, mostly in analytical and nutritional areas. More than a half of the functional foods available in the market nowadays include dietary fiber as an active component in their recipes (Macagnan et al., 2016). It is recommended that a person should consume 25-30 g dietary fiber daily (Saura-Calixto et al., 2009). Moreover, most diet experts and nutritionists suggest 20–30% of daily fiber intake should be in the form of soluble fiber.

In general, dietary fiber includes a mixture of carbohydrate polymers present in plants (oligosaccharides as well as polysaccharides) such as pectin, cellulose, gums, resistant starch, inulin and hemicelluloses, which might be associated with lignin as well as other non-carbohydrate constituents (such as waxes, polyphenolic compounds and saponins). As a matter of fact, dietary fiber is made up of such a complex and heterogeneous groups having different physiological, chemical and physical properties,
which makes it difficult to define as well as develop methods capable of reflecting its true biological effects in the humans (Westenbrink et al., 2013). Some researchers define it as non-digestible carbohydrate, lignin (intrinsic in plants) and isolated non-digestible carbohydrates; others propose that dietary fibers are non-digestible carbohydrates and lignins along with other synthetic non-digestible carbohydrates. Trowell et al. (1985) defined it as the remnants of plant cells which are resistant to digestion (hydrolysis) by the enzymes present in the alimentary canal of humans. Dietary fiber components are neither absorbed nor degraded when these pass through the upper part of the gastrointestinal tract but exert some nutritionally important effects in the small intestine such as slowing down of gastric emptying and affecting assimilation of nutrients. When these components pass into the large intestine, these are degraded by gut bacterial enzymes and then partially or completely fermented, producing short chain fatty acids as well as water and gases (Brownlee et al., 2006). The latest and widely accepted definition of dietary fiber has been given by the CODEX Alimentarius Comission in 2008. This commission defines it as the carbohydrate polymers having ten or more monomeric units, that are not hydrolyzed by the endogenous enzymes in small intestine of humans, belonging to three categories: first category includes carbohydrate polymers which are edible and naturally occur in food as consumed; second includes carbohydrate polymers, which are obtained from food raw materials by enzymatic, chemical or physical means and which show physiological effect of health benefits by generally accepted scientific evidences to the competent authorities; and the last category includes synthetic carbohydrate polymers, which show physiological effect of health benefit by generally accepted scientific evidences to the competent authorities (Cummings et al., 2009). This definition includes resistant starches, resistant oligosaccharides and resistant maltodextrins.

### 2.4.2.1. Sources of dietary fiber

The main sources of dietary fibers include cereals, legumes, fruits and vegetables. In particular, cereal polysaccharides are the main sources of dietary fiber used by the food industry. On the other hand, in the recent years non-conventional sources of dietary fiber that have gained importance are obtained from fruits and vegetables. Fruit and
vegetable by-products (peels, stems, cores, pips and skins) from the processing industries are growing in popularity as fiber sources, as these are inexpensive, undervalued and abundant. These by-products were considered an economic and pollution problem in the past but nowadays are considered as potential sources of functional components (Tejada-Ortigoza et al., 2015). Cereals are the main sources of cellulose, hemicelluloses and lignin, while fruits and vegetables are abundant in pectin (composed of galacturonic acid as well as some other monosaccharides such as galactose, rhamnose and arabinose), mucilage and gums. The effectiveness and beneficial effects of dietary fiber not only depend on the fiber intake but also on its physicochemical characteristics, composition, structural organizational and bioactive compounds associated with it (Elleuch et al., 2011). Dietary fibers are usually extracted using hot water (at 60 to 100 °C, pH 1.3 to 7.5, 0.3 to 12 h), which is the most commonly used methods for obtaining polysaccharides from fruits and vegetables (Tejada-Ortigoza et al., 2015). Other lesser used methods of extraction include mechanical, chemical and enzymatic ones.

2.4.2.2. Classification of dietary fiber and determination methods used

Total dietary fiber (TDF) is classified into soluble or insoluble, on the basis of whether it forms a solution when mixed in water (soluble) or not (insoluble). Dietary fiber solubility is related to the structure of polysaccharides as these can be set irregularly (soluble) or regularly (insoluble) as side chains or on the backbone. In addition, the presence of substitution groups such as carboxylic acids or sulfates increases solubility. Insoluble dietary fiber (IDF) includes cellulose as well as other polysaccharides along with non-carbohydrate constituents (cutin, lignin and other constituents of the cell wall). Soluble dietary fiber (SDF) includes beta-glucans, pectins, galactomannans, arabinoolxylans and other indigestible oligosaccharides and polysaccharides. SDF content is high in fruits and vegetables and is associated with colonic degradation, lower serum cholesterol levels, slow glucose absorption, enhanced immune functions and high fermentability (Abdul-Hamid and Luan, 2000). On the other hand, IDF is characterized by its low density, porosity and its ability to decrease intestinal transit and increase the fecal content (Roehrig, 1988). The proportion between SDF and IDF is an important nutritional parameter due to the different physiological effects exerted by them (Saura-
Calixto et al., 2009). It is usually observed that the proportion of SDF is considerably higher (although this might vary) in fruits and vegetables than cereals.

The methods used for the determination of TDF content can be divided into three categories, which are the non enzymatic-gravimetric methods, the enzymatic-gravimetric methods and the enzymatic-chemical methods. The enzymatic-chemical methods also include the enzymatic-chromatographic and the enzymatic-colorimetric methods. At present, the most commonly used methods for measuring the TDF content are the Association of Official Analytical Chemists (AOAC) enzymatic-gravimetric method (Prosky et al., 1992) and the enzymatic-chemical method (Englyst et al., 1994). Non enzymatic-gravimetric methods were the first devised ones and included crude fiber, neutral detergent fiber and acid detergent fiber. These methods did not measure the water soluble components present in the mixtures. Crude fiber was composed of residue that remained after the chemical decomposition by oxidative or hydrolytic treatment. The protocols for enzymatic-gravimetric methods contain enzymatic treatment for removal of starch as well as protein, the precipitation of soluble dietary fiber using aqueous ethanol and afterwards included dietary fiber residue filtration, weighing and lastly correction for ash and protein in the residue (Prosky et al., 1992). The most commonly used method for determining the TDF is the AOAC official method 985.29 enzymatic-Gravimetric method which includes removal of fat from the sample (if > 10%) and then gelatinization using heat stable \( \alpha \) amylase and enzymatic digestion using amyloglucosidase and protease for removing starch and protein. Afterwards, the steps involve precipitation, filtration and weighing of the residue as previously described. The contents of SDF and IDF can be determined using this method. On the other hand, the first step of the enzymatic-chemical methods is exactly similar to the enzymatic-gravimetric one involving removal of starch using enzymes and also protein at some occasions. Dialysis or precipitation using aqueous ethanol then separates the high molecular weight polysaccharides (mainly SDF) from products of starch hydrolysis and low molecular weight sugars. Neutral sugars of the hydrolyzed polysaccharides are afterwards quantified using HPLC or gas–liquid chromatography, providing the values of individual monosaccharides and the uronic acids are determined using a spectrophotometer (Englyst et al., 1994).
2.4.2.3. **Functional properties of dietary fibers**

Dietary fibers show few functional properties owing to their ability of modifying the structural properties of matrix wherein these are embedded. These properties have been more helpful in deciphering their physiological effects, notwithstanding, the chemical composition alone. Functional properties of these fibers depend on the source, the preparation method, ratio of IDF to SDF and the particle size (Figuerola *et al*., 2005). The main properties of dietary fibers which are nutritionally relevant are primarily the surface area characteristics, particle size as well as bulk volume, entrapment or adsorption of minerals or organic molecules and the rheological and hydration and properties. Viscosity as well as ion exchange capacity (due to pectin) contribute to the metabolic effects (lipid and glucose metabolisms), while bulking effect, particle size and fermentation pattern to the effects on colon functionality (Guillon and Champ, 2000). In comparison with IDF (in food processing), the SDF have many advantages such as having neither bad taste nor bad texture, having greater ability to form gels and act as emulsifiers, greater capacity to provide viscosity and are easier to incorporate into processed drink and foods.

Hydration properties of dietary fibers can be determined by measuring the water absorption, the water holding capacity and the swelling capacity. Water absorption parameter provides detailed information (mainly the substrate pore volume) about the dietary fiber (Guillon and Champ, 2000). Water holding capacity is the amount of water that is retained by 1 g of dried dietary fiber under some defined conditions (temperature, centrifugation speed and soaking time) and consists of the sum of physically trapped water, hydrodynamic water and bound water (Martínez *et al*., 2012). The limitation of this measurement is that some portion of soluble fibers is lost during analysis (Elleuch *et al*., 2011). Swelling capacity is directly associated with cellulose components of the dietary fibers and is determined by immersing these in water for overnight and afterwards measuring the volume change. In addition to the hydration properties, dietary fibers also possess the ability to absorb oil, known as the oil holding capacity. It is the amount of oil which is retained by the dietary fibers after they are mixed, incubated in oil and later centrifuged. This property is related with the surface properties of components, the hydrophilic nature, overall charge density and structure of polysaccharides (Martínez *et
al., 2012). Besides these properties, while studying the viscosity (resistance to flow) of dietary fibers, it observed that most of these exhibit a non-Newtonian flow i.e. increase in shear rate can decrease or increase the viscosity of the solution (Abdul-Hamid and Luan, 2000). SDF increases the viscosity of a solution. Viscosity also increases with an increase in the dietary fiber content but increase in the temperature decreases it. Usually fiber solutions behave as pseudoplastic fluids (where viscosity decreases suddenly by an increase in shear rate).

2.4.2.4. Health benefits of consuming diets containing high amounts of dietary fiber

It is nowadays well accepted that diets containing high amounts of dietary fibers (such as those rich in fruits, vegetables and cereals) have several positive effects on human health. The consumption of these fibers has been related to a decreased incidence of several types of cancer and these also have a significant role in the prevention of several other diseases (Jiménez-Escrig et al., 2001). Slavin (2001) reported the capacity of dietary fibers to adsorb carcinogenic agents (both in vivo and in vitro) and recommended to consume foods having suberized and lignified cell walls or ones having most effective compounds that could link to the hydrophobic carcinogenic agents. Regarding type 1 and 2 diabetics, clinically, several studies have shown that diets having high amounts of fiber (having low glycaemic index), improved the levels of glycated proteins (both fructosamine as well as haemoglobin A1c), which act as markers of glycaemic control (Kendall et al., 2010). There are also other many health benefits of consuming high fiber diets. Rodriguez et al. (2006) reported that fiber polysaccharides regulate the absorption of lipids by acting as strong inhibitors of pancreatic lipase which plays an important role in lipid metabolism. Additionally, Gallaher et al. (1992) documented that dietary fiber contributed to decrease the levels of low density lipoproteins and total cholesterol in the blood plasma, which was due to a greater excretion and dilution of bile acids. Moreover, fiber rich foods also have the capacity to bind metabolites of cholesterol, playing an important role in the small intestine by not only digesting but also absorbing lipids.
2.5. UTILIZATION OF JAMBOLAN, BLACK CARROT AND BEETROOT AS FUNCTIONAL FOOD INGREDIENTS

The design and development of functional food products should not only be carried out purely on the basis of nutritional function, the product properties such as texture, color and taste should also be taken into consideration. Moreover, before designing any functional food the target population (age wise or gender wise), health targets (biological functions that would be improved), target bioactive compounds, target dose, target delivery site and target claims should also be considered (Sun-Waterhouse, 2011). Day et al. (2009) suggested that if the source of bioactive components was a natural traditionally consumed food and involved no purification steps other than minimal concentration/drying, it was less likely to have regulatory restrictions on its use as a food ingredient. Sun-Waterhouse (2011) reported that low consumer acceptability of a functional product was possible, such as that found for food products that had high polyphenolic content and/or that had high IDF content. In addition, the author further suggested that it was feasible to add whole fruits and vegetables into food products that had balanced compositions of nutrients and most of times did not compromise with the taste.

2.5.1. Jambolan

Jambolan is a good source of anthocyanins which have been nowadays used as natural colorants in various food products. Jampani et al. (2014) successfully purified jambolan anthocyanins free from sugars using adsorption. Jambolan fruit concentrate has been widely used for various medicinal purposes, currently having a large market for the treatment of various enteric disorders including chronic diarrhea (Ayyanar and Subash-Babu, 2012). Sari et al. (2012) suggested that incorporation of jambolan anthocyanins, both in natural or co-pigmented forms (using co-pigments of caffeic, ferulic and sinapic acid, and rosemary extract) in the beverage models not only provided the benefits of colorants but also of antioxidants. Li et al. (2009) observed that jambolan fruit extract showed pro-apoptotic and antiproliferative effects against breast cancer cells.

Although jambolan fruits contain plethora of bioactive constituents, yet these fruits are highly perishable. Rai et al. (2011) investigated macro-perforated packaging
treatments for the extension of shelf-life as well as assessed the physiological, biochemical and microbiological changes in jambolan during storage. These authors reported that the qualitative parameters retained satisfactorily under macro-perforated packaging treatments, surmising that the fruits could be stored for long term using 1 macro-perforation packages. Mussi et al. (2015) assessed the effect of spouted bed drying on the drying kinetics and functional values of jambolan residues (peels and seeds). They observed a lowering in moisture and water activity from 63% to 4-9% and 0.6 to 0.3, respectively. These authors further reported that dried samples maintained the antioxidant activity independently of the process variables, despite of large reduction (up to 70%) in anthocyanin content. Another way of preservation may be incorporation of fresh or dried jambolan fruit pulps into food products. Bezerra et al. (2015) evaluated the properties of caprine frozen yoghurt having incorporated jambolan pulp or powder as a carrier to probiotic Bifidobacterium animalis subsp. lactis BI-07. Their results showed that this yogurt was able to maintain high probiotic survival rates along three months of frozen storage, indicating the successful production of a high quality dairy product having functional bioactive ingredients.

Ripe jambolan fruits can also be used for making jellies, squashes and wine (Banerjee et al., 2005). Lago-Vanzela et al. (2011) produced jambolan jellies using various formulations, reporting jellies formulated with saccharin and cyclamate had better sensory characteristics as compared to other formulations. Nuengchamnong and Ingkaninan (2009) identified and characterized major polyphenolic compounds present in jambolan wine as hydrolysable tannins and their derivatives (gallic acid, caffeoylquinic acid and ellagic acid).

2.5.2. Black carrots

Black carrots are other anthocyanin rich sources but have less consumer acceptance as vegetables. In many parts of the world (mainly the developed nations) black carrot juice/concentrate has been used as a natural food colorant (as a healthier alternative to synthetic colorants such as Red 40 and FD&C) owing to its light, pH and high heat stability (Suzme et al., 2014). However, these have not been tapped to their full potential in India (used only in making a fermented beverage named Kanji), in spite of
having higher amounts of antioxidants in comparison with commonly available orange carrot varieties. Suzme et al. (2014) indicated that processing raw black carrots into its concentrate led to a significant reduction in phenolic, flavonoid and monomeric anthocyanin contents.

Black carrots are good choice for coloring confectionery, jams, jellies, fruit juices and soft drinks (Downham and Collins, 2000). The production of black carrot jams and marmalades might be a good alternative to the ones traditionally available and can improve market competitiveness. Kamiloglu et al. (2015) investigated the influence of jam and marmalade processing, the effect of storage conditions on color, anthocyanin content and antioxidant activity of black carrots. They reported that anthocyanin content as well as antioxidant activity of black carrots decreased after jam and marmalade processing. They further reported that after 20 weeks of storage anthocyanin and antioxidant activity was better preserved at 4 °C than at 25 °C. Day et al. (2009) reported that black carrot concentrate could be successfully added in pasta as the final product color by concentrate addition (0.5-2% w/w) did not have any adverse effect on the consumer acceptance. These authors further reported that although cooked pasta with added black carrot concentrate had a softer texture and longer cooking time than cooked pasta without any addition, yet the difference was comparatively small within acceptable limits.

Other available black carrot product is shalgam juice, which is a fermented beverage (having black carrot, turnip, chili powder and extract obtained from the lactic acid fermentation as the main ingredients) produced and consumed in Turkey. Ekinci et al. (2016) studied the antiproliferative effect of shalgam juice on colorectal carcinoma cell lines and observed a dose-dependent inhibition of these cancerous cell lines as well as significantly higher inhibition compared to black carrot juice. Besides juice, black carrot pomace might be of interest to food processing industry due to the large amounts of waste produced in cut/peeled carrots and juice production. Nevertheless, black carrot pomace is yet very much underutilized despite having many advantages such as being easily added into food products without introducing functional issues/negative flavor, while still retaining bioactive components such as dietary fiber (Chantaro et al., 2008).
Moreover, Chantaro et al. (2008) reported that its water retention and swelling capacity was higher compared to other byproducts such as orange, pear and apple and pomace.

2.5.3. Beetroot

Beetroots are mainly utilized to form juices or are cooked to form salads as well as pickles (Vulić et al., 2012). The presence of bioactive compounds in beetroot makes it a suitable ingredient to be added into various food products. Moreover, these are rich sources of water soluble betalain pigments such as betacyanins (purple to violet in color) and betaxanthins (yellow to orange in color), that can be used as natural food colorants. However, these pigments have found limited use owing to their poor stability when exposed to light and heat (Serris and Biliaderis, 2001). Chranioti et al. (2015) reported maltodextrin as well as gum arabic to be effective agents for microencapsulation by freeze drying of beetroot coloring extracts, suggesting this method to feasible for producing food-grade colorants. Junqueira-Goncalves et al. (2011) used gamma-irradiated (with irradiation dose of 5.0 kGy) beetroot extract as a natural colorant in ham-flavored cream cheese and compared the results of this addition with similar cheese but having carmine cochineal (an expensive and difficult to extract natural stable colorant) as a coloring agent. They observed no significant differences in the overall appearance and flavor using sensory evaluation suggesting beetroot extract as a potential colorant for cheeses. Jagannath et al. (2015) reported that fermentation of beetroots by Lactobacillus delbrueckii or by Lactobacillus plantarum preserved betanins (10-15mg/100 mL beetroot extract) up to 32 days of refrigerated storage.

Beetroot juice obtained directly from the roots is hazy and turbid. Thakur and Gupta (2006) clarified beetroot juice using pectinases, fining agent (bentonite), centrifugation and ultrafiltration, while it was concentrated using reverse osmosis and thermovacuum evaporation. Mridula et al. (2016) optimized the levels of beetroot juice, groundnut meal and refined wheat flour with the goal of developing nutritious pasta using response surface methodology. These authors reported the best combination to be 18 mL beetroot juice and 20 g groundnut meal added to 83.5 g refined wheat flour. Vanajakshi et al. (2015) developed fermented moringa leaves based beetroot beverage (made using one part of moringa leaves paste and two parts of beetroot juice with Lactobacillus
plantarum and Enterococcus hirae as fermenting microorganisms) and reported that it could be used as a refreshing health drink having probiotic attributes as well as can be helpful in reducing malnutrition. Ozturk et al. (2014) studied the effect of addition of beetroot juice to mellorine (mainly composed of milk, sugar, vegetable oil, stabiliser and emulsifier) for enhance its bio-functional and sensory properties. They observed a decrease in the dry matter, apparent viscosity but an increase in ash content, TPC and melting rate with beetroot juice addition.

Zielińska-Przyjemska et al. (2009) examined the ability (using in vitro experimentation) of beetroot juice and beetroot chips to activate caspase-3 (a marker of apoptosis execution) in stimulated and non-stimulated neutrophils of healthy and obese women. These authors reported pro-apoptotic effects of non-stimulated neutrophils after 24 h incubation using beetroot chips and juice evidencing their anti-inflammatory therapeutic use. Račkauskienė et al. (2015) reported that the addition of powdered beetroot juice to milk model system lowered the formation of furosine as an early stage indicator of Maillard reaction more efficaciously than N(6)-Carboxymethyllysine as an advanced stage marker, thereby reducing the formation of harmful compounds. Stojceska et al. (2010) developed beetroot powder incorporated rice flour extrudates and these were reported to have higher dietary fiber content than extrudates with no beetroot addition. Apart from beetroot juice and powder, Vulić et al. (2014) reported that its pomace should be regarded as a potential nutraceutic resource and a functional food ingredient due its antiradical and hepatoprotective activities.

2.6. UTILIZATION OF FRUITS AND VEGETABLES IN VARIOUS FOOD PRODUCTS

2.6.1. Extruded products

Extrusion cooking is one of the widely used processing methods in breakfast cereal, confectionary, snack and pet food industries, owing to its significant number of advantages such as high productivity, no process effluents and continuous processing (Gamlath, 2008). Moreover, extrusion conditions have a minor effect on the natural flavors and colors of foods and these lower anti-nutritional factors, sterilize the product and inactivate undesirable enzymes (Guy, 2001). Many beneficial ingredients (such as
fruits and vegetables) are added to the extruded mixtures for the production of ready-to-eat products as well as with the need for consuming high-value products (Bisharat et al., 2013).

Altan et al. (2008) investigated the use of grape pomace in the production of barley based snack food using extrusion cooking with an aim of optimizing process conditions using response surface methodology. They used extrusion temperatures, screw speeds and pomace levels varying from 140 to 160 °C, 150 to 200 rpm and 2 to 10%, respectively and studied their effects on the physical characteristics of the extrudates. They reported that these characteristics were mostly affected by changes in the extrusion temperature as well as pomace levels but with a lesser extent to the screw speed. Their graphical optimization studies revealed that acceptable extrudates were produced at extrusion temperatures, screw speeds and pomace levels of 155-160 °C, 150-187 rpm and 4.47-6.57%, respectively. Bisharat et al. (2013) examined the addition of dehydrated broccoli (4-10%) and olive paste (4-8%) to corn flour for the producing corn extrudates having increased nutritional value as well as superior quality. They prepared extrudates using a twin screw extruder at different extrusion conditions, including extrusion temperatures, screw speed and moisture contents varying from 140 to 180 °C, 150 to 250 rpm and 14 to 19%, respectively. They reported that the increment of broccoli as well as olive paste ratio porosity of the extrudates owing to their high dietary fiber content. They also reported that increment of extrusion temperature and screw speed reduced the mixture viscosity and led to more porous extrudates, while the opposite effect was observed for increment in moisture content. Further, they reported that expansion of extrudates decreased as moisture content increased, while the opposite effect was observed with increase in the screw speed. In their subsequent study, Bisharat et al. (2015) examined the antioxidant activity, TPC and sensory characteristics of broccoli and olive paste enriched corn extrudates produced under identical extrusion conditions as described previously in their study. They reported that antioxidant activity and TPC of the extrudates increased with increase in broccoli and olive paste addition and extrusion temperature, while these decreased with increase in moisture content of the feed.

Selani et al. (2014) examined the utilization of pineapple pomace (0 to 21% level) in the preparation of corn extrudates at varying feed moisture levels (14 to 16%) and
extrusion temperatures (140 and 160 °C). They reported that pineapple pomace addition decreased expansion as well as lightness and increased redness, while had no effect on hardness, bulk density and yellowness when compared to extrudates prepared from without any pomace addition (at 14% moisture content and 140 °C extrusion temperature). O’Shea et al. (2014) investigated the effect of extrusion temperature, screw speed and examined the addition of apple pomace into a corn-based extrudates. They reported that screw speed had the greatest effect on extrudate quality (such increase in the bulk density with increase in screw speed), while increase in extrusion temperature and apple pomace decreased their expansion. Their study concluded that 150 °C extrusion temperature, screw speed of 69 rpm and 7.7 % apple pomace produced extrudates with best acceptability. Kaisangsri et al. (2016) examined the incorporation of carrot pomace in corn starch at replacement levels of 5 to 15% for the preparation of extrudates at varying feed moisture content levels from 15 to 30%. They reported that at 10 and 15% carrot pomace levels, expansion considerably decreased but surface improved when compared with extrudates without any pomace addition. Their study concluded that best extrudates were prepared using 5% carrot pomace level at 15% feed moisture content. Huang and Ma (2016) successfully applied extrusion technology to orange pomace in order to increase its SDF fraction. They reported that this increase was mainly contributable to the redistribution of IDF to SDF. They further reported that functional properties including water holding capacity, water solubility index of extruded orange pomace improved considerably compared to the unextruded pomace. All these studies supported the addition of fruits and vegetables as well as their by-products into traditional formulations for the preparation of nutritious extrudates which are not only rich in dietary fibers but also in polyphenolic compounds.

2.6.2. Muffins

Muffins are commonly consumed breakfast/evening snack foods, which are generally prepared from wheat flour, milk, eggs, sugar and fat/oil. These are sweet baked products (ranking third in breakfast products) having good taste, high volumes and typical porous structures conferring a spongy texture, which makes them highly appreciated by consumers (Matos et al., 2014). However, these baked products have low
nutrient values and high energy densities which make them non-recommendable from a nutritional point of view to be eaten every day. The satiating effect of muffins needs to be manipulated for controlling food intake and also for potentially regulating body weight. The addition of fruit and vegetables, especially their pomaces (owing to their high levels of polyphenolic compounds and dietary fiber) seem to be a promising approach towards achieving this goal.

There have been many reports in the literature on the addition of fruits and vegetables in muffin recipes for enhancing their nutritional values. In spite of this, Sedej et al. (2011) reported that functional food products were rapidly growing in popularity in dairy and confectionary sectors but in the bakery sector these were relatively underdeveloped. Rupasinghe et al. (2008) utilized apple skin (which is an underutilized food processing by-product) powder as a food ingredient in muffins. They reported that muffins incorporated with apple skin powder had higher TPC and dietary fiber than muffins without it. Moreover, they reported that antioxidant activity of muffins increased with apple skin incorporation and there was less or no impact of thermal processing on this activity. Rosales-Soto et al. (2012) evaluated the effect of freeze drying, mixing time and baking on the TPC and antioxidant activity of raspberry juice during the preparation of muffins. They reported that freeze drying of batters containing raspberry juice reduced their TPC as well as antioxidant activity regardless of mixing time. Their results suggested that utilizing non freeze dried raspberry juice in batters was better than using freeze dried one, as the muffins prepared from the former had higher TPC and antioxidant activity than the ones prepared from the latter. Moreover, they reported that optimal mixing time for batters was 5 min.

Bhaduri and Navder (2013) studied the effect of 10% freeze dried blueberry powder incorporation on the physicochemical characteristics, shelf life and antioxidant activity of muffins. They reported a decrease in specific gravity of the batter, muffin hardness as well as chewiness and an increase in the TPC and antioxidant activity with blueberry powder incorporation. Moreover, they also reported that exposure to high oven temperatures during the baking process decreased the total antioxidant activity and also lowered the TPC (around 10%) of the muffins compared to the batter. These authors further reported that incorporation of blueberry powder to muffins also significantly
lowered their water activity and dramatically increased their shelf life (with no microbial growth even after three months of refrigerated storage).

Walker et al. (2014) evaluated the use of wine grape pomace (winemaking by-product) as a source of antioxidant dietary fiber in muffins. They studied their physicochemical properties including TPC, antioxidant activity and sensory evaluation. They reported that TPC, antioxidant activity and dietary fiber increased with fortification and muffins prepared with 5 or 10% wine grape pomace flour replacement were the most acceptable. In addition, they reported that it was advantageous to use a small particle size of the pomace as it was less noticeable when chewed during sensory evaluation. Ramírez-Maganda et al. (2015) investigated the partial substitution of wheat flour and cane sugar (50 and 75%) by a mango processing by-product (mango peel and paste by-products) as a food ingredient in muffins. They reported that antioxidant activity, chemical composition and in vitro starch hydrolysis of muffins were significantly affected by the amount of mango processing by-product substitution in the recipe. They further reported that the substitution at 50 and 75% level did not affect consumer preference and had higher TPC, antioxidant activity and indigestible fraction content compared to muffins prepared by without any substitution. Bajerska et al. (2015) investigated the acceptability of muffins incorporated with sour cherry pomace (at 0% to 40% levels) and determined the effect of the most acceptable muffins on the human appetite sensations, postprandial glycemia and energy intake. The sensory analysis of their study showed that sour cherry pomace could be successfully added to muffin formulations at levels up to 30% without any reduction in the overall acceptability and also an increase in both TPC and dietary fiber was observed with the addition. Additionally, they reported that this addition also significantly affected the hunger, prospective food intake and postprandial glucose responses compared with the muffins without any addition.

2.6.3. Gluten-free products

Most of the studies involving addition of fruits and vegetables into muffin formulations contain wheat flour as an important ingredient. Wheat flour forms gluten network (with the addition of water in it) which is responsible for the elastic and extensible properties of the batter. Celiac disease is an autoimmune disorder resulting
from the consumption of gluten, causing an inflammation of the small-intestinal mucosa, further leading to malabsorption of nutrients (Murray, 1999). This disease affects one in hundred people worldwide and the only known available treatment at present is strict avoidance of foods that contain gluten (Mustalahti et al., 2010). There is therefore a need of substituting wheat flour with suitable ingredients in formulation that are able to mimic the properties of gluten. In recent years, the paradigm of research has shifted towards designing of gluten-free products not only for the people having this disease but also for people interested in consuming wheat free foods (Nachay, 2010). Moreover, there is a need for the preparation of eggless muffins which will allow the consumption by lacto vegetarians (especially in India, containing significant proportion of the vegetarian population).

Gluten-free products are mainly starch based and usually contain lesser amounts of nutrients as compared to products containing gluten. Some previous studies showed that strict adherence to gluten-free diet worsened the nutritional balance in the affected persons (Filipčev et al., 2015). Therefore, development of new, enriched gluten-free formulations containing bioactive components (such as fruits and vegetables) is necessary in the present times, but only limited studies have been done in this regard. Few studies have reported the use of fruits and vegetables in the preparation of gluten-free breads and cakes. Siqueira et al. (2013) evaluated the use of unripe banana flour (both as a source of resistant starch and dietary fiber) for gluten-free breads. They obtained good results with 100% unripe banana flour and also with formulations having rice flour and potato starch. Majzoobi et al. (2016) reported that addition of carrot pomace powder of different particle sizes (210 µm and 500 µm) in the gluten-free sponge cake recipes not only enhanced batter and cake qualities but also nutrient levels. O’Shea et al. (2015) optimized the level of orange pomace incorporation in gluten-free bread formulations using response surface methodology. They reported that the optimized bread formulation was the one that contained 94.6% water, 5.5% orange pomace and 49 min proofing time. Korus et al. (2012) used defatted strawberry and blackcurrant seeds as dietary supplements in gluten-free bread. They reported that the maximum addition of 10% strawberry seeds or 5% blackcurrant seeds in the resulting bread, significant increased the dietary fiber, polyphenolic compounds and protein content, while had no negative impact
on sensory parameters. Some researchers have also advised the use of gums apart from fibers (from fruits, vegetables and other sources) in designing of gluten-free cakes for improving their quality characteristics. Preichardt et al. (2011) reported that with the addition of 0.3-0.4% XNG in the formulation of gluten-free cakes (prepared using corn and rice flour), these cakes resembled in chemical, physical as well as sensory attributes with that of wheat flour cakes. Apart from breads and cakes, the use of fruits and vegetables in designing of gluten-free biscuits/cookies, pasta and spaghetti has been reported by many researchers. Filipčev et al. (2015) assessed the effect of sugar beet molasses in liquid and dry forms on the quality of gluten-free biscuits, reporting that fracturability increased with molasses addition (more pronounced with dry molasses). Additionally, they reported that liquid molasses had a softening effect but dry molasses hardened the biscuits. Šarić et al. (2016) successfully valorized blueberry and raspberry pomace by applying them as dried powders in the formulation of gluten-free cookies. They reported not only this addition increased polyphenolic content, minerals and dietary fiber content but also reduced the fat content (also improved fatty acid composition) when compared with commonly available gluten-free cookies in the market. Mirhosseini et al. (2015) investigated the effect of partial replacement of corn flour with pumpkin powder on the quality characteristics of gluten-free pasta. They reported that addition of 25% pumpkin powder to the pasta formulation improved its color and sensory attributes as well as textural properties. Flores-Silva et al. (2014) determined the chemical compositions and starch digestibility of gluten-free spaghetti developed with unripe plantain, chickpea and maize flour mixtures. These authors successfully developed gluten-free spaghetti having higher ash, protein as well as fat contents but reduced amount of glycaemic carbohydrates (a lower available starch content but higher resistant starch content) than control semolina spaghetti. Padalino et al. (2013) optimized the formulation of gluten-free spaghetti based on corn flour and different types of vegetable flours (yellow pepper, green pepper, red pepper, asparagus, fennel, pumpkin, broccoli, artichoke, tomato, spinach, zucchini, carrot and brinjal). They reported that the best gluten-free spaghetti was the one having yellow pepper flour addition from chemical composition, rheology and sensory viewpoint.
Review of Literature

The review of literature indicated that a limited work has been carried out in evaluating the variability in chemical composition, profiles of polyphenolic compounds, antioxidant and antimicrobial activities of commonly consumed fruits (peels and pulps) and vegetables in India. Jambolan, black carrot and beetroot are suitable choices for the production of novel functional food products, as these are rich sources of polyphenolic compounds and have been relatively underutilized than other commonly available fruits and vegetables in the market.