Chapter 2

Review of Literature
2.1. Global scenario of maize production and utilization:

The production of cereals has witnessed a fair increase of 13% from 2010 to 2013. In the year 2013 the total production of cereals was more than 2500 million metric ton (MMT), in which coarse grains alone consisted of 1300 MMT. Among the coarse grains, maize is an important cereal with annual global production of 1000 MMT of which United States contributed with 350 MMT and China with 200 MMT. India stood sixth in position with the production of 23 MMT (FAOSTAT, 2013). International Food Policy Research Institute (2003) proposed that global maize demand will increase by 58% from 585 MMT in 1997 to 927 MMT by 2025. The production of maize in India has increased considerably since last decade from 14 MMT in the year 2004-2005 to 23 MMT during 2012-2013. Andhra Pradesh and Karnataka are the two states in India which produces the nearly 38% of India’s maize. The detail maize producing areas are shown in Fig. 2.1.

Large groups of people in Latin America, North America, Asia and Africa consume maize as staple food. In contrast to rice and wheat, maize is mainly utilized for animal feeding although direct and industrial food uses are increasing (Serena-Saldivar, 2004). In the United States, maize is widely processed into various products, such as grits, flour, corn meal, starch, snacks, tortillas, and breakfast cereals, apart from its use as animal feed. In addition to its food and feed use, maize has a number of industrial applications, including the production of bioethanol and starch. The diverse uses of maize reflect its long history as a domesticated crop and its wide gene pool (Gewin, 2003). However, with increasing attention being drawn to the development of nutraceuticals in the last decade, the bioactive compounds derived from maize and their health properties have recently become the major focus of studies on this grain.
**Fig. 2.1:** State-wise production of maize in India in 2011-12
(Source: http://farmer.gov.in/cropstaticsmaize.html)
2.2. Variety of maize:

Several genotypically different types of maize are found. On the basis of kernel colour it can be classified as white, yellow, red, purple or black. White and yellow maize are the most common. Although, genetically white maize is similar to yellow maize, there is a difference in appearance due to the presence of carotene oil pigments in the kernel of yellow maize. The grain is classified into groups based on endosperm characteristics, kernel colour, maturity, and final uses. They may be grouped into two classes viz., high-yielding and specialty crops. In addition, mutant varieties e.g. QPM (quality protein maize) have also been developed. The followings are some popular maize genotypes grown worldwide for commercial and human consumption.

*Dent maize* is the most commonly grown crop in the USA. The kernel contains both corneous and soft starch, characterized by very hard, vitreous, horny endosperm at the sides and back (Singh *et al.*, 2009). The central core extends to the top, or crown of the kernel, which collapses on drying, resulting in the distinctive indentation (dent) (Paliwal *et al.*, 2000). Dent corn has a fairly wide range of colours, from yellow to white, but yellow is the most common and is extensively grown for seed, silage, biofuel, and other commercial uses in the US.

*Flint maize* is primarily vitreous, with little soft starch, and is enclosed by a corneous outer layer. Starch is more concentrated at the periphery than in the centre, which gives the endosperm hard external layers (Haros *et al.*, 2001). Flint corn exhibits an extended range of colours from white through yellow, orange, red, mahogany, blue, purple, and black (Boutard, 2012), and it is widely grown in Latin America, Northern Europe, and some parts of Asia for commercial purposes (Gujral *et al.*, 2001).

*Popcorn* kernel is characterized by a high proportion of hard endosperm. Popcorn is grown in a small scale.
Sweet corn is one among the specialty maize genotypes which has higher sugar content than all other genotypes due to recessive mutants blocking conversion of sugar to starch. Standard sweet corn at the immature, milky stage contains about 10% sucrose, while field corn in the same stage has about 4% sucrose.

Baby corn is premature maize kernel which is usually harvested within 2 days of emerging silks. The normal ears of baby corn are 4-10 cm long. Baby corn is consumed world-wide as an annexe of food preparations.

OPM is a mutant genotype with 2-3 times increased levels of two essential amino acids-lysine and tryptophan. This maize type has a balanced amino acid profile and hence the name ‘quality protein maize’.

Waxy maize is a genotype which contains only amylopectin as starch unlike other field maize with 75% amylopectin and 25% amylose. Waxy maize is consumed in some parts of East Asia and also used for producing starch in industrial level.

2.3. Classification of Maize:

Kingdom: Plantae
Sub-kingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Tracheobionta
Class: Liliopsida
Subclass: Commelinidae
Order: Cyperales
Family: Poaceae
Genus: Zea
Species: Zea mays L.

(Source: USDA/NRCS plant data base; http://plants.usda.gov/core/profile?symbol=zema)
2.4. Phytochemicals:
Phytochemicals are chemical compounds of plants which have protective or disease preventive properties. These include both primary and secondary metabolites. Sugar and protein components are the primary metabolites which are essential for plant growth. Phytochemicals derived from the secondary metabolites helps in plants defense mechanism and includes terpinoids, alkaloids, flavonoids, lignans, steroids, saponins, glucosides and phenolicss (Saxena et al., 2013)

Harborne (1999) identified the three major classes of plant chemicals as terpenoids, alkaloids, and phenolic metabolites.

2.4.1. Terpenoids:
The terpenes are the major class of phytonutrients and also known as isoprenoids (Dillard and German, 2000). Terpenoids are secondary metabolites with isoprene (2-methylbuta- 1, 3-diene) units as carbon backbones and as primary monomers. There are approximately 36000 terpenoids compounds which have been identified and made this group as the largest group of plant metabolites (Yamunadevi et al., 2011). Terpenoides are mainly found in green foods, soy plants and grains. It has a unique antioxidant activity. These molecules react with free radicals by dividing themselves into fatty membranes due to presence of their long carbon side chain. Plants synthesize huge amounts of diverse terpenoids, via the combination of the terpenoid biosynthetic route and other secondary metabolic pathways. Plants use mevalonic acis pathways to synthesize sterols and sesquiterpenes, the side chain of ubiquinone and sesquiterpenes (C_{15}) and methylerthyritol phosphate pathways for the synthesis of isoprene (C_{5}), monoterpenes (C_{10}), diterpenes (C_{20}), including gibberellins and the phytol tail of tocopherols and chlorophylls, and carotenoids (C_{40}) (Penuelas and Munne-Bosch, 2005).
Harborne (1999) defined the terpenoids as monoterpenoids, sesquiterpenoids, sesquiterpene lactones, diterpenoids, triterpenoid saponins, phytosterols, cucurbitacins, nortriterpenoids, other triterpenoids and carotenoids.

2.4.1.1. Tocotrienols and tocopherols:

Tocotrienols and tocopherols are most studied terpene antioxidants. These are the natural terpenes of grains. \(\alpha-\delta\)-Tocopherol (vitamin E) has been studied extensively. The tocotrienols (\(a\), \(\gamma\) and \(\delta\)) and RRR-\(\delta\)-tocopherol were effective apoptotic inducers for human breast cancer cells (Yu et al., 1999).

2.4.1.2. Carotenoids:

Carotenoids are highly pigmented yellow, orange and red coloured compounds. These are especially abundant in yellow-orange fruits and vegetables and dark green, leafy vegetables. Carotenoids are mainly two types viz. carotenes and xanthophylls. Carotenes include \(\alpha\)-carotene, \(\beta\)-carotene, \(\gamma\)-carotene, lycopene and lutein whereas, xanthophylls include zeaxanthin, cryoptoxanthin, astaxanthin (Dillard and German 2000).

2.4.1.3. Limonoids:

The limonoids are the tetranortriterpenoid. This was first obtained from the citrus fruits. The term limonoids was derived from limonin. This group has a series of biological activities
viz. insecticidal, antifeedant, antimicrobial, antimalarial, anticancer, antiviral etc. (Roy and Saraf, 2006)

2.4.1.4. Phytosterols:

Phytosterols are plant sterols structurally similar to cholesterol that act in the intestine to lower cholesterol absorption. Because they have very low systemic absorption and are already present in healthy diets, increasing the intake of phytosterols may be a practical way to reduce coronary heart disease with minimum risk (Ostlund, 2004). Two sterol molecules that are synthesized by plants are β-sitosterol and its glycoside.

2.4.2. Alkaloids and other nitrogen containing compounds:

Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Alkaloids are extremely toxic compounds. The use of alkaloids in herbal medicine is very limited but in modern medicine it is significantly used with strictly regulated dosage.

Alkaloids are mainly well-defined crystalline substances. They react with acids and form salts. In the plant they may exist freely or as salts. The elements of alkaloids are carbon, hydrogen and nitrogen. Alkaloids may also contain oxygen. Alkaloids are basically divided into two groups viz. non-heterocyclic and heterocyclic compounds. Different types of alkaloids are given in Table 2.1.
**Table 2.1: Different types of alkaloids and their structures**

<table>
<thead>
<tr>
<th>Non-heterocyclic alkaloids</th>
<th>Hordenine or N methyltyramine</th>
<th><img src="image" alt="Mescaline" /></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mescaline</td>
<td><img src="image" alt="Ephedrine" /></td>
</tr>
<tr>
<td></td>
<td>Ephedrine</td>
<td><img src="image" alt="Colchicine" /></td>
</tr>
<tr>
<td></td>
<td>Colchicine</td>
<td><img src="image" alt="Erythromycin" /></td>
</tr>
<tr>
<td></td>
<td>Erythromycin</td>
<td><img src="image" alt="Pachysandrine A" /></td>
</tr>
<tr>
<td></td>
<td>Pachysandrine A</td>
<td><img src="image" alt="Taxol" /></td>
</tr>
<tr>
<td>Heterocyclic alkaloids</td>
<td>Pyrrole</td>
<td><img src="image" alt="Pyrrole" /></td>
</tr>
<tr>
<td></td>
<td>pyrrolidine</td>
<td><img src="image" alt="pyrrolidine" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td></td>
</tr>
</tbody>
</table>
| Pyrrolizidine    | ![Pyrrolizidine](image)
| Pyridine         | ![Pyridine](image)
| piperidine       | ![piperidine](image)
| Purine           | ![Purine](image)
| Tropane          | ![Tropane](image)
| Quinoline        | ![Quinoline](image)
| Isoquinoline     | ![Isoquinoline](image)
| Aporphine        | ![Aporphine](image)
| Quinolizidine    | ![Quinolizidine](image)
| Indole or benzopyrrole | ![Indole or benzopyrrole](image)
2.4.3. Phenolic metabolites:

The phenolic compounds comprise large group of phytochemicals. The phenolics are secondary metabolites of plants and widely distributed in higher plants. Different classes of phenolic compounds are given in Table 2.2. The most important groups of dietary phenolics include flavonoids, phenolic acids, and polyphenols (King and young, 1999). Flavonoids are the largest group of plant phenols and the most studied (Dai and Mumper, 2010). Phenolic acids are diverse group including hydroxybenzoic and hydroxycinnamic acids and widely distributed in plants. Polyphenols or phenolic polymers are most commonly tannins. These are high molecular weight compounds and divided into two classes \textit{viz.} hydrolyzable and condensed tannins. Phenols protect plant cells from oxidative damage. They have also been studied widely as antioxidants for humans.

\textbf{Table 2.2:} The Major Classes of Phenolic Compounds

\begin{tabular}{|c|c|c|}
\hline
Sl No. & Basic skeleton & Class \\
\hline
1 & C\textsubscript{6} & Simple phenols \\
\hline
2 & C\textsubscript{6} & Benzoquinones \\
\hline
3 & C\textsubscript{6}-C\textsubscript{1} & Hydroxybenzoic acid \\
\hline
\end{tabular}
### Table 2.2 continued…..

<table>
<thead>
<tr>
<th></th>
<th>Chemical Structure</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>C₆-C₂</td>
<td>Acetophenones</td>
</tr>
<tr>
<td>5</td>
<td>C₆-C₃</td>
<td>Hydroxycinnamic acid</td>
</tr>
<tr>
<td>6</td>
<td>C₆-C₃</td>
<td>Coumarins</td>
</tr>
<tr>
<td>7</td>
<td>C₆-C₄</td>
<td>Naphthoquinones</td>
</tr>
<tr>
<td>8</td>
<td>C₆-C₁-C₆</td>
<td>Xanthones</td>
</tr>
<tr>
<td>9</td>
<td>C₆-C₂-C₆</td>
<td>Stilbenes</td>
</tr>
<tr>
<td>10</td>
<td>C₆-C₃-C₆</td>
<td>Flavonoids</td>
</tr>
<tr>
<td>11</td>
<td>(C₆-C₃)₂</td>
<td>Lignans</td>
</tr>
</tbody>
</table>
Table 2.2 continued……

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>(C$_6$-C$_3$-C$_6$)$_2$</td>
<td>Bi-flavonoids</td>
</tr>
<tr>
<td>13</td>
<td>(C$_6$-C$_3$-C$_6$)$_n$</td>
<td>Condensed tannins</td>
</tr>
</tbody>
</table>

### 2.4.3.1. Flavonoids:

This group of phenolic compounds are ubiquitous in nature. More than 4,000 flavonoids have been recognised till date. They are mainly found in vegetables, fruits and beverages like tea, coffee (Saxena et al. 2013). The flavonoid subclasses of phenols include flavanones, dihydro flavonols, flavones and flavonols. Naturally, most flavonoids occur associated with sugar and characterized as monoglycosidic, diglycosidic etc. The glycosidic linkage is normally located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose (Pretorius, 2003).

Flavonoids have wide range of biological activities against free radicals, free radical mediated cellular signalling, allergies, platelet aggregation, inflammation, ulcers, microbes, viruses, tumours and hepatotoxins (Kinsella et al., 1993). The flavonoids *viz.* quercetin, kaempferol, apigenin, myricetin and luteolin were quantitated in various foods by Hertog *et al.* (1993). They showed that the flavonoid intake is associated with reduced risk from coronary heart disease.

The flavonol kaempferol widely found in the diet. This has anti-inflammatory and antibacterial activities and is directly mutagenic (Harborne and Baxter, 1993).
2.4.3.2. Phenolic acids:

Phenolic acids are generally phenol with carboxylic acid as functional group. Phenolic acids are synthesized from L-phenylalanine or L-tyrosine through the Shikimate pathway. Phenylalanine and tyrosine are the common precursors for the majority of the natural phenolic products. Hence they are important amino acids in this pathway (Heleno et al., 2015). Naturally occurring phenolic acids are of two types depending on their carbon backbone, hydroxybenzoic acids and hydroxycinnamic acids. Plant phenolic compounds are characterized by hydroxylated aromatic rings (Balasundram et al., 2006). The study of phenolic acids are particularly important due to their properties against oxidative damage which further leads to various degenerative diseases, for instance cardiovascular diseases, inflammation and cancer. Cancer cells have higher levels of reactive oxygen species (ROS) than normal cells thus particularly sensitive to oxidative stress (Mandal et al., 2010). In the previous studies on bioavailability of phenolic acids, importance has been given on both the direct intake via food consumption and the indirect bioavailability through gastric, intestinal and hepatic metabolism (Battisti et al., 2008). The study of phenolic acid in plants as secondary metabolite is one of the many different facets of investigations. Phenolic acids are also associated with different qualities of food including colour, sensory qualities, as well as nutritional and antioxidant properties. Phenolics work as antioxidants, as a result of reactivity of the hydroxyl substituent on the aromatic ring. The stabilization of phenolic acid is affected by the presence of the substituents on the aromatic ring which in turn affects the radical-scavenging ability. Therefore different phenolic acids have diverse antioxidant capacity (Rice-Evans et al., 1996; Chalas et al., 2001).
2.4.3.3. Polyphenol or phenolic polymer:
Polyphenol or phenolic polymers are mainly tannins. Tannin includes a diverse group of oligomers and polymers. The tannins are a heterogeneous group of high molecular weight polyphenols with the capacity to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals. The tannins can be divided into following groups’ *viz.* Gallotannins, ellagitannins, complex tannins and condensed tannins (Saxena *et al.*, 2013).

2.5. Major cereals and phytochemicals:
Cereals are the most important food for human in the world. Rice, wheat and maize are the major agricultural cereal grains (Hung, 2014). Whole grains contain unique phytochemicals. Various classes of phenolic compounds in grains comprise phenolic acids, anthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (Lloyd *et al.*, 2000; Shahidi and Naczk, 1995). Phenolic acids for example ferulic acid and diferulates are predominantly found in grains while insignificant quantities may be present in some fruits and vegetables (Bunzel *et al.*, 2001; Shahidi and Naczk, 1995). Grains also contain tocotrienols, tocopherols, and oryzanols (Lloyd *et al.*, 2000). The concentrations of phenolic compounds in grains depends on grain types, varieties and the grain fraction (Adom and Liu, 2002; Adom *et al.*, 2003 and 2005). The most common phenolic compounds found in whole grains are phenolic acids and flavonoids (Liu, 2007).

Adom and Liu (2002) showed that ferulic acid can exist in different forms *viz.* free, soluble-conjugated and bound, in whole grains. Their study revealed the presence of higher proportion bound ferulic acid than free and soluble-conjugated ferulic acid in rice, maize, wheat and oats. The order of total ferulic acid content among the tested grains was maize > wheat > oats > rice (Adom and Liu, 2002). Ferulic acid content of whole grains differs among different varieties. Adom *et al.* (2003) evaluated varietal effects of 11 wheat varieties.
Their observation revealed that significant differences existed on total ferulic acid content among wheat varieties. They also showed that the ferulic acid exist mostly in the bound form in all varieties. Liyana-Pathirana et al. (2006) also indicated the presence of bound phenolic acids in wheat grains. The bound forms mostly exist in bran associated with cell wall materials.

Okarter et al. (2010) analysed phytochemical content of wheat in six different varieties. According to their findings ferulic acid was the predominant phenolic acid in whole wheat. The other phenolic acids detected by them include p-coumaric acid, syringic acid, vanillic acid and caffeic acid. The carotenoid and vitamin E content was also reported in their study. Lutein was the major carotenoid found in the whole wheat varieties. Zeaxanthin, β-carotene and β-cryptoxanthin were also detected.

More than 50 phenolic compounds were identified in several whole millet grains through high performance liquid chromatography (HPLC) and HPLC-tandem mass spectrometry. The phenolic compounds belong to different classes viz. phenolic acids and their derivatives, dehydrodiferulates and dehydrotriferulates, flavan-3-ol monomers and dimers, flavonols, flavones and flavanonols. Insoluble bound form of phenolics attached to the cell wall material of millet grains was found as major contributor to the total phenolic content of the grain (Chandrasekara and Shahidi, 2011a).

Shao et al. (2014) identified and quantified phenolic acid and anthocyanins in white, red and black rice kernels. They analysed bran, germ and endosperm part of rice varieties. Their study reveals the presence of cis-p-coumaric acid in bound form in bran and cis-sinapic acid in the free/conjugated form in germ and bran. Bound phenolic acids in rice bran accounted for 90% of total acids in whole grain. Cyanidin-3-O-glucoside and peonidin-3-O-glucoside were detected in black rice bran as the total anthocyanins.
Barley (*Hordeum Vulgare* L.) is best known to be used in the beer industry, malting and as animal feed (Baik and Ullrich, 2008). Lately, barley is considered as an ingredient for functional foods production (HoltekJolen *et al.*, 2006) owing to its high contents of glucans, tocopherols, tocotrienols and phenolic compounds including phenolic acid derivatives, proanthocyanidins, quinones, flavonols, flavones, flavanones etc. (Hung, 2014). The phenolic content of barley may serve as an excellent dietary source of natural antioxidants (Madhujith and Shahidi, 2009; Zhao *et al.*, 2008).

Sorghum (*Sorghum bicolor* M.) is one of the leading cereal food crop after rice, wheat, maize and barley in the world and is particularly important as a food resource in Asia and Africa (Rooney and Waniska, 2000). Sorghum is a rich source of various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols and policosanols (Awika and Rooney, 2004).

Oat (*Avena sativa* L.) and finger millet (*Eleusine coracana*) are proven as rich source of phenolic compounds and as good antioxidant. Phenolic acids found in the oat include *p*-hydroxybenzoic acid, ferulic acid, *p*-coumaric acid, caffeic acid and vanillic acid (Emmons *et al.*, 1999). Millet contains gallic acid, coumaric acid, syringic acid and vanillic acid as major phenolic acids (Viswanath *et al.*, 2009). Phenolic compounds in oat and millet are also concentrated in outer layer (bran) of grains (Hung, 2014).

Like other cereal grains, rye grain contributes significant quantities of energy, protein and selected micronutrients (Edge *et al.*, 2005). Phytochemical analysis of rye showed the presence of phenolic acids and ferulic acid dehydrodimers (Andreasen *et al.*, 2000), tocopherols and tocotrienols (Zielinski *et al.*, 2007) and alkylresorcinols (Gliwa *et al.*, 2011). Phytochemicals from different cereal grains are given in the Table 2.3.
### Table 2.3: Phenolic compound from cereals

<table>
<thead>
<tr>
<th>Sources</th>
<th>Phenolic compounds</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>Ferulic and <em>p</em>-coumaric acids</td>
<td>Abdel-Aal <em>et al.</em>, 2012.</td>
</tr>
<tr>
<td>Crop</td>
<td>Flavonoids</td>
<td>References</td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Finger Millet</td>
<td>Naringenin, kaempferol, luteolin glycoside, phloroglucinol, apigenin, catechin, epicatechin, trans-feruloyl-malic acid, dimer of prodelphinidin, diadzein, catechin gallates, trimers and tetramers of catechin, gallic, protocatechuic, vanillic, ferulic, p-hydroxybenzoic, chlorogenic, caffeic, trans-cinnamic, catechin, epigallocatechin, epicatechin, taxifolin, myricetin, kaempferol, quercetin.</td>
<td>Subba Rao and Muralikrishna, 2002; Chethan and Malleshi, 2007; Shobana et al., 2009; Chandrasekara and Shahidi, 2011a</td>
</tr>
<tr>
<td>Little millet</td>
<td>Gallic, gentisic, vanillic, p-coumaric, ferulic, chlorogenic, caffeic acids.</td>
<td>Pradeep and Guha, 2011; Chandrasekara and Shahidi, 2011a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>Ferulic, caffeic, p-coumaric, sinapic acids, galangin, kaempferol, gallic acid, quercetin</td>
<td>Chiremba et al., 2012; Deng et al., 2012; Luthria and Liu, 2013</td>
</tr>
<tr>
<td>Oats</td>
<td>Catechin, gallic acid, galangin, protocatechuric acid, kaempferol.</td>
<td>Zieliński and Kozłowska, 2000; Deng et al., 2012</td>
</tr>
<tr>
<td>Barley, Wheat and yellow maize</td>
<td>Carotenoides</td>
<td>Ndolo and Beta, 2013</td>
</tr>
</tbody>
</table>
Table 2.3 continued...

<table>
<thead>
<tr>
<th>Yellow maize, wheat, barley and oats</th>
<th>Ndolo and Beta, 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, sinapic, isoferulic, and diferulic acids,</td>
<td></td>
</tr>
</tbody>
</table>

Other than providing the major caloric and protein source for human, recent researches indicate that cereal grains contain significant amounts of phenolic metabolites which are related to reduced risk of chronic diseases viz. anti-allergenic, antiatherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects (Manach et al., 2005; Farnoche et al., 2005; Liyana-Pathirana and Shahidi, 2006b; Aguilar-Garcia et al., 2007; Liyana-Pathirana and Shahidi, 2007; Hung and Hatcher, 2011). Among the different health benefits the antioxidant activity of the phenolic compounds is most important as this property of phenolic compounds attributed to the beneficial effect against degenerative diseases such as heart disease and cancer involved in ROS (i.e., superoxide anion, hydroxyl radicals and peroxy radicals) (Heim et al., 2002).

2.6. Extraction of phenolic metabolites from grain (cereals and pulses) sources:

Whole grains have been assessed for the presence of different phytochemicals. Among the phytochemicals, phenolic compounds are of particular interest due their wider health benefits such as antioxidants, anti-inflammatory, anti-cancerous, anti-diabetics and prevent cardiovascular diseases (Acosta-Estrada et al., 2014). Simple phenolic acids and flavonoids are the most common phenolic
compounds and they generally occur as soluble conjugated (glycosides) and insoluble bound forms (Nardini and Ghiselli, 2004).

The extraction of phenolic compounds is usually performed with the aid of various organic solvents under ideal temperature and pH conditions. For extraction of phenolic compounds it is important to consider the structure as these compounds have single or more hydroxyl groups (-OH) and can create bonding with sugars, acids or alkyl groups. Hence, there are several techniques for the extraction of phenolic compounds.

2.6.1. Conventional extraction techniques:

Various classical extraction techniques have been used for extraction of phenolic compounds from various plant sources. Most of these techniques are based on the choice of solvent coupled with the application of heat and/or agitation. Selection of solvent for the extraction of phenolic compounds depends upon the solubility in solvent. Large number of organic, inorganic, polar and non-polar solvents alone or in combinations has been used for the extraction of varied range of phenolic compounds. The conventional extraction techniques of phenolics include:

2.6.1.1. Soxhlet extraction:

In 1879, German chemist Franz Ritter Von Soxhlet had first proposed soxhlet extractor for extraction of lipid. Later it became widely-used apparatus for extraction of various bioactive compounds from different natural sources. Soxhlet extraction is standard and model technique for evaluating the performance of new extraction methods. Selection of suitable solvent for the extraction of targeted phenolic compound using the soxhlet extraction is important because the yield of extracts and extract compositions changes with different solvents (Zarnowski and Suzuki, 2004). Different solvents *viz.* water, hexane, isopropanol, ethanol, methanol are used as extraction of various phenolic compounds.
Soxhlet extraction is a well-established technique with great advantages of industrial applications, better reproducibility, efficiency and less extract manipulation in contrast to the other non-conventional extraction methods for instance ultrasound-assisted, microwave-assisted or pressurized solvent extractions. However, disadvantages of Soxhlet extraction include excess consumption of solvent and time.

2.6.1.2. Maceration:

Preparation of plant extract using maceration is one of popular, cheap and well established procedures. This technique is widely used for extraction of volatile products such as aroma compounds and colour pigment, tannin etc. (Canals et al., 2005; Del Llaudy et al., 2008). The main disadvantage of this process is time and solvent consumption.

2.6.1.3. Hydrodistillation:

Hydro-distillation is the oldest and most common method of extracting essential oil since it is economically viable and safe. Hydro-distillation is a traditional method for extraction of bioactive compounds and essential oils from plants. This is the simplest and usually the cheapest process of distillation. Hydro-distillation seems to work best for powders and very tough materials like roots, wood, or nuts; hence this procedure is easy to use for extraction of bioactive compounds such as phenolics from plant matrices. The main advantages of this method include reduced steam usage, shorter processing time and higher yield and involvement of no organic solvents. There are three types of hydrodistillation viz., water distillation, water and steam distillation and direct steam distillation (Vankar, 2004). In hydrodistillation, the plant material is heated, either by placing it in boiling water or by passing steam through it. The heat and steam cause the cell structure of the plant material to burst and break down, thus releasing the bioactive compound and essential oils. Indirect cooling by water condenses the vapor mixture of water and oil. Condensed
mixture flows from condenser to a separator, where oil and bioactive compounds separate automatically from the water (Silva et al., 2005). Hydrodiffusion, hydrolysis and decomposition by heat are three main physicochemical processes in hydrodistillation process. Extraction using high temperature affects recovery of volatile components; this drawback limits its use for extraction of thermo-labile compound.

2.6.1.4. Solvent system for extraction of phenolics:

Extraction efficiency of any conventional method mainly depends on the choice of solvents to be used. The polarity of the targeted compound is the most important factor for choosing the solvent. Structural affinity between solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and cost of solvent are also important in selection of solvent for the extraction of bioactive compounds. Some examples of phenolic compounds extracted using different solvents are given in Table 2.4. The phenolics are usually extracted with organic solvents which include two phase solvent extraction systems. Commonly used solvents are methanol, ethanol, acetone, ethyl acetate and diethyl ether (Del Pozo-Insfran et al., 2006; Gutierrez-Uribe et al., 2010). However, polar phenolic acids, of benzoic and cinnamic derrivatives need mixtures of alcohol–water or acetone–water for better extraction (Stalikas, 2007). Moreover, acidified or chilled solvents are commonly used (Rochin-Medina et al., 2012; Singh-Gujral et al., 2012).

The extractibility of phenolic compounds in whole oats using 80% methanol was found to be substantially higher than water (Zieliński and Kozłowska, 2000). In a comparison of extraction of phenolic antioxidants from wheat bran with 70% methanol or ethanol, absolute ethanol and 50% acetone, it was found that 50% acetone was most effective (Zhou and Yu, 2004). Both 80% methanol and ethanol was found to be efficient at extracting phenolic compounds from barley (Bonoli et al., 2004a; Madhujith et al., 2006).
Bonoli et al. (2004b) extracted free phenolic compounds from barley flour by simple acetone-based solid–liquid extraction method which led to higher extraction yields of flavan-3-ols and proanthocyanidins which was nearly twice than that of other samples. The use of alcohol-based methods (aqueous ethanol or methanol) produced a higher recovery index for catechins and hydrolysable tannins. The prolonged alkaline hydrolysis resulted in extraction of higher amounts of hydroxycinnamic acids and flavonols. Pressurized liquid extractions did not produce a satisfactory recovery of the free phenolic compounds in barley.

Rao and Muralikrishna (2004) isolated non-starch polysaccharide–phenolic acid complexes from native and germinated cereals, including rice, maize, wheat and millet (ragi) designated as water extractable (WEPs) and water-unextractable (WUPs) non-starch polysaccharides having yield of 0.60–3.56% and 7.49–37.8%, respectively. Ferulic and coumaric acids were the main bound phenolic acids predominantly found (90%) to WUPs. The content of ferulic acid is several-fold higher than that of coumaric acid and their contents decreased upon germination.

### 2.6.1.5. Acid and alkali hydrolysis:

Generally, the acidic hydrolysis treatment used to hydrolyze polysaccharides for chemical analysis of carbohydrates, which disrupts the cell wall structure and may allow the release of phenolics bound to cell wall constituents mainly polysaccharides and protein. In the extraction of phenolics, an alkali or weak acidic hydrolysis treatment of cereals may allow a complete hydrolysis of phenolics esterified or bound to soluble carbohydrates, protein and other constituents to release free phenolics. However, these treatments may obtain a low and partial release of insoluble or non-extractable phenolics (Adom and Liu 2002).
Increased extraction efficiency were found for phenolic extraction from wheat flour and bran using successive acidified methanol/water (50:50, v/v, pH 2) and acetone/water (70:30, v/v) than 70:30 (v/v) of either ethanol:water or methanol:water (Pérez-Jiménez and Sauro-Calixto, 2005).

Chethan and Malleshi (2007) reported the phenolics contents of finger millet which are concentrated in the seed coat and the acidic methanol is an effective solvent for the extraction of millet phenolics. The phenolics of the millet are heat-stable but pH-sensitive and are largely unstable under alkaline conditions.

Arranz and Saura-Calixto (2010) observed the use of enhanced sulfuric acid hydrolysis produced a high yield of phenolics that were trapped within cores or bound to cell wall constituents (dietary fibre, proteins) and found hydrolysable phenolics (p-hydroxybenzoic, caffeic, cinamnic, ferulic and protocatechuic acids) content increased up to 9 folds for wheat bran and 2 folds in the case of wheat flour compared to the content in methanol/acetone extracts.

Solid substrate fermentation using microorganisms such as Saccharomyces cerevisiae etc. could be a promising technology to enhance the production and extraction of phenolic compounds for the design of different functional foods and for the specific use as nutraceuticals (Dey and Kuhad, 2014).

Wang et al. (2015) reported the bound phenolic compounds in rice bran were released while extracted with ethyl acetate based on alkaline digestion. Their investigation led to the isolation of a new compound, p-hydroxy methyl benzoate glucoside, along with other nine known compounds, cycloeucalenol cis-ferulate, cycloeucalenol trans-ferulate, trans-ferulic acid, trans-ferulic acid methyl ester, cis-ferulic acid, cis-ferulic acid methyl ester, methyl caffeate, vanillicaldehyde and p-hydroxy benzoaldehyde.
Table 2.4: Extraction of phenolic compounds using different solvent system

<table>
<thead>
<tr>
<th>Source</th>
<th>Solvent system</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats</td>
<td>80% methanol and water</td>
<td>Zieliński and Kozłowska, (2000)</td>
</tr>
<tr>
<td>Wheat</td>
<td>70% methanol or ethanol, absolute ethanol and 50% acetone</td>
<td>Zhou and Yu (2004)</td>
</tr>
<tr>
<td>Barley</td>
<td>80% methanol or ethanol</td>
<td>Bonoli et al. (2004a), Madhujith et al. (2006)</td>
</tr>
<tr>
<td>Wheat</td>
<td>0–100% (v/v; water/ethanol, methanol or acetone)</td>
<td>Liyana-Pathirana and Shahidi (2005)</td>
</tr>
<tr>
<td>Finger millet</td>
<td>1% HCl–methanol</td>
<td>Chethan and Malleshi (2007)</td>
</tr>
<tr>
<td>Wheat</td>
<td>Acid hydrolysis</td>
<td>Hartzfeld et al. (2002)</td>
</tr>
<tr>
<td>Wheat</td>
<td>Methanol/H$_2$SO$_4$ 90:10 (v/v) at 85 °C for 20 h.</td>
<td>Arranz and Calixto (2010)</td>
</tr>
<tr>
<td>Wheat</td>
<td>Alkali hydrolysis (2 M NaOH for 4 h) shaking under nitrogen gas.</td>
<td>Adom and Liu (2002); Pellegrini et al. (2006).</td>
</tr>
<tr>
<td>Wheat</td>
<td>Solvent composition (water, methanol, 70% methanol, ethanol, 70% ethanol, acetone and 70% acetone), extraction temperature (30–60 °C), extraction time (15–90 min) and solid-to-solvent ratio (1:2.5 to 1:20, w/v)</td>
<td>Dey and Kuhad (2014)</td>
</tr>
<tr>
<td>Barley</td>
<td>acidified methanol (HCl/methanol/water, 1:80:10, v/v/v)</td>
<td>Lahouaret et al. (2014)</td>
</tr>
<tr>
<td>Rice</td>
<td>Alkaline hydrolysis, extraction with 5 times ethyl acetate</td>
<td>Wang et al. (2015)</td>
</tr>
</tbody>
</table>
2.6.2. Non-Conventional or modern extraction techniques:

Disadvantages of conventional extraction techniques *viz.* increased consumption of energy and harmful organic solvents; longer extraction time; low extraction selectivity and thermal decomposition of thermo-labile compounds have forced the food and chemical industries to find new extraction techniques which typically use lesser solvent and energy. These techniques are considered as non-conventional extraction techniques such as ultrasound extraction, microwave assisted extraction, enzyme assisted extraction etc. (Azmir *et al.*, 2013). Extraction and separation under extreme or non-classical conditions is currently a dynamically developing area in applied research and in industry.

Number of novel extraction techniques has been developed for phenolicss from plants to shorten the extraction time, minimum solvent consumption, enhance extraction yield and quality.

2.6.2.1. Ultrasound-assisted extraction (UAE):

Ultrasonic radiation is a special type of sound wave beyond upper limit of human detection, which has frequencies higher than 20 kHz (20 kHz to 100 MHz). The main benefit of UAE can be observed in plant samples because ultrasound energy facilitates leaching of organic and inorganic compounds from plant matrix facilitating the extraction of organic and inorganic compounds from solid matrices using liquid solvents (Herrera and Luque de Castro, 2005). Ultrasonic baths or closed extractors fitted with an ultrasonic horn transducer are two general designs used for UAE of phenolics. Probable mechanism of this technique is that the ultrasound induces a greater penetration of solvent into cellular materials and improves mass transfer. It also disrupts biological cell wall and facilitates the release of the contents. Hence, the enhancement of extraction with ultrasonic power depends on efficient cell disruption and effective diffusion across the cell wall. Other important factor for efficient extraction
includes moisture content, particle size of sample, temperature, pressure, frequency and time of sonication.

Wang et al. (2008) extracted the phenolic compounds from wheat bran by UAE. The solvent concentration, extraction temperature and extraction time parameters were well considered for maximum extraction of phenolic compounds. It was found that extraction with 64% ethanol at 60 °C for 25 min yielded maximum phenolics. Rodrigues et al. (2008) observed maximum extraction of phenolic compounds (22.44 mg/g) by ultrasound using a 50% (v/v) ethanol:water solution and 15 min of sonication at ultrasound intensity of 4870 W/m². The UAE of mustard seed varieties produced higher recoveries of total phenolic content in comparison with the conventional solid–liquid extraction (Szydlowska-Czerniak et al., 2015).

2.6.2.2. Microwave-assisted extraction (MAE):

The MAE appears to be one of the novel methods for extracting soluble phenolics from a wide range of materials using microwave energy (Paré et al., 1994). Microwaves are made up of two oscillating fields that are perpendicular such as electric field and magnetic fields and so called electromagnetic fields and are in the frequency range from 300 MHz to 300 GHz. In MAE system, electromagnetic energy is rapidly converted to heat. Different chemical substances have different capacity to absorb microwaves which makes MAE an efficient method for extractions of selectively target compounds from complex food matrices (Barbero et al., 2006; Hemwimon et al., 2007, Liazid et al., 2011). The efficiency of the MAE process depends on extraction time, extraction temperature, solid–liquid ratio and the type and composition of solvent used (Pizarro et al., 2006; Rostagno et al., 2007; Song et al., 2011). Rapid heating for the extraction of bioactive substances from food matrices; reduced thermal gradients; reduced equipment size and better recovery than conventional extraction.
processes are some of the advantages of MAE system (Cravottoa et al., 2008).

Mandal et al. (2007) explained the phenomenon of increase in total phenolic content using MAE, after exposure of plant cells to microwave heating. Plant material absorbs microwave energy and subsequently converts into heat and the moisture begins to evaporate. This process of water vaporization generates pressure within the cell wall that eventually leads to cell rupture, thus facilitating the leaching out of active constituents into the surrounding solvent and improves extraction yield.

Extraction using solvent viz. methanol, acetone, and hexane for both conventional and microwave-assisted methods were studied for total phenolic and tocopherol contents and free radical scavenging capability of wheat bran (Oufnac et al., 2007). In comparison to conventional method, microwave-assisted solvent extraction using methanol significantly increases the total phenolic content from 241.3 to 467.5 μg of catechin equivalent; total tocopherol content from 15.8 to 19.5 μg/g of bran at extraction temperatures of 100 and 120°C, respectively.

The MAE method has been applied for the first time to the extraction of the phenolic compounds from rice grain. The effects of microwave power, temperature, extraction time and solvent treatment were investigated. The extraction variables were optimized by the response surface methodology. It was shown that at 185 °C extraction temperature, 1000 W microwave power, 20 min of extraction time and 10:1 solvent-to-sample ratio, resulted higher yield of 15 phenolic compounds. The results demonstrated that MAE was more effective in terms of both yield and time consumption. MAE to any number of materials can significantly reduce extraction time compared to conventional extraction methods. The MAE method is capable of extracting nearly 30% more phenolic compounds from
peanut skins in $1/12^{\text{th}}$ of the time required for SLE. (Kerem et al., 2005; Martino et al., 2006).

Phenolic compound extraction were carried from oat bran concentrate by Stevenson et al. (2008) using integrated treatment of supercritical carbon dioxide, then microwave-irradiation at 50, 100 or 150 °C for 10 min in water, 50% or 100% ethanol. Defatted oat bran concentrate in 50% ethanol and microwave-irradiation at 150 °C extracted higher phenolic content than any other combination.

Moreira et al. (2012) reported a novel application of MAE on phenolics from brewer’s spent grains and extraction yield of ferulic acid was investigated through response surface methodology. At optimal conditions (15 min extraction time, 100 °C extraction temperature, 20 mL of solvent, and maximum stirring speed), the yield of ferulic acid was $1.31 \pm 0.04\%$ (w/w), which was 5 fold higher than that obtained with conventional solid–liquid extraction techniques.

Chiremba et al. (2012) performed the extraction of bound phenolic acids from sorghum and maize bran and endosperm with MAE (45 s, and 1400 W) in 2M sodium hydroxide with the aim of releasing ferulic and coumaric acids at 190°C and found that this temperature is sufficient to break ether bonds which are labile at 170 °C.

Ndolo and Beta (2014), extracted phenolic acid from yellow maize, wheat, barley and oats fraction as well as whole grains using microwave-assisted alkaline aqueous extraction.

During extraction, particle size of the plant matrices plays important role. Smaller the size, larger will be the surface area to get exposed to the solvent resulting in increased extraction yield. In a study conducted by Povilaitis et al. (2015), rye and wheat brans were ground to different particle size fractions and extracted at 10.3 MPa pressure and 80 °C temperature by consecutive application of hexane,
acetone and methanol:water (80:20%). The highest extract yield was obtained from rye bran using methanol-water. In most cases, particle size had a significant effect, as smaller particle size gives higher extraction yield.

2.6.2.3. Enzyme-assisted extraction (EAE):

EAE of bioactive compounds from plants matrices is a potential alternative to conventional solvent based extraction methods. These methods are gaining more attention because of the need for eco-friendly extraction technologies. Some phytochemicals in the plant matrices are dispersed in cell cytoplasm and some compounds are retained in the polysaccharide-lignin network by hydrogen or hydrophobic bonding, which are not accessible with a solvent in a routine extraction process. Enzymatic pre-treatment has been considered as a novel and an effective way to release bound compounds and increase overall yield by breaking the cell wall and hydrolyzing the structural polysaccharides and lipid bodies (Rosenthal et al., 1996; Singh et al., 1999). Application of enzymes in extraction improves the effect of solvent pre-treatment by increasing the yield of extractable compounds and reducing the amount of solvent needed for extraction. Various factors including enzyme composition and concentration, particle size of plant materials, solid to water ratio and hydrolysis time are recognized as key factors for extraction (Niranjan and Hanmoungjai, 2004).

Recent studies on enzyme assisted extraction have shown faster extraction, higher recovery, reduced solvent usage and lower energy consumption when compared to non-enzymatic methods. Various enzymes such as pectinases, cellulases and hemicellulases are widely used in alcoholic beverage and juice processing industries for clarification to degrade cell walls and improve juice extractability. During processing the disruption of the cell wall matrix also releases components such as phenolic compounds into the juice, thus
improving product quality. Enzyme-assisted extraction methods have been shown to achieve high extraction yields for compounds including polysaccharides, oils, natural pigments, flavours and medicinal compounds (Barzana et al., 2002; Passos et al., 2009; Sowbhagya and Chitra, 2010).

2.7. Importance of phenolics in nutrition:

The richest source of antioxidants in our diet is phenolics (Manach et al., 2004). Phenolics also have other specific biological activities which include cell signalling (Wheeler et al., 2004), cell adhesion (Williams et al., 2004) and regulating gene expression (Yuan et al., 2005). The biological properties of phenolics in the prevention of age-related diseases such as cardiovascular disease and cancer are gaining interest among food technologists (Williamson and Manach, 2005). Among phenolics, phenolic acids are mainly responsible for increasing bile secretion, reducing blood cholesterol and lipid levels whereas flavonoids are particularly important for antioxidant activity (Saxena et al., 2013).

The phenolic compounds associated with whole grains are mostly present as insoluble bound forms (Acosta-Estrada et al., 2014). About 85%, 75%, and 62% of the total phenolics are present as the insoluble bound forms present in maize, wheat and rice, respectively (Adom and Liu, 2002). Brown rice contains around 88% of bound phenolics (Zhou et al., 2004) and barley might contain between 55% and 90% bound phenolics depending on variety (Abdel-Aal et al., 2012).

The insoluble phenolics are covalently bound to cell wall structural components for instance cellulose, hemicellulose, lignin, pectin and rod-shaped structural proteins (Fig. 2.2). The phenolics play an important role in providing both physical and chemical barriers against pathogen invasion and astringency that prevents attack by insects and animals (Sancho et al., 2001; Liu, 2007).
Phenolic acids through their hydroxyl groups in the aromatic ring form ether linkages with lignin and through their carboxylic group form ester linkages with structural carbohydrates and proteins (Liyana-Pathirana and Shahidi, 2006; Liu, 2007; Bhanja et al., 2009).

![Diagram of primary cell wall structure and cross linking](image)

**Fig. 2.2:** Representation of primary cell wall structure of plant material and cross linking between structural components and phenolic compounds. (A) Cellulose, (B) Hemicellulose, (C) Structural protein, (D) Pectin, (E) Phenolic acids, (F) Lignin. (Source: Acosta-Estrada et al., 2014).

The bound phenolics are absorbed in the gastrointestinal tract through various pathways which involve microorganisms, enzymes and glucose transporters (Fig. 2.3). Partial release of bound phenolics takes place within the gastrointestinal lumen.

Kroon et al. (1997) showed that the existing microflora of colon releases esterase and xylanase enzymes which cause the release of feruloyl groups from wheat fibre. According to their report over 95% of the total release of feruloyl groups takes place during fermentation in the colon. The gastric and small intestinal treatments release only 3% of ferulic acid. As the cell wall fibrous materials are difficult to digest the insoluble bound phenolics survive stomach and intestinal digestion and reach the colon (Adom and Liu, 2002). Selma et al. (2009) reported the colon microflora comprising *Clostridium* spp., and *Eubacterium* spp., degrade flavonols to simpler phenolic compounds.

<table>
<thead>
<tr>
<th>Link</th>
<th>Cell wall structural component</th>
<th>Functional group of phenolics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ether</td>
<td>Lignin</td>
<td>-OH group in the aromatic ring</td>
</tr>
<tr>
<td>Ester</td>
<td>Carbohydrates, proteins</td>
<td>-COOH group</td>
</tr>
</tbody>
</table>
Liu (2007) proposed that the health benefits of bound whole grain phenolic phytochemicals are more effective in the colon. These studies as well as the epidemiological studies partly explain the mechanism of grain consumption in the prevention of colon and other digestive cancers (Adom and Liu, 2002). Chitindingu et al. (2015) revealed that the bio-accessibility of phenolic compounds of certain cereal grains is higher in large intestine than the small intestine. The lower bio-accessibility in the small intestines attributed to the incapability of human digestive enzymes to digest many food constituents to which phenolic compounds were conjugated. In large intestine, colonic fermentation releases significant amount of condensed tannins hence they are bioaccessible.

![Absorption pathways of bound phenolic compounds in the gastrointestinal tract.](image)

**Fig. 2.3:** Absorption pathways of bound phenolic compounds in the gastrointestinal tract. (A) Hydrolysis of bound soluble conjugated forms by mucosa cells cinnamoyl esterases. (B) Soluble conjugated forms transport into enterocytes by the sodium-dependent glucose transporter SGLT1. (C) Lactase phloridzine hydrolase (LPH) (β-glucosidase) of the brush border hydrolysis soluble conjugated phenolic compounds. (D) Epithelial cells cytosolic β-glucosidase hydrolyzes glycosides and aglycones are formed after absorption. (E) Esterase and xylanase activities of colon microorganism (e.g. Clostridium spp., Eubacterium spp and Bifidobacterium adolescentis). (Source: Acosta-Estrada et al., 2014).
There are different mechanisms of release and absorption of phenolic compounds in the small intestine. Andreasen et al. (2001b) proposed that the major path for release and in vivo absorption of hydroxycinnamic acid is the hydrolysis by intestinal esterases. The activity is mainly located in the mucosa epithelial cells of small intestine.

Lactase phloridzin hydrolase, a β-glycosidase found on the brush border of mammalian small intestine capable of hydrolyzing various flavonol and isoflavone glycosides (Day et al., 2000). Certain phenolics can also be transported through the brush border by sugar transporters as glycosides. Glycosides could be transported into enterocytes by the sodium-dependent glucose transporter SGLT1 (Manach et al., 2004). The glycosides and aglycones which are formed after absorption, are hydrolysed by β-glucosidase in the epithelial cells (Selma et al., 2009).

Through the dietary intake the phenolic compounds are absorbed in the stomach and small intestine and then distributed throughout the body. These compounds provide health benefits such as inhibition activities against oxidation of LDL cholesterol and liposomes (Chandrasekara and Shahidi, 2011b).

After ingestion and absorption, phenolic acids are conjugated by methylation, sulfation and glucuronidation reactions through specific enzymes (Fig. 2.4). In methylation reaction Catechol-O-methyltransferase catalyses the transfer of a methyl group from S-adenosyl-L-methionine to phenolics that have an o-diphenolic moiety. This enzyme is most active in liver and kidney.
Sulfation reaction catalyse by the sulfotransferases enzyme which transfer the sulphate moiety from 30-phosphoadenosine-50-phosphosulfate to a hydroxyl group on the phenolics. This conjugation reaction occurs in the liver. The membrane-bound enzymes UDP-glucuronosyltransferases located in the endoplasmic reticulum in many tissues catalyse the glucuronidation reaction. In glucuronidation reaction glucuronic acid transfers from UDP-glucuronic acid to phenolics (Heleno et al., 2015). The presence of glucuronidated metabolites in the mesenteric or portal blood after perfusion of phenolics in the small intestine of rats shows that glucuronidation of phenolics first occurs in the enterocytes before conjugation in the liver (Crespy et al., 2001).

2.8. Phenolic compounds from maize and antioxidant capacity:
Maize is a major cereal used as staple food in various parts of the world after rice and wheat. The maize kernels can be of different colours such as white, yellow, red and black. Depending on the
chemical compounds deposited or stored in the kernel, maize grain colour differs. Although white and yellow maize is biologically and genetically alike, there is a difference in appearance owing to the absence of carotene oil pigments in the kernel. The presence of carotene causes the yellow colour of the grain.

Phenolic compounds, vitamins and carotenoids act as natural antioxidants and are the effective nutrients in the prevention of these oxidative stress related diseases. ROS are chemically reactive molecules containing oxygen in form of singlet oxygen, superoxide anion radicals, peroxide anions or hydroxyl radicals and responsible for many diseases such as cancer, Alzheimer disease, cardiovascular disease, kidney disease etc. ROS can damage vital cellular components including lipids, proteins, amino acids and DNA (Thannickal and Fanburg, 2000).

Adom and Liu (2002) reported that the total phenolic compound is much higher in maize than wheat, rice and oats. The bound phenolic was also found higher in maize than the other cereals. The phenolic and ferulic acid content was highly correlated with total antioxidant capacity of bound extracts whereas a low correlation between parameters measured for free extracts.

Li et al. (2007) stated that the phenolic contents of the mutant maize genotypes were higher than that of the typical corn. The total antioxidant activity of maize was found to be the highest than the other grains analysed which include wheat, oat and rice. Free phenolic compounds were attributed to the total antioxidant activity, while bound phytochemicals were accountable for the free radical scavenging capacity.

Pedreschi and Cisneros-Zevallos (2007) analysed the phenolic compounds of Andean purple maize from the water and ethyl acetate fractions. The water fraction was found to be high in anthocyanins including cyanidin-3-glucoside, pelargonidin-3-glucoside and
peonidin-3-glucoside. The other fraction that is ethyl acetate, was rich in phenolic acids such as \textit{p}-coumaric, vanillic acid, protocatechuic acid and flavonoids such quercetin derivatives and a hesperitin derivative.

Maize is the richer in starch and protein contents compared to other major crops such as rice and wheat. It also contains a high amount of carotenoids, tocopherols, and oils (Chander \textit{et al}., 2008). HPLC analysis of 87 elite maize inbreds, a major heterotic group in China revealed the presence of high carotene (\textit{\beta}-carotene, \textit{\beta}-cryptoxanthin, \textit{\alpha}-carotene, lutein and zeaxanthin) and tocopherol (\textit{\alpha}-tocopherol, \textit{\gamma}-tocopherol, \textit{\delta}-tocopherol) level. All the traits also showed high level heritability (Chander \textit{et al}., 2008). Although maize is a good source of sugar, protein and phytochemicals, during maturation of grains, the content of reducing sugar and crude protein decrease and the starch and total lipids increase. The total carotenoid content behaves differently. In the first stage of development it remains in decreased condition then with maturation it increases and again decreases to minimum at fully mature stage. The phenolic and lutein content decreased during maturation while \textit{\beta}-cryptoxanthin level increased (Xu \textit{et al}., 2010).

In another study Lopez-Martinez \textit{et al}. (2009) showed that in Mexican maize, the phenolics are present mainly in bound form. The free phenolics mainly comprise of anthocyanins. Their study also revealed that the orange maize contained the highest amount of total ferulic acid (1.62 mg/g of whole flour grain) and the white maize contained the least amount (1.39 mg/g).

In the study of phytochemical analysis of coloured maize genotypes Zilic \textit{et al}. (2012) showed that the antioxidant capacity of maize kernel is dependent on the concentration of phenolic compounds. Several parameters had been used to study the phytochemistry including total phenolics, flavonoids, anthocyanins, \textit{\beta}-
carotene, and lutein. Free, conjugated and insoluble bound phenolic acids were also analysed. The light blue maize showed highest ABTS [2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging capacity which was in accordance with high total phenolic, flavonoid and ferulic acid content.

Ndolo et al. (2013) reported the presence of highest ferulic acid content in bran and aleurone fraction of yellow maize than wheat and barley varieties. Fluorescence intensity profiles and fluorescence microscopy showed that ferulic acid distribution varied across the grains and fluorescence intensity values were highest in the outer layers and lowest in the endosperm.

2.9. Processing of maize and its effect on phenolic compounds:

Maize is consumed as whole grain or processed to various products viz. corn meal, grits, starch, flour, snacks, tortillas, tortilla chips and breakfast cereals etc. Different processing methods are used to prepare these products. Different processing technique of cereals includes decortication or de-hulling or milling, thermal processing, malting, fermentation, nixtamalization etc. Thermal processing and nixtamalization techniques are mainly used for maize grain processing.

Thermal processing is the most common method of food preservation and processing. This also has impact on quality aspects for instance, sensory, nutritive and phytochemical quality (Duodu, 2011). Heat processing can be done by cooking, roasting, microwave heating or extrusion cooking. Studies showed that the different thermal processing of the maize grains can increase or decrease the phenolic content, antioxidant capacity and change the phytochemical profile.

Thermal processing by autoclaving increases the phenolic content and DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical
scavenging capacity of maize (Kwon et al., 2007). Randhir et al. (2008) also reported similar finding on maize sprouts and seedlings. Thermal processing of grains releases the bound phenolics upon breakdown of cellular components and cell walls. This process is known as phenolic aglycosylation. This phenolic aglycosylation could be the probable cause of increasing antioxidant activity (Hopia and Heinonen, 1999). Oboh et al. (2010) showed that the ferric reducing antioxidant capacity of yellow and white maize increased upon roasting although the extractible phenolic and flavonoid content reduced significantly. Micronisation, a high temperature short time (HTST), process sometime used to increase storage stability of whole grain flour also affects the bioactive compounds. Although the antioxidant activity of the grains increased upon micronisation, the procedure incurred a negative effect on tocopherols and β-carotene content of the grain (Zilic et al., 2010). The infrared heating for short time period also reduced the phenolic content but increased the antioxidant capacity. The antioxidant capacity increased upon high temperature treatment due to the Maillard reaction (Zilic et al., 2013).

In a recent study micro-fluidization process was used to increase the antioxidant capacity of maize bran (Wang et al., 2014a). Alkali hydrolysis followed by micro-fluidization increased the release of ferulic and p-coumaric acids, which in turn resulted in higher antioxidant capacity. The greater release of ferulic and p-coumaric acids are due to the reduction in particle size and loosening of the tight microstructure of the maize bran due to micro-fluidization.

Maize tortillas are salted snack of Mexico and Central America. Tortillas are produced by nixtamalization. Nixtamalization is a thermal alkaline treatment (Serna-Saldivar et al., 1990). During this procedure the maize kernels cooked in a lime or calcium hydroxide solution and soaked overnight. The cooked maize or the nixtamal is washed and ground into dough. The dough so obtained is called ‘masa’. Masa’ is further processed to tortillas (Serna-Saldivar et al., 1990). This
process affects the physico-chemical, nutritional and sensory properties of the maize kernels and consequently, the food products. During nixtamalization various changes can occur to the grains such as removal of pericarp, incorporation of calcium into the kernels, improvement of bioavailability of niacin and formation of flavour and colour compounds which can influence the sensory quality (Duodu, 2011). During nixtamalization the fiber components of the maize grain such as hemicelluloses and lignin are hydrolyzed. The phenolics which are generally bound to these fiber components of the cell wall (Gonzalez et al., 2004; De La Parra et al., 2007) are released into the alkaline solution and leached out. This phenolic rich alkaline solution is known as ‘nejayote’. The ‘nejayote’ is considered to be even richer in phenolics than the original maize kernels and ‘masa’ (Gutiérrez-Uribe et al., 2010). Hence, a significant loss in phenolic antioxidants occurs during the whole procedure of nixtamalization (Del Pozo-Insfran et al., 2007). Anthocyanin also degrades due to instability in alkaline condition during this procedure (Fossen et al., 1998; Del Pozo-Insfran et al., 2007; De La Parra et al., 2007; Mora-Rochin et al., 2010). Though nixtamalization reduces the total phenolic content, studies revealed that lime cooking increases the free ferulic acid content of the grains (Del Pozo-Insfran et al., 2007; Mora-Rochin et al., 2010).

In order to overcome the phenolic loss Carrera et al. (2012) used ecological nixtamalization process instead of traditional process for the production of tortillas. Calcium salts (calcium chloride, calcium carbonate, calcium sulphate and calcium acetate) were used in ecological nixtamalization in place of lime or calcium hydroxide of traditional process. Their result showed that the dry matter loss of ‘nejayote’ was less in ecological process (1.2% to 1.4%) than the traditional process (3.2%). The loss of total dietary fibre and soluble fibre also found to be considerably less in ecological process. In a similar study Rodríguez-Mendez et al. (2013) reported that ecological nixtamalization maintains the acidic or neutral medium during the
process thus conserves a higher proportion of the pericarp which in turn decreased the release of ferulic acid into the ‘nejayote’ and also inhibit the anthocyanin degradation.

Aguayo-Rojas et al. (2012) showed that the tortillas produced from lime-cooking extrusion technique retained more phenolic compounds. The effect of nixtamalization process on phenolic compounds of different maize products is presented in Table 2.5.

**Table 2.5:** The effect of nixtamalization process on the phenolic compounds of maize products

<table>
<thead>
<tr>
<th>Type of maize</th>
<th>Effect on phenolic compound</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>White and blue maize</td>
<td>Total phenolic content, anthocyanin content and antioxidant capacity decreased after nixtamalization and further on ‘masa’ and ‘tortilla’ production. Increase in free ferulic acid content after lime cooking.</td>
<td>Del Pozo-Insfran <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>White, yellow, blue and red maize</td>
<td>The total phenolics and anthocyanin content decreased after nixtamalization. The free and soluble-conjugated ferulic acid increased while bound ferulic acid content decreased. As a result hydrophilic and lipophilic antioxidant capacity of free phenolics increased whereas that of bound phenolics decreased.</td>
<td>De La Parra <em>et al.</em>, 2007</td>
</tr>
<tr>
<td>White, yellow, blue and red maize</td>
<td>Reduction of the total phenolic content of tortillas upon nixtamalization process. Nixtamalized white maize tortillas showed lowest retention of phenolic compounds. Nixtamalized flours showed higher free ferulic acid content in prepared tortillas in comparison to extruded flours tortillas. A reduction in anthocyanin levels was found on blue maize tortillas upon nixtamalization.</td>
<td>Mora-Rochin <em>et al.</em>, 2010</td>
</tr>
</tbody>
</table>
Table 2.5 continued.....

<table>
<thead>
<tr>
<th>Maize Type</th>
<th>Process Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>White, blue, red and purple maize</td>
<td>The nixtamalization process reduced total phenolics, anthocyanins and antioxidant capacities. The quinone reductase induction ability was also reduced with respect to raw grain. Processing of masa into tortillas had negative effect on total phenolic, anthocyanin and antioxidant capacities. The quinone reductase induction capacity was also reduced in the coloured maize varieties.</td>
<td>López-Martínez et al., 2011</td>
</tr>
<tr>
<td>Blue maize</td>
<td>In nixtamalization process two types of calcium sources, calcium hydroxide [Ca(OH)(_2)] and calcium lactate (C(<em>6)H(</em>{10})O(_6)Ca) were used. Cyanidin-3-glucoside concentration was increased by nixtamalization using both calcium sources, while pelargonidin-3-glucoside concentration decreased.</td>
<td>Sánchez-Madrigal et al., 2014</td>
</tr>
<tr>
<td>Blue maize</td>
<td>Calcium-salt nixtamalization in blue tortilla presented a higher ferric reducing antioxidant capacity than traditional tortilla. The dietary fibre content also increased after calcium-salt nixtamalization.</td>
<td>Bello-Pérez et al., 2015</td>
</tr>
</tbody>
</table>

The studies showed that the whole grain maize was mainly used for the preparation of tortillas and has not been utilized for the development of convenience foods such as pasta, noodles etc. Although maize starch mixed at certain percentage with wheat flour was used to prepare noodles (Yousif et al., 2012), however, to the best of our knowledge, there are no reports available on development of whole grain maize noodle.
2.10. **Noodles from other cereal grains:**

Noodles are the staple foods of several Asian countries since long. It is prepared in different formulations and shapes in different places. Noodles can be made from wheat, rice, buckwheat, and starches derived from potato, sweet potato, and pulses (Fu, 2008). The origin of noodle is a debatable issue between Chinese, Italian and Arab countries. According to early Chinese literature, hand-made noodle was already well developed technology during the Yuan dynasty (1279–1368 AD). During that period people used to prepare noodles with different shape, size and taste. Very thin hand-stretched noodles are also the creation of that era. Many noodles available in today’s market may be evolved from the products developed at that period (Miskelly, 1993). The Japanese regular salted noodles were developed from the modified noodle processing techniques of the Chinese hand-made noodle (Nagao, 1996). Most noodles prepared in present time are machine made, though manufacturing process differs among countries. The modern noodle preparation technique originates from the Chinese hand-made noodles (Fu, 2008).

The basic procedure of noodle making through machine includes dough mixing, sheet forming, compounding, sheeting/reduction and cutting. Noodle strands after cutting are further processed to produce different types of noodles namely fresh, dried, steamed, boiled, frozen-boiled, sterilized-boiled, steamed and deep-fried noodles, steamed and hot air-dried noodles etc. (Fu, 2008).

The demand of gluten free products is increasing because of their beneficial health aspects such as low glycemic index for diabetic patients, reduced risks of celiac diseases and allergic reactions, as caused by eating wheat based products (Torbica et al., 2010).

Bhattacharya et al. (1999) showed noodles prepared with rice of different genotypes showed good texture, cooking loss and cooking quality. Rice based noodles are prepared from rice cultivars containing
high amylose, low gelatinization temperature and high gel consistency (Yoenyongbuddhagal and Noomhorm, 2002).

Hypoallergenic wheat flour, a special type of wheat flour in which gluten is partially hydrolyzed by enzymes is also used in preparation of noodles. Oishi et al. (2009) evaluated the physical properties of hypoallergenic wheat flour noodle prepared in Japanese manufacturing condition. They recommended that hypoallergenic wheat flour in combination with sodium alginate, starch and curdlan can be useful for preparing noodles for gluten allergic patients.

Hydrothermal treatments such as annealing and heat-moisture treatment of rice flour or starch increase the cooking and texture quality of noodles nearly equal to commercial noodles (Hormdok and Noomhorm, 2007; Cham and Suwannaporn, 2010) Rheological and cooking properties of the gluten-free noodles prepared with dry- and wet-milled rice flours were studied by Heo et al. (2013). Wet-milled rice flour showed greater swelling power and high elongational viscosity over dry-milled flour.

Although noodle products are usually prepared from fine wheat flour by a process of sheeting and cutting, mixture of cereal grains are also being used to prepare noodles now-a-days in order to improve its rheological, physico-chemical and nutritional qualities.

Pigeon pea starch blend with 30% rice starch can produce noodles with superior quality compared to native pigeon pea and rice starch noodles. The amylose content, solubility and freeze thaw stability of pigeon pea starch was found to be higher while cooking time and solid loss was lesser in rice starch. Hence the blend resulted in good quality noodles particularly in terms of higher transparency, slipperiness, overall acceptability and cohesiveness values (Yadav et al., 2011).
Incorporation of maize starch in wheat flour showed better results. Native or pregelatinized maize starch did not cause any negative effects on sensory attributes of noodles in comparison to the control sample (Yousif et al., 2012).

Ahmed et al. (2015) investigated the effect of blending broken rice flour with wheat flour at different percentage. Though 20% broken rice blended flour showed better structure and texture among the different blended flours, the study revealed that the native wheat flour noodles are of superior sensory qualities over the blended noodles.

Bharath Kumar and Prabhasankar (2015a) studied the influence of low glycemic index ingredient in fresh and dried noodles. They used pea flour as low glycemic index material and mixed at 20% and 40% level to the durum semolina flour to prepare noodles. They evaluated the physico-chemical, rheological and noodle making characteristics, *in-vitro* starch digestibility and microstructure of noodles. The texture of the pea-flour-incorporated noodles was found to be firmer than the control product which contained only durum semolina flour.

Incorporation of *rajma* bean (*Phaseolus vulgaris*) at 10%, 20% and 30% with the durum semolina (*Triticum durum*) flour to prepare noodle dough, resulted in decreased peak viscosity while the protein content, dietary fibre content and water absorption capacity increased (Bharath Kumar and Prabhasankar, 2015b).

### 2.11. Aim and scope of the present study:

Since the major cereal grains *viz.* rice and wheat have been studied in length for their health beneficial attributes, literature on maize is limited in these aspects. Maize, being the most high-yielding cereal among the coarse grains, needs closer examination due to their potential health benefit in the prevention of chronic diseases. A more complete analysis of the phytochemical content and antioxidant properties of a range of diverse whole maize samples is needed.
Emphasis on the distribution of free and bound phenolic content and the distinction between free and bound antioxidant properties are keys to understand the potential health benefits of whole maize consumption. In none of the reports on maize phytochemical research, the profile of accumulation of secondary metabolites in the botanical fractions of the grain was shown keeping in view the diversity of kernel structure of Dent and Flint maize. In addition India produces various specialty maize among which QPM, Baby corn, Popcorn and Sweet corn are being popularized and cultivated by a large number of farmers. These specialty maize are usually consumed as whole grain and hence, their health beneficial attributes ought to be known. However, data on detailed phytochemical profile of specialty maize is scarce more so, from this subcontinent.

Likewise every cereal, maize is processed to products after milling which results in removal of the outer layers of the grain (pericarp and germ) containing the key components such as micronutrients and phytochemicals. Hence, to retain the health-promoting properties of cereals (maize in particular) the bioactive rich botanical fractions of the kernel need to be incorporated. On the contrary, incorporation of fiber rich pericarp disrupts the protein-starch matrix within the product’s microstructure resulting in undesirable texture and palatability of the products. Additionally, maize lacks in gluten,a polamin protein which hydrates, swells and forms elastic dough, making it even more challenging to develop any product with desirable consistency. Hence, an approach towards developing maize based products needs closer investigation on employing traditional technology to overcome those challenges.

The present study is an attempt to augment the diversity of phytochemicals in Indian maize-crops, which in turn would bring knowledge to the Indian breeders for bio-fortification of the grain. Technologically, the study is an endeavour of formulating a consumer-
preferred functional food from whole-grain maize. The objectives of the study have been enumerated below:

1. Evaluation of phenolics, carotenoids and tocopherol of maize genotypes *viz.* high-yielding crops and specialty crops.

2. Characterization of phenolic acids and flavonoids of the suitable genotypes and their milling fractions.

3. Evaluation of antioxidant properties of the genotypes and their milling fractions.

4. Preparation of maize-noodle and determination of the processing effect on the phytochemical profile.