CHAPTER II

LITERATURE REVIEW

2.1   Cow's Milk

2.1.1   Composition and nutritional value of cow's milk

Milk and dairy products can form important components of a balanced diet because they provide a wide range of important nutrients. Cow's milk may contain about 3.3- 4.0% protein, 3.65- 4.35% fat, 82.55-88.0% moisture, 0.77-0.81% ash and 4.0-4.5% minerals (Yadav, Jain, Sinha, & Marrota, 2007). Among the minerals present in cow's milk, calcium and phosphorous are the most abundant (122.2 and 76.3mg/100g respectively). The carbohydrate content of cow's milk is exclusively lactose sugar. The cholesterol content is about 14 mg/100g and it does not contain any dietary fiber. Various processing methods were shown to retain a great portion of the nutrients including the vitamins (A, B, D, E, K) in the milk. Milk is also regularly fortified with vitamins (e.g. Vitamin D) during processing.

2.1.2   Supplementation of milk or milk products with polyphenols

Polyphenols in most fruits are recognized as the major class of phytochemicals with antioxidant activity (Seeram, Ahanng, Reed, Krueger, & Vaya, 2006). Addition of antioxidants particularly from natural sources into processed food is an emerging trend for the development of functional foods (Gad & Abd El-salam, 2010). Although maximum antioxidant capacity and hence better health benefit could be gained by ingesting milk proteins-phenols complex (Leenen, Roodenburg, Tijburg, & Wiseman, 2000) other studies reported reduced bioavailability of phenolic compounds after ingestion with milk (Lorenz et al., 2007; Serafini et al., 2009).

2.1.3   Milk polyphenols and antioxidant activity

Since many antioxidants can be found in milk and several reactions are possible the oxidative reactions in milk may be affected by a complex interplay of pro- and anti-oxidants Superoxide dismutase and catalyse are amongst common antioxidant enzymes demonstrated in milk (Ito & Akuzawa, 1983). Naturally occurring molecules in milk such as the iron-binding protein lactoferrin, vitamins C and E (tocopherols and tocotrienols) can also act as antioxidants. Some carotenoids have provitamin A action as well as antioxidant functions. In addition non-enzymatic antioxidants can also act as radical scavengers in the lipid (e.g. Vitamin E, carotenoids and ubiquinol) or water (e.g. Vitamin C) phases. Interestingly some flavonoids may operate both as radical scavengers and metal ion binders (Lindmark-Mansson & Akesson, 2000). Sonmez, Ertas, Okur, & Guzel-Seydim (2010) determined the antioxidant activity (AA) and TPC content of 6 different brands of UHT milk (plain and flavoured with chocolate or strawberry) using ABTS and F-C methods and found higher AA and TPC in flavoured milk samples. They reported a background AA of

4.31 ± 0.51 mm/ml TEAC and TPC of 1030.10 ± 19.30 mg/L GAE in plain UHT milk. These background polyphenols were determined to be a consequence of several factors, namely, the consumption of particular fodder by cattle, the catabolism of proteins by bacteria, contamination with sanitizing agents, process-induced incorporation or deliberate addition as specific flavoring or functional ingredients (O'Connell & Fox, 2001). Interestingly the consumption of polyphenol-rich foods by cattle can affect ruminant health (reduces the incidence of pasture bloat in the reticulorum - (Haslam, 1998)) and the yield and quality of milk (fat content and non-casein nitrogen content (Blauwiekel et al., 1997)). Therefore the specific polyphenols profile of milks from different ruminant species can play a significant role in the distinct sensory traits of these milks and their products. Taken together these reports established the ability of polyphenols to enhance functional properties of milk and dairy products (i.e., microbiological stability, oxidative stability and heat stability).

2.2 Yogurt

2.2.1 Definition and history of yogurt

Yogurt is defined as a coagulated milk product resulting from the fermentation of milk sugar lactose into lactic acid by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Adolfsson, Meydani, & Russell, 2004). Other lactic acid bacteria (LAB) may also be added to achieve special yogurt preparation. To be considered a healthy food the LAB used for yogurt making is expected to be alive and present in large amounts at the time of consumption.

The earliest yogurt was made by wild fermentation of milk by the Bulgars in the 2nd century where it remained primarily a food for the South, Central and Western Asia, South

Eastern Europe and Central Europe. The observation that frequent consumption of fermented milk containing *L. bulgaricus* increased health and longevity was only made about a hundred years ago by Metchnikoff, who claimed that the intake of yogurt decreases the toxic effect of the putrefactive bacteria in the colon by decreasing their growth (Metchnikoff, 1908; Wollowski, Rechkemmer, & Pool-Zobel, 2001). During milk fermentation, flavor substances can be produced by the lactic acid bacteria (Zourari, Accolas, & Desmazeaud, 1992), such as non-volatile acids, volatile acids, carbonyl compounds and other compounds (Serra, Trujillo, Guamis, & Ferragut, 2009).

2.2.2 Manufacture of yogurt

The manufacture of yogurt consists of the following six basic steps:

a) Filtration of milk for the removal of debris,

b) Checking the presence of antibiotics that may affect the activity of the starter bacteria,

c) Standardization of milk for making good quality yogurt,

Firstly, the milk fat in yogurt can vary from 0.5 to 4.5 % according to the market demand. Hence milk fat can be separated or fortified to the desired value for final product. Secondly, non-fat milk solid (NFMS) is raised to the desired value. The NFMS value in milk differs from 8.5 to 9% consisting of 4.5% lactose, 3.3% protein (2.6% casein and 0.7% whey protein) and 0.7% mineral salts. The NFMS is vital for good quality product. Protein and mineral content contributes to gel structure and lactose is the energy source for bacteria. However the NFMS value in liquid milk is not high enough for strong gel structure and it should be raised to 13-18% value by adding skimmed milk powder or by partial moisture evaporation under vacuum.

d) Homogenization of milk,

Homogenization is the size reduction of fat globules to 1-2μm. It prevents the segregation of fat during production and improves the incorporation of other ingredients such as skimmed milk powder. After the homogenization, the membrane of the fat globule is destroyed and lipase enzymes attack the destroyed globules. Hence the lipase enzymes should be deactivated by heat treating the milk immediately after homogenization to prevent lypolysis (Serra et al., 2009).

e) Heat treatment,

The processed milk is pasteurized at 90-95°C for 5-10 min by passing it through plate heat exchanger or at 80-85°C for 30 min in process vessel. Heat treatment destroys all the pathogenic bacteria, and inactivates enzymes such as lipase. It also denatures whey proteins, β-lactoglobulin and α-lactalbumin. This phenomenon is very important for gel strength, because the complex is formed between κ-casein and denatured β- lactoglobulin, which increases the hydrophilic nature of the casein. The complex between κ-casein and denatured β-lactoglobulin also decreases the syneresis and improves the stability of coagulum. Optimum temperature value is 85°C, since heat treatment at higher temperatures decreases the hydrophilicity of casein micelles (Sodini, Remeuf, & Haddad, 2004).

f) Inoculation and incubation,

After the heat treatment, the milk is cooled to 43°C prior to inoculation with the starter culture. The bacilli to cocci ratio in yogurt starter culture is 1:1 (Sokolinska & Pikul, 2004). The inoculation amount can vary between 0.5-5 percent (%) but the optimum value is 2 %. The starter cells are mixed with milk by stirring. Then milk is dispensed into containers and incubated at 42-43°C for 3-4h until the end of the incubation time which is industrially set at pH4.5.

g) Cooling and storage,

The first alternative to end incubation is cooling the product as quickly as possible at an acidity of 1.2-1.4% (pH 4.3) to avoid over acidification, shrinkage of the protein gel and whey syneresis on the surface. The second alternative for cooling is a controlled two stage cooling whereby the temperature is initially reduced to about 37°C at ~pH4. 6 followed by further cooling to ≤10°C and slowing of acidification to pH 4.3. During refrigerated storage at 4°C the viscosity of yogurt increases for 1-2 days. Hydration leads to much firmer gels and casein micelles continue to stabilize (Tamime & Robinson, 1999).

2.2.3   Factors affecting physical properties of fermented milks

Although the moisture content of fermented milks is similar to that of milk, they behave like solid materials. The structure of fermented milk is a gel consisting of a network of casein entrapping milk serum and fat. The major factors influencing milk gel characteristics are total solids, amount of fat, amount and type of protein, processing parameters (e.g. Heat treatment), pH, type and amount of starter culture used, addition of stabilizer, temperature of incubation and subsequent storage, and amount and type of exoplysaccharides (EPS). For instance, yogurt made from milk heated for 10 minutes at 90°C had higher viscosity and hardness than that made from UHT-treated milk (Mottar, Bassier, Joniau, & Baert, 1989). The gel strength of yogurt made from unheated milk was also shown the be lower than that made using heated milk (Tamime, Hassan, Farnworth, & Toba, 2007). Yogurt made with homogenized milk is firmer and shows less syneresis than that made using unhomogenized milk (Tamime et al., 2007). Various stabilizers such as gelatin, starch, α-carrageenan, locust bean gum and pectin are used in yogurt manufacture. In general, stabilizers strengthen and stabilize the casein network and increase the viscosity of the continuous phase thereby reducing syneresis and increasing viscosity of yogurt

(Everett & McLeod, 2005). Modifying milk composition and bacterial culture may also used to manipulate yogurt's physical properties as demonstrated by the improved body and texture and reduce syneresis by increasing milk solids for the former and higher storage modulus yogurts with less fermentation time (higher inoculation rate) for the latter (Lee & Lucey, 2004).

2.2.4   Types of yogurt

Yogurts in the market can be classified according to their chemical composition (full-fat, reduced-fat or low-fat yogurt), manufacturing method (set-type and stirred-type), flavoring and the type of post incubation process (Shah, 2003). Set-yogurt is fermented in a retail container whereas for stirred yogurt (e.g. "Ayran") fermentation occurs in tanks and the gel is broken before cooling and packaging by stirring resulting in low viscosity stirred yogurt. In terms of flavoring, yogurt can be classified into several subgroups. The traditional plain yogurt may be added with fruits or flavored by adding sweetener and coloring compounds.

2.2.5   Nutritional value of yogurt

Yogurt, by virtue of milk is rich in proteins, several B vitamins and essential minerals (Reid et al., 2003). Yogurt is also a rich source of calcium and contains as much fat as the milk it is made from. Factors affecting the nutritional value of yogurt include the milk constituents, species and strains of bacteria used in fermentation, source and type of milk solids, temperature and time of fermentation (Adolfsson et al., 2004).

Yogurt can be easily fortified with a range of vitamins, antioxidants, probiotics and other beneficial nutrients. Nutrients added to yogurt include lycopene, omega-3

eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and CoQIO which is one of the most powerful antioxidants known to science (Gerdes & Wagner, 2007). Most of these ingredients have well-recognized health claims (Ozer & Kirmaci, 2010). Probiotics, prebiotics and phytosterols may also be added to the yogurt mix. The prebiotics function as dietary fibers and are mostly polydextrose. Examples of such prebiotics are inulin and oligofructose (Aryana, Plauche, Rao, McGrew, & Shah, 2007; Vasiljevic, Kealy, & Mishra, 2007). Inulin is commonly present in plants such as artichokes, leeks and garlic and is made up of glucose and fructose chains. Oligofructose is produced from enzymatic hydrolysis of inulin or partial enzymatic hydrolysis of sucrose (transfructosylation by P-fructofuranosidase). Both inulin and oligofructose provide nutritional and functional benefits in foods which ultimately promote the growth of probiotics such as bifidobacteria and lactobacillus and hence improve digestive efficiency and health (Bruno, Lankaputhra, & Shah, 2002). The addition of sweeteners is important to attract certain market component particularly young children. It is of advantage that the addition of most sweeteners had little inhibitory effect on the growth of the cultures used (Riazi & Ziar 2011).

The viability of the lactic acid bacteria and probiotics may be as high as 85% and 90% respectively, except in the yogurt sample sweetened with sourwood honey. Vasiljevic et al. (2007) showed that the addition of β-glucan from oat and barley to yogurt improved probiotic *(Bifidobacterium animalis ssp. lactis)* viability and stability.

An increasing popular ingredient in yogurt is phytosterols. These are sterol compounds, which occur naturally in plants but have long been used to treat hypercholesterolemia (Monu et al., 2008). Phytosterols have become important ingredients in functional foods because of perceived health potentials in association with its ability to block cholesterol absorption and thereby reduce coronary heart disease. For instance the

consumption of a low-fat yogurt (0.7% fat) containing 3g/day of plant sterols reduced LDL cholesterol by 13.7% (Noakes et al., 2005).

2.2.6 Health benefits of yogurt

There are many health benefits of yogurt and these are attributed to either the yogurt bacteria or the products of microbial fermentation of milk. Lactic acid aids in calcium absorption and digesting some of the lactose for people with lactose intolerance (Tarakci & Kucukoner, 2004). Yogurt bacteria can produce some B vitamins particularly which are needed by body (Hugenholtz & Kleerebezem, 1999).

Yogurt may also acts as antibiotic, strengthening immune system (Adolfsson et al., 2004), protecting against gastrointestinal upset, reducing risk of cancer, lower blood cholesterol especially low density lipoprotein cholesterol, and help the body to assimilate protein, calcium and iron (Andronoiu et al., 2011).

2.2.7 Biodefense properties of yogurt

The benefits of yogurt have been shown in both animal and human studies. These promising health benefits include potential reduction of lactose intolerance, constipation, diarrheal diseases, colon cancer, inflammatory bowel disease, *Helicobacter pylori* infection and allergies (Adolfsson et al., 2004). Yogurt and fermented milk have bio defensive properties attributed to protective proteins and peptides found in cow's milk yogurt with unique biological activities (Adolfsson et al., 2004). Bioactive peptides such as casokinins or angiotensin-converting enzyme (ACE) I peptides play a role in reducing blood pressure by inhibiting ACE and blocking the conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor. Milk peptides may also have anti-carcinogenic properties as

demonstrated by their ability to retard the development of colon tumors and tumor precursors by virtue of the   biologically active methionine and cysteine (Korhonen, Marnila, & Gill, 2000). This is because both amino acids are involved in the cellular methylation and DNA stabilization as well as cellular synthesis of gluthathione, which plays a crucial role in the defense mechanisms that protect against cancer. Bacterial predigestion of the milk proteins in the yogurt releases free amino acids (Shahani & Chandan, 1979) resulting in higher content of amino acids in yogurt than in milk, thus supporting the argument that proteins from yogurt are more digestible than proteins from milk.

Yogurts are usually sold as low-fat or non-fat varieties which implicate lipid hydrolysis makes little contribution to yogurt products attributes. However yogurt contains more conjugated linoleic acid (CLA), a long-chain biohydrogenated derivative of linoleic acid than fresh cow's milk. CLA has both immune stimulatory and anti carcinogenic properties (Whigham, Cook, & Atkinson, 2000). Due to the lower content of lactose in yogurt, the bioavailability of minerals such as calcium, magnesium and zinc may be reduced since lactose enhances the absorption of these minerals (Bronner & Pansu, 1999). Nevertheless since yogurt is acidic (low pH), calcium still exists in the ionic form and thus improves intestinal calcium uptake (Bronner & Pansu, 1999). Bacterial cultures used during yogurt making (fermentation process) influence the vitamin content of the final product. Despite B vitamins are not required for the growth of LAB, some of the strains do synthesize such vitamins, and as such vitamin losses as a result of processing could be corrected by utilizing such cultures (Winkler, Davidson, & Mwakasonda, 2005).

2.2.8   Quality of yogurt

The quality of yogurt can be accessed through its chemical, microbiological and physical properties. The chemical and microbiological parameters are controlled under food legislation of each country. For example, the Australian Food Standard Code (Standard 2.5.3, 2004) requires that the viable counts of yogurt starter cultures be no less than 10^6 cfu/g of a product throughout the storage period. The protein content must be no less than 30 g/kg while the pH of product must be less than 4.50. In some European countries, the use of stabilizers is prohibited (Degeest, Van Den Ven, & DeVust, 1999). However, there is no legal requirement for the physical properties of a product. In general, set yogurt should be firm, smooth in texture, free from lump or graininess and spoonable without any syneresis on the surface of the product (Tamime & Robinson, 2007). Several methods including sensory and instrumental assessments have been used for assessing physical properties of yogurts.

2.2.9   Rheological properties of yogurt

Rheology is the study of the flow and deformation of matter. In food research, the term is often used inter-changeably with texture, which refers to the flow, deformation, and disintegration of a sample under force (Shaker, Jumah, & Abu-Jdayil, 2000). Technically, texture relates to solid foods, whereas viscosity, the tendency to resist flow, relates to fluid foods. However, certain food can exhibit both solid and liquid characteristics, and thus the rheological characteristics can identify the properties of such food (Tunick, 2000). Rheological properties of foods are important in the design of flow processes, quality control, storage and processing and in predicting the texture of foods (Shaker et al., 2000). The texture of food products is related to sensory properties of food because it represents

all the rheological and structural attributes perceptible by means of mechanical, tactile, and, when appropriate, visual and auditory receptors. The assessment, rheology and structure of a product may be objectively evaluated by instrumental methods because they give relevant information on its textural properties. This attribute is of great use since the sensory and instrumental data are not always easily correlated (Sodini et al., 2004).

In a dynamic rheological experiment, small amplitude oscillatory tests, a sinusoidal oscillating stress or strain with a frequency ω is applied to the material and the phase difference between the oscillating stress and strain as well as the amplitude ratio is measured (Rao, 2003). As a result of the applied strain, two stress components are generated in the viscoelastic material, an elastic component that is in line with the strain and the viscous component that is out-of-phase (90°). For deformation within the linear viscoelastic range, the generated stress can be measured in terms of an elastic or storage modulus G' and a viscous or loss modulus G". The storage modulus expresses the magnitude of the energy that is stored in the material or recoverable per cycle of deformation whereas the loss modulus is a measure of the energy which is lost as viscous dissipation per cycle of deformation (Rao, 2003).

2.2.10 Separation of whey

Whey or serum separation, which is also called wheying off, 'is the appearance of whey on the surface of a gel' (Lucey, 2002), and is a common defect during gelation and subsequent storage of fermented products such as yogurt. 'Spontaneous syneresis is the contraction of a gel without the application of any external forces (e.g. centrifugation)' (Lucey, 2002). Casein gels are dynamic by nature. Excessive rearrangements of particles making up the gel network before and during gelation have been considered to be

responsible for whey separation. Hence, whey separation is related to the instability of the gel network that has a strong tendency to undergo further rearrangement of network structure resulting in loss of the ability of the gel to entrap all the serum phase. Conditions under which considerable whey separation could occur include high incubation temperature (45 °C), excessive pre-heat treatment (> 80°C for 30 min), disturbances while the gel is still weak, low acid production (pH 4.9 instead of 4.6) and low total solids content (Xu, Emmanouelidou, Raphaelides, & Antoniou, 2008).

Although heat treatment increases the rigidity of yogurt gels (which is an important textural attribute), it is not very effective in preventing the wheying-off that occurs in milk incubated at very high temperatures (Lucey, Tamehana, Singh, & Munro, 1998). In acid gels made from heated milk, there is an increase in loss tangent during gelation even at much higher frequencies and a reduction in fracture strain both of which could assist in rearrangements and whey separation (Lucey, 2001). However, Al-Kadamany, Khattar, Haddad, & Toufeili (2003) reported that samples of labneh stored at 5 °C syneresed to a lower degree, and displayed marked fluctuations in the degree of syneresis, as compared to those stored at 15 and 25 °C. Thus, the susceptibility of yogurt gels to whey separation varies markedly and is not fully understood (Al-kadamany, Khattar, Haddad, & Toufeili, 2003).

2.3   Sensory evaluation

Sensory evaluation is defined as the science of judging and evaluating the quality of a food by the use of the senses, i.e. taste, smell, sight, touch and hearing. Sensory testing has been developed into a precise, formal, structured methodology that is continually being updated to refine existing techniques. The developed methods serve economic interests and

can establish the worth or acceptance of a commodity. Before a product reaches the market, it has to pass through many tests to give reasonable impression how well the public will accept it. This is especially true in the food industry because of the many taste and social preferences (Meilgaard, Civille, & Reveco, 1991). Thus subjecting a product to sensory evaluation before making a serious investment is a simple and effective way to answer these questions with relative certainty. Consumer testing by affective testing is a useful tool employed to try to answer questions about the success of a new product. Although there are many different types of consumer tests, the affective test is the most popular for basic consumer tasting of food.

Affective tests aim to achieve the followings:

a) Allow different treatments to be judged to find the optimum accepted product.

b) Break the masses of consumers down into smaller groups to allow an understanding of who will buy the product and how to market it to them.

c) Assess the market share potential for the new product.

In addition, other products can be improved upon by testing results. Information is obtained by asking specific questions about a person's age, sex, geographic location, nationality, religion, education and employment along with their preferences on the product being tested. To put it more simply, it stereotypes user groups based on these variables and learns the preferences of particular groups' eating habits. This is not done because of prejudicial motivation, but simply due to consumer preferences which tend to be much grouped, based on such factors listed above. This type of testing is a very accurate tool in understanding consumer preferences. Sensory tests offer a course to select a product that optimizes value for money. Sensory evaluation is used as a practical application in product development by aiding in product matching, improvements, and grading. Research is

another area where sensory evaluation is frequently used. Evaluation of a product may be needed to determine the effects an experiment had on its subject. Finally, quality control and marketing is yet another application of sensory testing (Stone & Sidel, 2004; Meilgaard et al., 1991).

Simply stated, sensory evaluation is divided into two methods, subjective and objective testing. Subjective tests involve objective panellists, while objective testing employs the use of laboratory instruments with no involvement of the senses. Both tests are essential in sensory evaluation and necessary in a variety of conditions (Meilgaard et al., 1991).

2.4   Problems in low-fat yogurt

Traditionally the solids content of milk is increased up to 18% for yogurt production. Increasing total solids by fortification with dairy ingredients increases the concentration of proteins by 4-5% and results in improved yogurt texture. However, high level of fortification with milk solids can lead to certain problems such as powdery taste in the yogurt and cause excessive acid development, especially during storage (Mistry & Hassan, 1992). Additionally, heat treatment of milk at temperatures above 70 °C prior to fermentation is common for increasing the gel firmness and reducing the level of syneresis. This effect is a consequence of whey protein denaturation during the heat treatment as these denatured proteins are more susceptible to inter-protein aggregation with other denatured whey proteins or with casein micelles.

2.5   Probiotics and health

A lot of studies have indicated the potential therapeutic effects of LAB including the probiotics and yogurt. Some of these effects include immune stimulation due primarily to yogurt or LAB-induced changes in the gastrointestinal microecology (Fiander, Bradley, Johnson-Green, & Green-Johnson, 2005). Probiotic bacteria are defined as "live microorganisms which when administered in adequate amounts confer health benefits to the host" (FAO/WHO, 2002). These probiotics must meet certain requirements (Isolauri, Sutas, Kankaanpaa, Arvilommi, & Salminen, 2001): (1) have the ability to colonize the host's intestine; (2) have the ability to survive and withstand exposure to low pH and bile acids; (3) have the ability to adhere to intestinal epithelium; (4) be non pathogenic and nontoxic; (5) host must be able to benefit from it; (6) must be human specific organisms (except, veterinary probiotics); (7) must be stable during storage.

Probiotic administration could be used to alleviate gut diseases and also prevent and treat other forms of diseases such as allergies and immune related diseases (Gill & Guarner, 2004). In fact most short-term human studies have shown that probiotics such as lactobacilli and bifidobacteria have the ability to modulate a host's immunity and thus making fermented dairy products with probiotics very popular due to their health benefits (Shah, 2006). Consumption of yogurt or lactic acid bacteria modulates the production of cytokines that play different roles in regulating immune functions. For example, the use of fermented foods and cultured milk products containing live microbes has been in existence a long time ago and is believed recently, to offer a possible means of controlling allergies. The observation that frequent consumption of sour milk containing *Lactobacillus bulgaricus* increased health and longevity was only made in the 20th century by Metchnikoff, who claimed that the intake of yogurt decreases the toxic effect of the

putrefactive bacteria in the colon by decreasing their growth (Metchnikoff, 1908; Wollowski et al., 2001). Probiotics have shown some potential in stopping the food-borne infections such as salmonella and *E. coli*. The antimicrobial effects of probiotics have extensively been studied and their effects on the microflora of the gut cannot be overemphasized. Some probiotics produce short chain fatty acids, which contribute to the low pH of the colon, thus inhibiting the growth of pathogenic microorganisms and favoring the growth of the less virulent microorganisms (Rolfe, 2000). The health benefits of these probiotics are dose dependent, judging from the fact that probiotic bacteria and the bifidobacteria were using different sugars for metabolism when grown in cow's milk (Farnworth et al., 2007). Studies on the growth and metabolism of selected strains of probiotic bacteria in milk emphasized the importance of fermentation time since probiotic strains produced different amounts of metabolic products at various fermentation times (Ostlie, Helland, & Narvhus, 2003). Clinical studies in adults showed that consumption of probiotic drink containing *L. casei, L. bulgaricus* and *S. thermophilus* could decrease the incidence of antibiotic associated diarrhea (Hickson et al., 2007). In this regard traditional yogurt cultures may be considered as probiotics since these cultures are able to eliminate symptoms of lactose intolerance hence the health benefit of improved digestion (Guarner et al., 2005). Some potential clinical effects of probiotics are presented in Table 2.1.

Table 2.1 Potential clinic targets of probiotic intervention (Isolauri, Salminen, & Ouwehand, 2004)

Effect	Potential mechanism	Potential risks
Nutritional management of diarrhea	Reduction in the duration of rotavirus shedding, normalization of gut permeability and microbiota	Risk related to host and strain characteristics
Nutritional management of Allergic disease inflammatory bowel disease	Degradation/structural modification of enteral antigens, normalization of the properties of aberrant indigenous microbiota & of gut barrier functions, local & systemic inflammatory response, increase in the expression mucins	Strains with proinflamofmatory effects/adverse effects on innate immnity. Translocation/infection
Reducing the risk of infectious disease	Increase in IgA-secreting cells against rotavirus, the expression of mucins	Risk related to host & strain characteristics
Reducing the risk of allergic/inflammatory disease	Promotion of gut barrier functions, potential, regulation of the secretion of inflammatory mediators & promotion of development of the immune system	Directing the microbiota towards other adverse outcomes/directing the immune responder type to other adverse outcomes

2.5.1   Interaction between LAB in milk

Probiotic bacteria in general are enteric flora which grow slowly in milk and often show a loss of viability during refrigerated storage. Their viability is affected by many

factors (Lucas et al., 2006) including pH, hydrogen peroxide, dissolved oxygen in fermented milk, strains used, interaction between species present, culture condition, chemical composition of the fermentation medium, availability of nutrient, incubation temperature, fermentation time and storage temperature.

2.5.2   Type of probiotics

2.5.2.a   *Lactobacillus delbrueckii ssp. Bulgaricus*

The genus Lactobacillus is quite diverse and consists of a number of different species that have little in common. A measure of their diversity can be estimated by the range of guanine plus cytosine (G + C) % content among the lactobacilli. Members of the species have G + C content of 32-53%, which is much wider than is encountered with other LAB. The lactobacilli include over 25 unique species, and the first level of differentiation is based on end product composition: homofermentators – classified as organisms that produce >85% lactic acid as their end product from glucose (e.g. *L. delbrueckii ssp. Bulgaricus, L. acidophilus*), and heterofermentators – classified as organisms that produce approximately 50% lactic acid as the end product, with considerable amounts of carbon dioxide, acetate and ethanol (e.g. *L. brevis, L. casei*) (Teixeira, 2000). Although they all produce lactic acid as a major end product they differ in the isomeric composition of lactic acid produced. Some produce exclusively L (+) lactic acid and these include *L. salivarus* and *L. casei*. Others, for example *L. delbrueckii ssp. Bulgaricus* and *L. jensenii* produce just D (-) lactic acid, and finally *L. acidophilus* and *L. helveticus* produce a mixture of D (+) and L (-) lactic acid. Their optimum growth temperature is in the range of 30-40 °C. They are also aciduric with an optimum growth pH of 5.5-5.8 but in general they can grow at a pH of less than 5.0 (Batt, 2000).

Lactobacillus delbrueckii ssp. *Bulgaricus* is also Gram-positive, but it occurs in milk as chains of 3 to 4 short rods, each 0.5-0.8 × 2.0-9.0 μm, with rounded ends. The optimum growth temperature is 45 °C. Its basic metabolism is homofermentative, to give D (-) lactic acid to a level of 1.7-2.1% in milk. It converts hexoses into lactic acid via the EMP pathway. Although lactic acid is the major end product of fermentation, secondary end products such as acetaldehyde, acetone, acetoin and diacetyl can also be produced in very low concentrations. *Lactobacillus delbrueckii* ssp. *Bulgaricus* can, like *S. thermophilus*, utilize lactose, fructose and glucose, and some strains can utilize galactose (Robinson, 2000).

2.5.2.b   *Streptococcus thermophilus*

The genus *Streptococcus* consists of Gram-positive, spherical-ovoid or coccobacillary cells. The G + C content of the DNA of species of this genus is 34-46 mol%. The 39 currently classified species of the genus *Streptococcus sensu stricto* are grouped as: a) oral (*S. salivarius*, *S. mutans*, *S. mitis*, *S. thermophilus*); b) pyogenic (*S. pyogenes*, *S. agalactiae*) and c) other streptococci (*S. bovis, S. equinus* and *S. alactolyticus*) (Gobbetti & Corsetti, 2000). *S. thermophilus*, like most LAB, is non-spore-forming, catalase-negative and facultatively anaerobic. These spherical or ovoid cells (0.7-0.9 μm diameter) occur in pairs or chains when grown in liquid media and in milk it occurs in long chains of 10-20 cells. It grows best at a temperature of about 42-45°C. They are heterotrophic and generally fastidious, requiring simple carbohydrates as energy source, and preformed amino acids as a nitrogen source. It ferments lactose homofermentatively, to give L (+) lactic acid as the principal product (Zirnstein & Hutkins, 2000). Lactose is actively transported across the cell membrane of *S. thermophilus*, by means of a membrane located enzyme, galactoside

permease. Inside the cell, the enzyme β-galactosidase hydrolyses the lactose to glucose and galactose. The glucose is metabolized to pyruvate via the Embden-Meyerhof-Parnas (EMP) pathway, and lactic dehydrogenase converts the pyruvate to lactic acid. In most strains of *S. thermophilus*, the galactose and lactic acid produced leave the cell and accumulate in the medium, but some strains possess a galactokinase, that converts the galactose to galactose-1-phosphate which is converted via the Leloir pathway to glucose-1-phosphate, that is further metabolized via the EMP pathway (Robinson, 2000).

2.5.3 Probiotics in yogurt

Probiotic bacteria are adjuncts added to fermented milks such as yogurt. These bacteria belong to the genera *Lactobacillus* and *Bifidobacterium*, which are common but non-dominant members of the indigenous microbiota of the human GIT (Vasiljevic & Shah, 2008). Some of the potential health benefits of functional foods containing probiotic bacteria include improved digestibility, improved nutritional value, improved lactose utilization, antagonistic action towards enteric pathogens, colonization in gut, anticarcinogenic effect, hypocholesterolemic effect, immune modulation, prevention of allergy and prevention of inflammatory bowel disease (Gomes & Malcata, 1999). *Lactobacillus acidophilus*, *L. casei*, *L. paracasei* and *Bifidobacterium* species are predominantly used in yogurt (Holzapfel, Haberer, Geisen, Björkroth, & Schillinger, 2001). Manufacturers of therapeutic fermented milk products commonly use five species of *Bifidobacterium* (*B. adolescentis*, *B. bifidum*, *B. breve*, *B. infantis* and *B. longum*) (Arunachalam, 1999).

The characteristics of probiotic strains vary, and each strain has to be studied individually. Some probiotic strains are sufficiently proteolytic to grow excellently in milk,

but others need growth stimulants. Those that do not ferment lactose need monosaccharides. Sometimes the texture or the taste of a milk product fermented with a probiotic does not meet with consumer approval or is technologically impractical. For this reason it is common to use probiotic bacteria together with standard starter cultures as in yogurt (Saxelin, Korpela, & Mayra-Makinen, 2003). Most *Bifidobacterium* species cannot ferment milk by themselves because they require low redox potential and peptides generated from the breakdown of casein, a milk protein. Moreover, when co-cultured with lactobacilli, they become inhibited as the pH drops (Klaver, Kingma, & Weekamp, 1993). Several factors such as strain characteristics, food matrix, temperature, pH and accompanying microbes affect the viability of probiotics (Fondén, 2003). A synbiotic product containing the probiotic bacteria and prebiotic in a single food can improve the survival of bifidobacteria during the storage of the product and during the passage to the intestinal tract, and also reduce the competition with microorganisms in the GIT. The combined use of two or more probiotic species is common in commercial probiotic foods, as these strains are believed to act synergistically on each other. Thus, the trend is to use yogurt bacteria as the main starter culture and probiotic bacteria as an adjunct starter (Shah, 2006). The most common probiotic dairy products worldwide are various types of yogurt, cultured buttermilks, various LAB drinks such as Yakult, and mixtures of probiotic fermented milks and fruit juice.

2.5.4   Characteristics of common probiotics

Lactobacillus acidophilus: This organism, first isolated by Moro in 1900 from infant faeces, has undergone many transformations in the description of its metabolic, taxonomic and functional characteristics. It is isolated from the intestinal tract of humans and animals

and is also reported in the faeces of milk-fed infants and older persons consuming high milk-, lactose- or dextrin-diets (Holzapfel et al., 2001). These Gram-positive rods (0.5-1 × 2-10 μm), with rounded ends, occur in pairs or short chains. It is non-flagellated, non-motile and non-spore-forming, and is intolerant to salt. It was initially categorized in the thermobacteria classification of LAB based on their homofermentative metabolism and ability to grow at 45°C. L. acidophilus was recognized as a heterogenous group by DNA hybridization studies. This group, known as the L. acidophilus complex, is composed of the six distinct species of L. acidophilus, L. crispatus, L. amylovorus, L. gallinarum, L. gasseri and L. johnsonii. Although these are regarded as separate species, they are closely related and have been suggested as belonging to one phylogenetic group or branch. Cultures of L. acidophilus are microaerophilic and capable of aerobic growth in static cultures without shaking. They prefer anaerobic conditions and growth is stimulated in broth or agar under a standard anaerobic gas mixture of 5% carbon dioxide, 10% hydrogen and 85% nitrogen (Klaenhammer & Russell, 2000). The nutritional requirements of *L. acidophilus* reflect the fastidious nature of these bacteria. Currently, members of the *L. acidophilus* complex are classified as obligate homofermenters. Hexoses are fermented by this group primarily to lactic acid by EMP pathway. All species produce L and D isomers of lactic acid, the yield being 1.8 mol/mol glucose. Additionally, they produce a variety of antimicrobial compounds, including lactic acid, hydrogen peroxide and a variety of bacteriocins (Gomes & Malcata, 1999).

Lactobacillus casei is a typical cheese bacterium isolated mainly from silage, sourdough, cow dung, human intestinal tract, mouth and vagina. It is a Gram-positive, non-motile, non-sporulating and catalase-negative bacterium having an optimum growth temperature of 30 °C. These cells, which are rods of 0.7-1.1 × 2.0-4.0 μm, often with

square ends with tendency to form chains, may come from four subspecies: *casei*, *pseudoplantarum*, *rhamnosus* and *tolerans* (Bergey's Manual of Systematic Bacteriology). The latest grouping of lactobacilli based on chemical-physiological criteria includes *L. casei* in the facultatively heterofermentative group. Hexoses are almost entirely converted into lactic acid via EMP pathway and pentoses are used by induced phosphoketolase, to produce lactic acid and acetic acid (Gobbetti, Ferranti, Smacchi, Goffredi, & Addeo, 2000).

Bifidobacterium were unknown by people working in the area of food science and technology, but since the mid-1980s there has been a revival of interest due to the expanded use of bifidobacteria in products that are now marketed as functional foods. Organisms of the genus *Bifidobacterium* are short, regular, thin rods (0.5-1.3 × 1.5-8 μm) that are slightly bifurcated club-shaped elements in star-like aggregates or disposed in 'V' or 'palisade' arrangements (Hoover, 2000). They are Gram-positive, usually catalase-negative, non-spore-forming, non-motile cells. They are anaerobic but some species are aero-tolerant. The optimum growth temperature is in the range of 37 to 41°C and the optimum pH for initial growth is 6.5-7.0. They metabolize glucose exclusively by heterolactic fermentation by the fructose-6-phosphate shunt also know as bifid shunt, to form L (+) lactic acid and acetic acid in the molar ratio of 2:3. Besides glucose, all bifidobacteria from human origin are also able to utilize galactose, lactose and fructose as carbon sources. A proton symport has been identified as the lactose transport system for *B. bifidum* DSM 20082 (Krzewinski, Brassart, Gavini, & Bouquelet, 1996).

2.5.5   Factors affecting viability of probiotic bacteria

Several factors, including the strains selected, interactions between species present, acidity, pH and hydrogen peroxide due to bacterial metabolism, have been identified to

affect the viability of probiotic microorganisms during manufacture and storage of yogurt (Dave & Shah, 1997). Other factors such as storage temperature, oxygen content, concentrations of acetic and lactic acids, nutrients limitations in milk/soymilk to sustain growth, growth promoters and inhibitors, inoculation level, fermentation time and post-acidification have also been suggested to affect viability of probiotic organisms in yogurt (Tamime, 2005). The improvement of survival and viability may be achieved by either appropriate culture selection (Lourens-Hattingh & Viljeon, 2001; Tuomola, Crittenden, Playne, Isolauri, & Salminen, 2001), microencapsulation (Capela, Hay, & Shah, 2006), supplementation of milk with nutrients (McComas & Gilliland, 2003) or use of growth enhancers such as prebiotics (Capela et al., 2006).

2.6   Tea

2.6.1   Tea plant

Botanically all the tea plants belong to the Camellia family. Tea plants can be divided into two species, the Chinese plant (*Camellia sinensis*) and the Assam plant (*Camellia assamica*). Tea leaves are dark green and the blossom is cream colored and aromatic. Only the top two leaves and a bud are used in tea processing. Tea plants can grow to a maximum height of about 30 feet, but under cultivation they are pruned to 3-5 feet to maximize leaf production and facilitate harvesting (Tea, 2011).

Figure 2.1 Tea plant with two leaves and a bud on the top along with blossoms (Botanical Tea Plant, 2010).

2.6.2   Types of tea

Based on the method of processing, teas are classified into four types: black, oolong, green and white tea. The different processing steps for each type of tea are shown in the Figure 2.2.
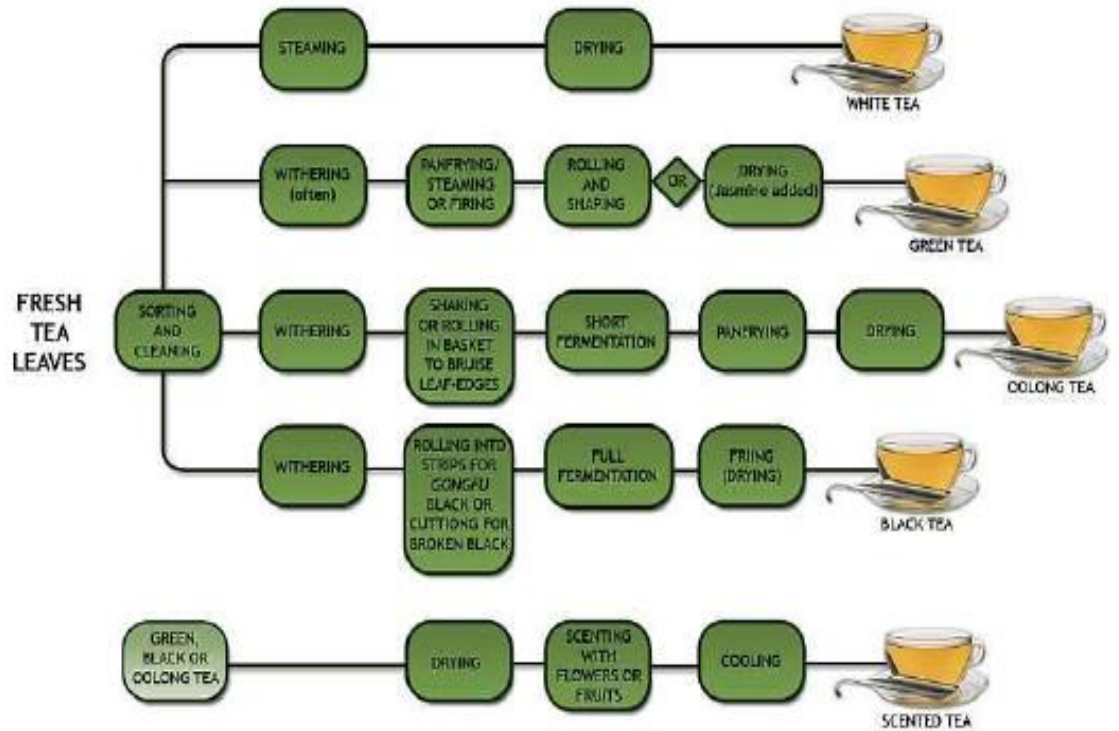
Figure 2.2 Processing steps in different types of tea (Tea Processing, 2005)

2.6.3   Manufacture of tea

For manufacturing green, oolong and black teas, two leaves and a bud are used whereas for white tea, buds or young leaves are used. The steps used for manufacturing green tea are described further in the following sections.

2.6.3.a   *Withering*

Withering reduces the moisture content of tea leaves. Leaves are spread on trays or fine meshed screens, and hot air is passed through them for 12-17 hours (Tea production process, 2001). Air temperature is 26.7- 32.2 °C and relative humidity is 70-80%. At the end of withering, the final moisture is reduced to 70%, and tea leaves are flexible enough to

be rolled (Tea production process, 2001). The withering step can be omitted for green tea, but it is always used to process black tea.

2.6.3.b *Steaming*

Tea leaves are steamed at 95-100°C for about 30-45 seconds to inactivate the polyphenol oxidase enzyme thus preventing the loss of polyphenols through oxidation. Steaming also helps prevent loss of vitamins and is the reason why green teas have higher vitamin content than the fermented teas (Chee & Juneja, 1997).

2.6.3.c *Rolling*

The objective of rolling is to break up the leaf cells so that essential oils are released, giving particular teas their unique aroma. Rolling also gives a particular shape and distinguished twist to the tea leaves. During rolling the temperature of the leaves must be kept below 29.4 °C to prevent fermentation and damage to the final product.

2.6.3.d *Drying*

Rolled tea leaves are dried immediately using hot air dryers to reduce moisture content to 3% and stop the oxidation process. Dried tea leaves are ready for packing and distribution.

2.7 Green tea and chemical composition

Green tea is one of most widely consumed beverages in Asian countries and has been familiar in China and Japan from centuries (Zaveri, 2006). The chemical composition of tea varies with the growing conditions like climate, season, agricultural practices, variety, age and position of the leaf (Aherne & O'Brien, 2002; Lin, Tsai, Tsay, & Lin,

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2003). Regarding the proximate composition; moisture, protein, lipids, sugars, fiber, ash and caffeine in different kinds of green tea range from 2.2-5.0, 18.2-30.7, 3.5-5.3, 28.6-39.2, 10- 19.5, 5.4-7.4 and 1.9-3.5g/100 g, respectively, while minerals like Ca, P, Fe, Na and K are 390-740, 210-350, 10.4-38, 3-11 and 1,900-2,800 mg/100g, respectively (Chee & Juneja, 1997). Tea on dry weight basis contains 15-20% protein and 5% minerals (Cabrera, Artacho, & Gimenez, 2006). In addition to minerals, green tea also contains alkaloids including caffeine, theobromine and theophylline which vary from 0.6 to 28.6 mg/g (Friedman, Levin, Choi, Kozukue, & Kozukue, 2006). Caffeine content of green tea increases anxiety and impairs sleep (Potawale et al., 2008).

Polyphenols are the main constituents of green tea, accounting for 25–35% on dry weight basis (Shaheen et al., 2006; Yao et al., 2006). Like the minerals, polyphenols content also varies. mentioned that TPC contents could range from 11.52 mg/g (Soysal, 2009) to 21.02±1.54% (Anesini, Ferraro, & Filip, 2008). Most of the polyphenols are flavanols commonly recognized as catechins (Fernandez, Pablos, Martin, & Gonzalez, 2002) the major components of which are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG).

Catechin and epicatechin both are monomeric flavanol with epicatechin having an ortho-dihydroxyl group in the B-ring at carbons 3' and 4' and a hydroxyl group at carbon 3' on the C-ring. EGC has trihydroxyl group at carbons 3', 4', and 5' on the B-ring. ECG has gallate moiety esterified at carbon 3 of the C-ring. However, EGCG has both trihydroxyl groups at carbons 3', 4', and 5' on the B-ring and a gallate moiety esterified at carbon 3' on the C-ring (Yilmaz, 2006). In green tea, catechins are present in higher amounts than that of black or oolong tea, because of the processing differences (Zaveri, 2006). Some other

sources of catechins are red wine, fruits like plum, apples, peach, strawberry, cherry, broad bean, lentil and cocoa (Scalbert, Manach, Morand, & Remesy, 2005).

The typical levels of catechins in tea are EC 9.90, ECG 12.4, EGC 40.6 and EGCG 71.7 mg/g on dry weight basis (Yoshida, Kiso, & Goto, 1999). Among catechins, EGCG is the abundant fraction (Bettuzzi et al., 2006) accounting for 65% of the total catechins (Zaveri, 2006). Ho et al. (1997) highlighted that epigallocatechin gallate constitutes 48–55% of total polyphenols and is responsible for majority of the health benefits of green tea (Nagle, Ferreira, & Zhou, 2006).

2.7.1   Polyphenols

The average intake of polyphenols from food is about 1g/day. In plants, polyphenols serve as secondary metabolites by providing anti-microbial and anti-fungal functions, as well as protection from insect and UV-radiation damage (Stevenson & Hurst, 2007). The structure of polyphenols is characterized by a benzene ring with two or more hydroxyl groups attached (Stevenson & Hurst, 2007). The main groups of polyphenols are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (Figure 1.6). The flavonoid group is of importance to this review as it contains the main polyphenolic component of GT, epigallocatechin-3-gallate (EGCG).
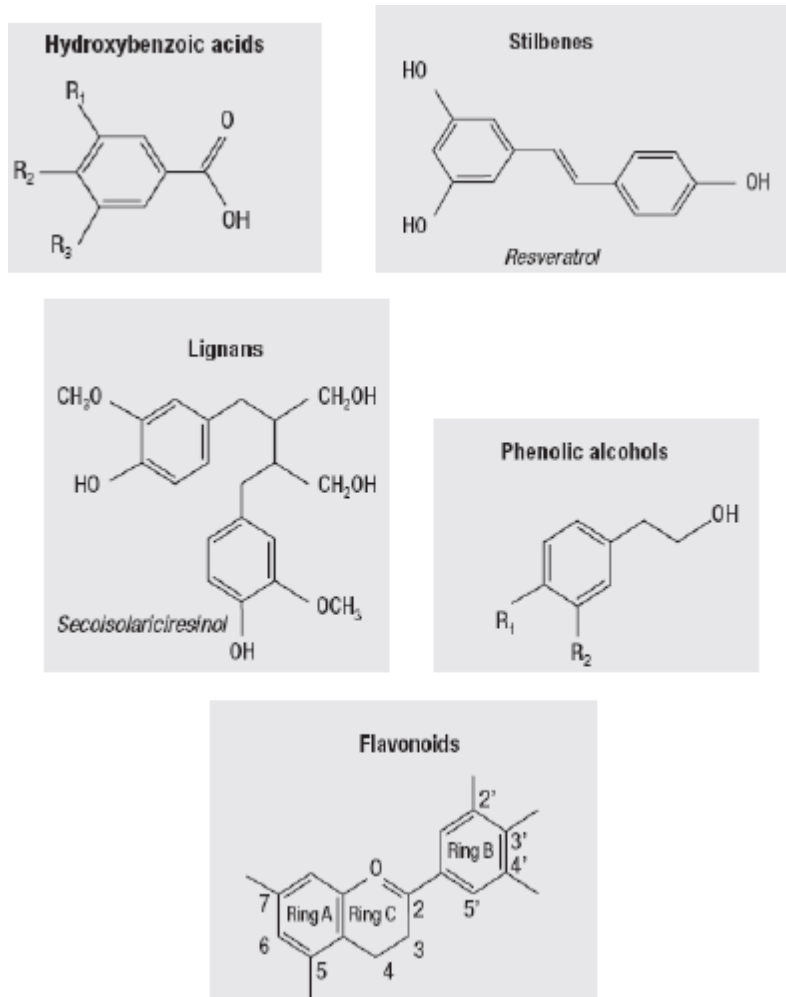
Figure 2.3 Structures of polyphenols (D'Archivio et al., 2007)

2.7.2   Flavonoids

Over 6,000 types of flavonoids have been identified in plants, with the list continuously growing (Geleijnse & Hollman, 2008). Flavonoids have a diphenylpropane skeleton structure ($C_6C_3C_6$) (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). The aromatic "A" ring is attached to heterocyclic benzopyran "C" ring and the "B" ring is a phenyl constituent (Aron & Kennedy, 2008). The flavonoids can be further divided into 6 subclasses depending on the oxidation state of the central pyran "C" ring, which include:

flavonols, flavones, flavanones, isoflavones, anthocyanidins and flavanols (Figure 2.4) (D'Archivio et al., 2007). Flavanols include catechins like epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC) and epigallocatechingallate (EGCG) molecules, which can be found in foods like red wine, dark chocolate, green tea (Figure 2.4).
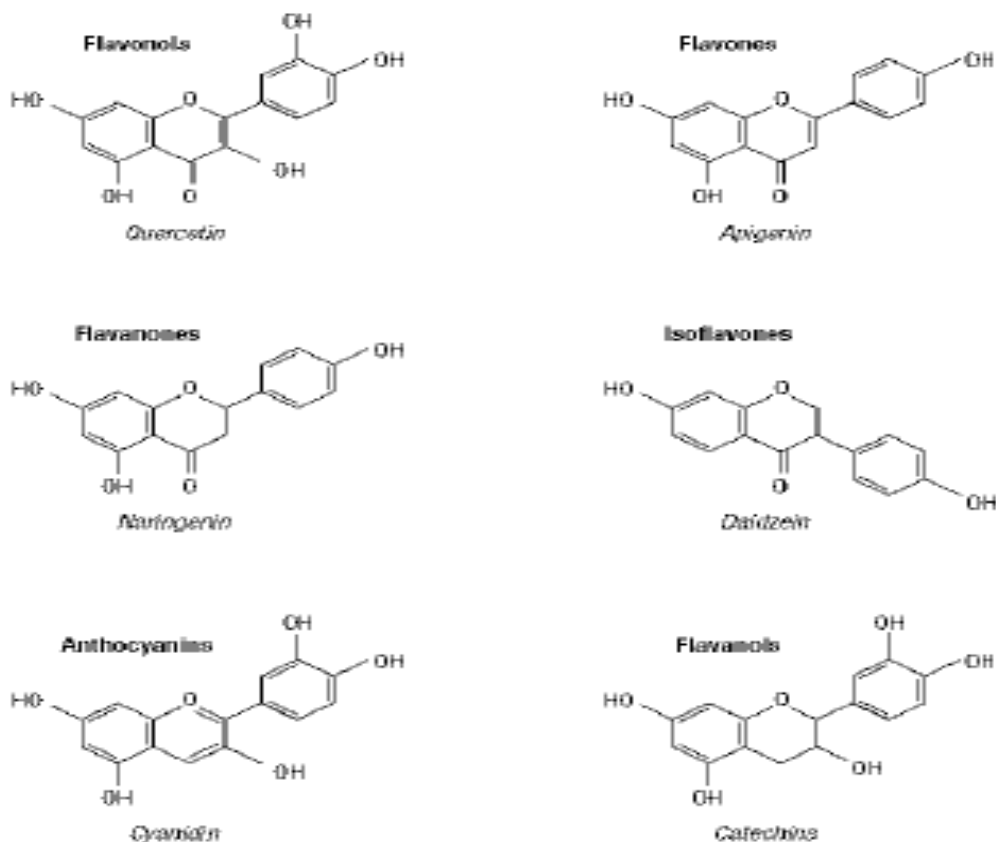

Figure 2.4 Structures of flavonoids (D'Archivio et al., 2007)

2.7.3 Catechins

Catechins are the main polyphenolic components of GT, comprising approximately 30% of the dry leaf weight (Graham, 1992). The structure of a catechin consists of a benzopyran skeleton substituted with a phenyl ring at the 2-position and a hydroxyl (or ester) at the 3-position (Chen, Ho, Chang, Hung, & Wang, 2005). Of the catechins present in GT, EGCG is the most abundant comprising 50-80% of the total catechins.
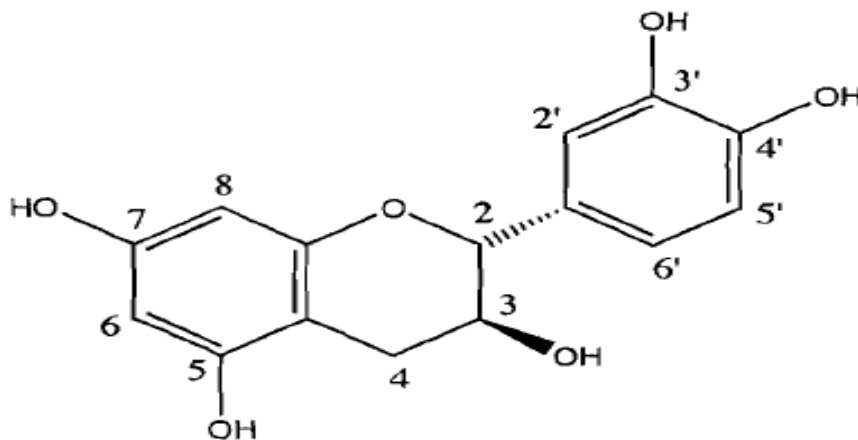
Figure 2.5 Green tea catechins

It is important to identify the manner by which green tea (GT) catechins, like epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and EGCG, are metabolized and absorbed in order to understand how these metabolites function *in vivo* to elicit beneficial health effects. In humans and animals the metabolism of GT catechins depends on intestinal metabolism, microbial metabolism, hepatic metabolism, transporters and chemical stability (Yong Feng, 2006). Once ingested, GT catechins can be found in the forms of methylated, sulfated and glucuronidated metabolites, microbial metabolites or degradants (Yong Feng, 2006).

The gastrointestinal (GI) tract plays an important role in the metabolism and bioavailability of GT catechins. After GT catechins are ingested, the metabolites that are formed in the small intestine have two possible metabolic pathways. Firstly, the metabolites could be transported to the intestinal lumen, which leads to the large intestine where the gut microflora further metabolize them into small phenolic acids and valerolactones (Yong Feng, 2006). These metabolites can then be either reabsorbed by the portal vein to be

further metabolized by the liver or excreted in fecal matter. Secondly, the metabolites formed in the small intestine are methylated, sulfated, glucuronidated or degraded and then further metabolized by the liver. Once metabolites reach the liver, they can either be excreted through urine or absorbed by cells and neurons (Yong Feng, 2006).
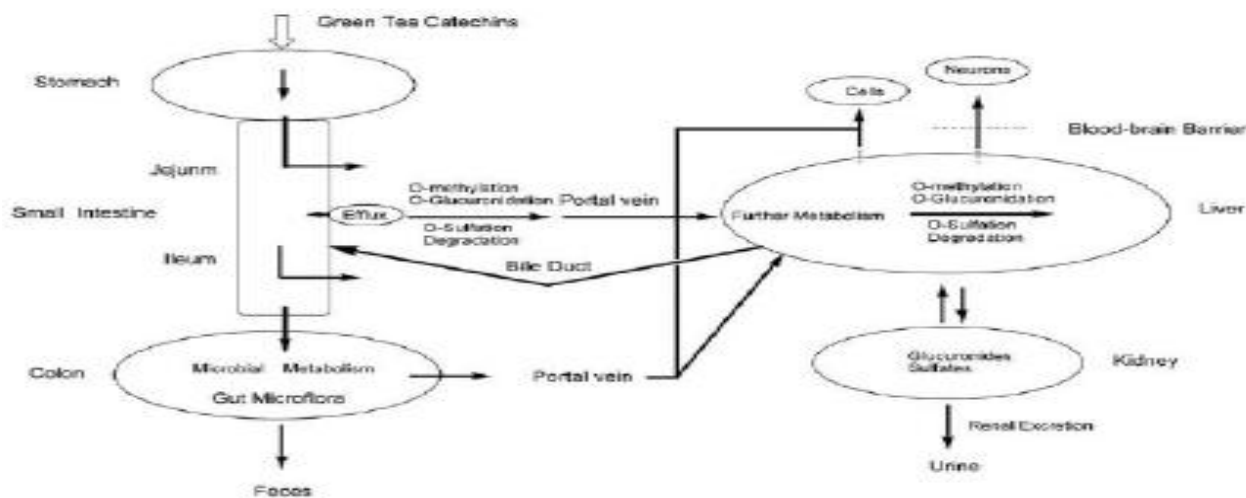

Figure 2.6 Proposed metabolic pathway of green tea catechins (Yong Feng, 2006)

2.7.4   Extraction of catechins

Extraction process is key to the withdrawal of active components of green tea (Danrong, Yuqiong, & Dejiang, 2009). Methodology and efficiency of catechins extraction is critical while studying their functionality behavior (Yoshida et al., 1999) as extraction conditions like solvent, temperature, time, pH and ratio of solvent to material affect catechins quantity and quality (Perva-Uzunalic et al., 2006). The method must facilitate complete extraction of the compounds of interest and evade their chemical modification (Zuo, Chen, & Deng, 2002). Among popular solvents, water, aqueous mixtures of ethanol, methanol and acetone are frequently used to extract plant bioactive molecules (Obanda,

Owuor, & Mangoka, 2001; Pomilio, Trajtemberg, & Vitale, 2002; Filip & Ferraro, 2003; Liang, Lu, Zhang, Wu, & Wu, 2003; Sun & Ho, 2005).

Druzynska, Stepniewska, & Wolosiak (2007) used four solvents i.e. water, 80% ethanol, 80% methanol and 80% acetone for extraction at room temperature for 15, 30 and 60 min and have found that the type of solvent and time have significant influence on the polyphenols extraction from green tea. In another study, Perva-Uzunalic et al. (2006) also performed extraction of catechins using different aqueous and pure solvents (acetone, ethanol, methanol, acetonitrile and water), temperature (60, 80, 95 and 100°C) and time (5 to 240 min). The extraction efficiency of major catechins varies from 61 to 100% however water gives maximum extraction rate i.e. 80°C after 20 min and 95˚C at 10 min. Water extraction could be performed in two steps to obtain EGC and EGCG enriched extracts (Labbe, Tetu, Trudel, & Bazinet, 2008).

Increasing extraction time is positively correlated to polyphenols extraction (Druzynska et al., 2007). Conversely, prolonged extraction procedure with higher temperatures results in degradation of catechins (Perva-Uzunalic et al., 2006). Temperature is a critical factor, if catechins are epimerized during extraction process, the resultant extract would not reflect the actual health claims (Wang, Provan, & Helliwell, 2003; Yao et al., 2004).

Maximal crude catechins (EGC, EGCG, ECG, EC) extraction from green tea was reported possible with water at 80˚C for 40 min followed by partitioning the resulting extract with water/chloroform to remove caffeine and subsequent purification of the aqueous layer with ethyl acetate (Row & Jin, 2006). Commercial extraction of catechins using water as solvent is routinely carried out at 80˚C because heating of green tea leaves above this temperature may cause partial epimerization of epigallocatechin gallate (EGCG)

and epicatechin gallate (ECG) in gallocatechin gallate (GCG) and catechin gallate (CG), respectively. Alternatively, the epimerization could be prevented by heating tea leaves in 50% ethanol for 10 min that gives optimum extraction efficiency with real catechins profile (Liang, Liang, Dong, & Lu, 2007).

2.7.5   Health benefits of green tea

Green tea is widely consumed worldwide due to its potential health benefits mostly from polyphenolics and flavonoids, which exhibit potent antioxidant capacity in both *in vitro* and *in vivo*. The polyphenolic compounds found in green tea may reduce the risk of various illnesses such as cancer and coronary heart disease (Negishi et al., 2004). Cancer, which is a group of diseases caused by uncontrolled growth and spread of abnormal cells, is a leading killer after cardiovascular disease (Chung, Schwartz, Herzog, & Yang, 2003). Evidence of the anticarcinogenic potential of tea polyphenols is attributed to their binding property to carcinogen and inhibiting heterocyclic amine 6 formation (Bhattacharya, 2012).

Epidemiological studies in Asian country where green tea is regularly consumed in large amount showed drinking green tea is directly related to prevention of cancer. For example, in a 9-year study of 8.552 Japanese adults, high green tea consumption (10 cups or more) delayed cancer onset by 8.7 years as compared to lower consumption (3 cups or lower) (Lambert & Yang, 2003). It was noted that black tea consumption was not as effective as green tea consumption on cancer prevention which can be attributed to green tea catechins such as epicatechin gallate, epigallocatechin gallate, and epigallocatechin that show higher anticarcinogenic property than theaflavin The occurrence of breast cancer, which is the fifth most common cancer death after lung, stomach, liver, and colon cancer and the most common cancer among women (Chen & Han, 2000), was inversely related to

the consumption of green tea because of estradiol (female hormone) and sex hormone-binding globulin (glycoprotein binding to testosterone and estradiol), which cause breast cancer onset, are affirmatively transformed (Inoue et al., 2001). Tea flavonoids such as epigallocatechin gallate, epigallocatechin and theaflavin-3-3'-digallate may also sufficiently inhibited the growth of lung tumor and the increase of 8-hydroxydeooxyguanosine formation in mouse lung DNA (Yang et al., 1998).

One benefit of consuming green tea is that carcinogenesis in the digestive tract is postulated to be inhibited by ECGC as demonstrated in *in vitro* studies whereby the polyphenols inhibited the growth and disintegration of a human stomach cancer cell line KATO III, and also inhibited tumor necrosis factor-a (TNF-a) release from the cells. Gastrointestinal tract cancer has big chance to develop when one consume an excess intake of protein and fat. The polyphenols of green tea may have a protective effect on adenomatous polyps and chronic atrophic gastritis formations. The inhibitory effect of green tea polyphenols was studied on the human lung cancer cell line, PC-9 and ECG and EGC were shown to have the same potency as EGCG whereas EC showed less inhibitory effect (Yang et al., 2000). Breast carcinoma is considered to be one of the most common cancers in women. Breast cancer is more prevalent in western countries compared to Japan and this could be attributing to daily intake of green tea as part of the diet.

Drinking green tea is believed to inhibit certain cancers, such as lung, skin, esophagus, liver, and stomach (Mandel, Weinreb, Amit, & Youdim, 2004). Non-melanoma skin cancer is considered to be the most common malignancy in humans. Polyphenols of green tea have been observed to have chemopreventive effects and also inhibitive effects such as carcinogenic activity against ultraviolet (UV) radiation. Thus, ingesting green tea in conjunction with sunscreen use could potentially protect the skin against adverse effects

caused by UV radiation. Along with the antioxidant activity, green tea is shown to exhibit tumour suppressing activity *in vitro* (Lee et al., 2004). In addition, polyphenols of green tea added to human gastric cancer cells induced apoptosis, and other tumour cells, and on mouse skin tumour agenesis. The possible health benefits are due to the respond to the negative effects of free radicals by utilizing defensive antioxidants present in green tea.

2.7.6 Antioxidant activity in green tea

Antioxidant activity is defined as the amount of sample to decrease the initial concentration of DPPH radical by 50% as efficient concentration (Buyukbalci & El, 2008). Antioxidant potential of catechins is two to four times greater than that of α–tocopherols (Tang, Sheehan, Buckley, Morrissey, & Kerry, 2001). Green tea extract holds higher protective effect against free radicals than oolong and black tea (Yokozawa et al., 1998). DPPH method is a major test to determine antioxidant potential (Parejo et al., 2003) and green tea DPPH scavenging activity could be as high as 84.0±1.3 (Yoo, Lee, Lee, Moon, & Lee, 2008). Scavenging ability of green tea against DPPH radical is due to galloyl moiety attached to the flavanol at 3-position along with ortho-trihydroxyl group in B ring (Nanjo et al., 1996). Among four major catechins, epigallocatechin gallate (EGCG) has the highest scavenging ability (Meterc, Petermann, & Weidner, 2008) and is the most effective scavenger of superoxide anions, hydroxyl radicals and 1, 1-diphenyl-2-picrylhydrazyl radicals (Nanjo, Mori, Goto, & Hara, 1999). EGCG is at least 100 times more effective than vitamin C and 25 times than vitamin E (Meterc et al., 2008).

The antioxidant activity of various catechins fractions increases in the following order: EC < ECG < EGC < EGCG (Meterc et al., 2008). DPPH radical scavenging activity is affected by solvent type and extraction time (Druzynska et al., 2007). The antiradical

activity of ethanolic extract of green tea is in fact better than water extract (Gramza, Pawlak-Lemanska, Korczak, Wasowicz, & Rudzinska, 2005).

Various studies were carried out to evaluate the antioxidant capacity of plants consumed as beverage. Katsube et al. (2004) performed extraction from fifty two kinds of edible plants with 70% aqueous ethanol solution and subjected to antiradical test, highest radical scavenging activity was shown by akamegashiwa and green tea. Similarly, Buyukbalci & El (2008) narrated that among ten herbal tea samples (green tea, absinthium, sage, blackberry peppermint, relaxtea, black tea, roselle, oliveleaves, thyme, shrubby), green tea possesses highest antioxidant activity followed by peppermint and black tea, whereas shrubby and blackberry are the weakest. Earlier, Aoshima, Hirata, & Ayabe (2007) highlighted that among 14 herbal tea samples antioxidant activity of green tea determined through DPPH assay was higher but lesser than rose. In a similar manner, Ohmori et al. (2005) assessed antioxidant activity of tea samples, including aqueous extract of green, oolong and barley tea against DPPH radical and elucidated that green tea has highest radical scavenging activity. Likewise, Quan, Hang, Ha, & Giang (2007) described that among thirty types of commercial Veitnamese-brand tea samples (21 green tea, 5 black tea, 4 oolong tea), green tea contains highest total polyphenols contents (TPC), total catechins contents and DPPH scavenging activity. Gadow, Joubert, & Hansmam (1997) compared antioxidant activity of green, oolong, black and rooibos tea samples through β- carotene bleaching method and DPPH radical scavenging activity. The results depicted that for both tests highest antiradical ability was shown by green tea. Scavenging effect of catechins is not dependent on their steric structure as no significant differences exist between the scavenging activity of catechins and their epimers (Nanjo et al., 1996). In a related study, Xu, Yeung, Chang, Huang, & Chen (2004) performed DPPH free radical assay to examine

antioxidant activity of green tea extract (GTE) epimers (gallocatechin gallate, catechins gallate, gallocatechin, and catechin) and compared with their corresponding precursors (epigallocatechin gallate, epicatechin gallate, epigallocatechins and epicatechin). Antioxidant activity of CG and GCG was similar to their precursor ECG and EGCG, respectively whilst C and GC were less potent than their precursors, EC and EGC. It is concluded that epimerization reaction occurring does not affect green tea antioxidant activity significantly.

2.8   Antioxidant activity

2.8.1   Action of antioxidant

Phytochemicals with antioxidant activity include phenolics, flavonids, cinnamic acids, proteins, carotenoids and lignans as well as sterols (Ou et al., 2001). Like many phytochemical, phytosterols exhibit antioxidant activity (Wang, Hicks, & Moreau, 2002). These compounds are receiving increased attention because consumers desire products with "all natural" labels. There has also been safety concern over the use of common synthetic antioxidants such as BHA (butylated hydroxinasole), BHT (butylatecl hydroxytoluene) and TBHQ (tert-butyhydroquinone). Antioxidants are necessary in many fat-containing foods to combat the damage to sensory and nutritional quality caused by lipid oxidation. Antioxidants are capable of donating hydrogen radicals and, therefore, reducing primary radicals to non-radicals (Ou, Hampsch-Woodill, & Prior, 2001). Once the hydrogen atom is donated, a radical with low reactivity is formed so that further reactions with lipids are halted. Antioxidants are classified as either primary or secondary. Primary antioxidants react with peroxide radicals prior to their reaction with unsaturated lipid molecules and convert them to more stable compounds. Secondary antioxidants slow the rate of chain

initiation (the autoxidation of a fat due to the contact of an unsaturated lipid with oxygen leading to the formation of free radicals) by methods such as scavenging oxygen; absorbing UV radiation, binding metal ions and deactivating singlet oxygen (Jadhav, Nimbalker, Kulkani, & Madhavi, 1996).

2.8.2   Antioxidants as antimicrobials

Antioxidants may be used to inhibit fungal arid bacterial growth and in some cases toxin production. Essential oils obtained from herbs and spices as well as extracts from the leaves and bark of traditional medicinal plants are natural antioxidants that have been investigated for their antimicrobial activity (Rajalakshmi & Narasimhan, 1996). The impetus for the development of natural antimicrobials is the same as that for natural antioxidants: consumer concern about synthetic ingredients. In addition, some organism has developed resistance to antibiotics and antimicrobials that are commonly used. Several natural antioxidants have been shown to exhibit antimicrobial activity. The mechanism of action differs between compounds, but activity has been shown to be dependent on the plant from which the extract has been produced and the bacterial strain against which it was tested. Flavonoids act as primary antioxidants, as well as chelators and superoxide anion scavengers. Their antioxidant activity is enhanced by the presence of a 3-hydroxyl group in their structure as well as a 2-3 double bond in their C ring (Blumberg, 2002). Spices and herbs contain many antioxidant compounds and some have yet to be identified and isolated (Rajalakshmi & Narasimhan, 1996). Phenolic acids are a group of compound found in parts whose antioxidant activity is correlated with the number of hydroxyl group in the molecule (Blumberg, 2002). Carotenoids are a group of antioxidants found in plants that prevent the formation of hydroperoxides in the presence of single oxygen by quenching the

single oxygen. This ability is related to the number of double bonds ill the molecule (Blumberg, 2002).

Flavonoids reportedly have antibacterial activity against microorganisms including *vuilgaris, Staphylococus aureus* and *Staphylococcus* epidermis (formerly Staphylococcus albus) (Tereschuk, Riera, Castro, & Abdala, 1997). Flavonoids extracted from *Tagetes minuta* tea leaves at low concentrations was shown effective against *Escherichia coli* growth in comparison to the antibiotic chloramphenicol (Tereschuk et al.,1997). Extract of the tea leaves were Rho effective in inhibiting *Bacillus subtilis* and *Pseudomonas aeruginosa*. However, *S. cerevisiae*, *Lactobacillus* rahmnosus, *L plantarum* and *Zymomanas* mobilis were not inhibited by the extracts.

The antimicrobial activities of phytosterol may not be effective againsta all range of microorganisms. For instance the *B-sitosterol* antimicrobial activity of selected herbs may inhibit the growth of *B. subtitles* at a concentration of 50 mg/ml although higher concentration (100 mg/ml) may be required against *Staphylococcus* aureus, *E. coli*, and *Psudomonas aeruginosa* (Beltrame et al. (2002). While investigating the antimicrobial activity of the Tanzanian plant *Uvaria schefflerii.* Moshi & Joseph (2004) extracted a mixture of stigmasterol and B-sitosterol the Ieaves. This 1:1 mixture of the phytostemls exhibited antifungal activity against *C albicants*, but failed to inhibit the growth of *Aspergillus niger, A. fumigatus .E. coli, S. aureus, P. aeruginosa and a Penicilium* species.

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