abstract

In the present study, eight cultivars of ripe mango fruits viz., *Alphonso*, *Banginapalli*, *Neelam*, *Raspuri*, *Rumani*, *Sindhura Totapuri* and *Mulgoa* grown in Andhra Pradesh, South India, were procured from the local market, Tirupati. Fresh edible portion of the fruits was used for the study. Mango (*Mangifera indica* L.) juice and wine samples were irradiated at a doses of 0, 0.5, 1.0 and 3.0 kGy and phsico-chemical properties, microbial load (bacteria, yeast and mold), total polyphenols, flavonoids, ascorbic acid contents, antioxidant activities, colour, aroma volatiles and sensory properties were evaluated. Variability was observed in different physico-chemical properties of mango juice and wine at different dose levels. The colour of mango juice and wine was improved in the irradiated samples, which is essential for maintaining the quality during storage. Microbiological assay of the mango juice and wine showed better quality after γ-irradiation. A significant ($P \leq 0.05$) reduction in ascorbic acid was observed in all the irradiated mango juice samples, however, dehydroascorbic acid was stable with increase in irradiation dose. The total polyphenols and flavonoids were significantly ($P \leq 0.05$) increased in all irradiated samples leading to increased free-radical scavenging activities (DPPH, FRAP, NO, ABTS and DMPD) in all cultivars of mango juice and wine samples. Mango juice and wine were found to be rich source of antioxidants and possessed strong *in vitro* radioprotective activity. γ-Irradiation appeared to be a suitable method for improving some defects and producing higher taste quality in mango wine, without the presence of irradiation residues. It revealed that γ-irradiation could be an effective method for improving the aroma volatiles and for enhancing sensory attributes of all cultivars of mango juice and wine samples.

The present study also revealed noticeable improvements of several hepatic and kidney parameters by following non-irradiated and γ-irradiated mango juice (GMJ) and mango wine (GMW) consumption in contrast with the rats with similar ethanol intake. Gamma irradiated mango products can be considered safe as they did not cause any lethality or adverse changes in the general behavior, and also no detrimental effects caused by these irradiated beverages in biochemical and histopathological changes in rat model could be observed. This shows the non toxic nature of the GMJ and GMW. Thus these may be safely administered for further *in vivo* studies to evaluate their antioxidant activities in humans.

1. GERERAL INTRODUCTION

Mango (*Mangifera indica* L.) is the most economically important fruit in the Anacardiaceae family, which comprises more than 70 genera. The word 'mango' originated as early as 16th century from the ancient Tamil word '*mangos*'. Historical records suggest that its cultivation as a fruit tree originated in India around 4000 years ago. In the early period of domestication, mango trees probably yielded small fruits, but folk selection of superior seedlings over many hundreds of years would have resulted in the production of larger fruits. Before 1970, mangoes were little known to consumers outside the tropics and the trade involving fresh fruit was non-existent. There was, nevertheless, in the subsequent years, a rapid expansion of mango production into nontraditional areas and the mango trade became well established as fresh fruit and processed products. With a growing world production, the mango represents one of the most important tropical fruits and is produced worldwide. Mango production is, however, quite concentrated, since Asia accounts for approximately 77% of global mango production, and America and Africa account for the remaining 23%. The world production of mangoes is estimated to be over 26 million tons per annum. India ranks first among the world's mango producing countries, accounting for 54.2% of the total mangoes produced worldwide and it is commercially the most important fruit crop in India, with more than a thousand varieties known to date. Other prominent mango producing countries include China, Thailand, Indonesia, Philippines, Pakistan, and Mexico.

Mango is an important fruit for human nutrition in several parts of the world. It is a tropical fruit widely accepted by consumers throughout the world for its succulence, sweet taste and exotic flavor, being called the 'king of fruits' (Tharanathan *et al*., 2006). Mango pulp is consumed in varied forms in both ripe and unripe stages. It is mostly eaten fresh, but a vast range of processed foods and drinks can be prepared, such as pickles, beverages, vinegar, chutneys, and desserts, as well as dessert flavoring and meat tenderizer. Industrially, there are three parts of interest from the mango fruit viz., the pulp, peel, and the seed. Conventionally the mango pulp is the main focal point in mango utilization both domestically and industrially. Along with the trade expansion of fresh mangoes, there has been an increase in world demand for processed mango products.

1.1 Mango processing: Fruit processing is one way to reduce losses at peak harvest periods and to maximize the fruit's great potential through varied products, including juices, nectar, flesh, and others. Mango is perhaps one of the most important fruits of the world which can be utilized by the processing industry during the different stages of its growth, development, maturity and ripening. Besides, since it is a fruit with high nutritive value, it is quite advantageous from the human health standpoint to enable fruit supply throughout the year via processed products. The products prepared both from ripe and green mangoes are highly popular in India and abroad. India dominated the world trade of processed mango products, even-though hardly 1% of the total mango production in India is processed. Mango processing also allows the use of less appealing varieties which cannot be sold on the fresh fruit market. Not only can the mango flesh be used for human food, but the waste originating from mango processing is a source of both macro- and micronutrients.

Export of processed mango products is continuously increasing. The major export product is canned mango pulp, which has increased over the past decade by about three times in volume and five times in value. Various processed products which can be prepared from both green and ripe mangoes. Although mango is a very popular tropical fruit, it is seasonal in nature. Due to this property, it is desirable to process it for future use. The result of the study on preparation of mango wine will inspire mango growers because it will add to the marketability and value of their product.

1.2 Mango as a fruit with high functional potential: The concept of a healthy diet has changed over the years. It was believed that a healthy diet provided all nutrients at adequate levels. Currently, besides supplying nutrients at adequate quantities and quality, it is believed that a healthy diet should have additional attributes, contributing to protection against diseases. Such protection is achieved by the presence of bioactive compounds contained in 'functional foods,' which are defined as 'a food that may provide a health benefit beyond the traditional nutrients it contains' (Scrinis, 2008).

1.2.1 Nutritional value of mango fruit: The mango can be included in the category of functional foods, since it provides the human diet with macro-and micronutrients and contains a large pool of bioactive compounds that are relevant to improving health and reducing the risk of disease. Furthermore, other parts of mango are also rich in bioactive compounds and nutrients, and could be exploited as nutraceuticals or active ingredients in the provision industry. Industrially, there are three parts of interest from the mango fruit namely—the pulp, peel, and the seed. Conventionally the mango pulp is the main focal point in mango utilization both domestically and industrially. The chemical composition of mango pulp varies with the location of cultivation, variety and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, minerals, pigments, tannins, vitamins and flavor compounds (Table 1.1). The Nutritional value per 100 g of fresh mango fruit is detailed in Table 1.2.

Table 1.1 The chemical composition of mango pulp

Food value per 100 g of ripe mango pulp	
Calories	62.1-63.7 Cal
Moisture	78.9-82.8 g
Protein	0.36-0.40 g
Fat	0.30-0.53 g
Carbohydrates	16.20-17.18 g
Fiber	0.85-1.06 g
Ash	0.34-0.52 g
Calcium	6.1-12.8 mg
Phosphorus	5.5-17.9 mg
Iron	0.20-0.63 mg
Vitamin A (Carotene)	0.135-1.872 mg
Thiamine	0.020-0.073 mg
Riboflavin	0.025-0.068 mg
Niacin	0.025-0.707 mg
Ascorbic acid	7.8-172.0 mg
Tryptophan	3-6 mg
Methionine	4 mg
Lysine	32-37 mg

Source: Tharanathan *et al*. (2006)

Table 1.2 Nutritional value per 100 g of fresh mango fruit

Principle	**Nutrient Value**	**RDA (%)**
Energy	70 Kcal	3.5%

Carbohydrates	17 g	13%
Protein	0.5 g	1%
Total Fat	0.27 g	1%
Cholesterol	0 mg	0%
Dietary Fiber	1.80 g	4.5%
Vitamins		
Folates	14 mcg	3.5%
Niacin	0.584 mg	3.5%
Pantothenic acid	0.160 mg	1%
Pyridoxine	0.134 mg	10%
Riboflavin	0.057 mg	4%
Thiamin	0.058 mg	5%
Vitamin C	27.7 mg	46%
Vitamin A	765 IU	25.5%
Vitamin E	1.12 mg	7.5%
Vitamin K	4.2 mcg	3.5%
Electrolytes		
Sodium	2 mg	0%
Potassium	156 mg	3%
Minerals		
Calcium	10 mg	1%
Copper	0.110 mg	12%
Iron	0.13 mg	1.5%
Magnesium	9 mg	2%
Manganese	0.027 mg	1%
Zinc	0.04 mg	0%
Phyto-nutrients		
Carotene-β	445 mcg	--
Carotene-α	17 mcg	--
Crypto-xanthin-β	11 mcg	--
Lutein-zeaxanthin	0 mcg	--
Lycopene	0 mcg	--

Source: USDA National Nutrient data base

1.3 Bioactive compounds in mango fruit: Apart from being important as a food, mango fruits as well as other parts of the plant are a source of bioactive compounds with potential health-promoting activity. All parts of mango trees have been used in traditional South Asian medicine: kernels, flowers, leaves, gum, bark, and peel. Diseases commonly treated with herbal remedies obtained from parts of the mango tree include dysentery, diarrhea, urinary tract inflammation, rheumatism, and diphtheria. A number of these uses are supported by scientific evidence (Ross, 2003). Bioactive compounds present in fruits have attracted attention from both the consumer and the scientific community, considering strong epidemiological evidences that show the benefits of fruit intake in human disease prevention (Ames *et al*., 1993). Mangoes contain several constituents which are included in the category of bioactive compounds with a great potential to modulate risk factors of diseases.

1.3.1 Ascorbic and dehydroascorbic acids: The term ‘vitamin C’ comprises the sum of ascorbic acid (AA) and dehydroascorbic acid (ADA) because ADA can be converted to AA in humans (Linster and Van Shaftingen, 2007). Similar to other fruits, mangoes differ in their ascorbic acid content because of genotype variations, climatic factors, agricultural practices, and ripening stage (Lee and Kader, 2000). Literature reports indicate great variation in ascorbic acid contents, ranging from 9.79 to 186 mg/100 g of mango pulp (Ribeiro *et al*., 2007). Besides other factors, such variation can be partially attributed to ripening stage, since ascorbic acid content declines during the maturation process. Therefore, products made from unripe or half-ripe mangoes usually have higher ascorbic acid content than those produced from ripe fruits.

1.3.2 Carotenoids: Mango pulp contains a wide pattern of carotenoids, including β-carotene, violaxanthin, cryptoxanthin, neoxanthin, luteoxanthin, and zeaxanthin. There is also a wide variation in β-carotene (550-3210 μg/100 g) and total carotenoid (1159-3000 mg/100 g) contents in pulp of different mango varieties (Ornelas-Paz *et al*., 2007; Ribeiro *et al*., 2007). However, since many of these studies do not differentiate the maturity level of the fruit tested, it cannot be concluded whether the differences are attributable to varietal characteristics or related to other factors, including ripening stage. For the first time, Varakumar *et al*. (2011) reported various carotenoid compounds in seven different cultivars of mango puree and wine (Table 1.3).

Table 1.3 Total carotenoid content in mango puree and wine (µg/100g).

Mango cultivar	Neoxanthin		Violaxanthin		Lutein		β-Carotene		Total carotenoids	
	Puree	Wine	Puree	Wine	Puree	Wine	Puree	Wine	Puree	Wine
Alphonso	41±2.42	12±1.4***	35 ± 2.1	9±0.9***	258±15	55±7.8***	5385±142	4251±138**	5720±240	4330±176*
Banginapalli	56±3.02	12±1.3***	42±1.54	13±0.97***	326±24	20±5.5***	3531±92	2898±101*	3955±151	2943±141*
Neelam	14±3.42	4±0.95*	37±1.21	12±1.08***	288±13	19±6.4***	641±159	543±53	980±152	578±101
Raspuri	12±0.9	2±0.55***	16±2.06	8±0.51*	212±19	42±3.5***	840±114	617±59	1080±132	634±113
Rumani	123±1.7	90±1.84***	30±1.14	12±0.67***	270±15	28±4.6***	3550±121	2753±91**	3970±151	2857±104**
Sindhura	102±3.6	31±0.84***	62±1.43	21±0.51***	452±21	37±4.1***	5192±109	4012±163**	5810±216	4101±163**
Totapuri	52±2.94	25±0.51***	15±1.01	7±0.49**	213±12	13±5.4***	1642±97	645±74***	1920±147	690±125**

***, **, * significantly varied when compared with the carotenoids in wine to puree at $p \leq 0.0001$, 0.001 and 0.01 respectively.

Source: Varakumar *et al*. (2011)

1.3.3 Polyphenolic compounds: The presence of the phenolic compounds glucogallin and gallotanin in mango flesh and seeds, and mangiferin, isomangiferin, homomangiferin, fisetin, quercetin, isoquercitrin, astragalin, gallic acid, methyl gallate, digallic acid, β-glucogallin, and gallotanin in leaves, twigs, seeds, and fruits of 20 local varieties was described by El Ansari *et al*. (1971). Peels and kernels contain large amounts of extractable phenolics, and there are varietal differences in their contents (Ribeiro, 2006). A wide pattern of phenolic compounds has been described in the pulp, peels, and kernels of mangoes. In particular, flavonols and xanthones have been identified and quantified (Berardini *et al*., 2005; Ribeiro *et al*., 2008; Barreto *et al*., 2008). The flavonols (quercetin, kaempferol, and rhamnetin) are present mostly as O-glycosides, whereas mangiferin is a C-glycoside and occurs both in its non-esterified form and conjugated with gallic acid. Also, Biological actions of mangiferin (1,3,6,7-tetrahydroxyxanthone-2-glucopyranoside) have been exhaustively studied, and several investigations have confirmed the bioactivity of xanthones. Studies conducted with VIMANG®, a formulation manufactured in Cuba that contains mangiferin as the main active ingredient, demonstrated protective effects in hepatic and brain tissues under oxidative stress in rats, inhibitory effect on carcinogenesis in rats, and against induced oxidative stress in cardiac and renal tissues of rats.

1.3.3.1 Polyphenolic composition of mango pulp: It has been reported that the total content of phenolic compounds in mango pulp ranges from 9.0 to 208.0 mg/100g (Ribeiro *et al*., 2007; Gil *et al*., 2006). Gallic acids, (*m*-digallic and *m*-trigallic acids), gallotannins, quercetin, isoquercetin, mangiferin, ellagic acid, and *b*-glucogallin are amongst the polyphenolic compounds identified in the mango pulp (Schieber *et al*., 2000). Gallic acid has been identified as the major polyphenol present in mango fruits, followed by six hydrolysable tannins and four minor compounds, *p*-OH-benzoic acid, *m*-coumaric acid, *p*-coumaric acid, and ferulic acid (Kim *et al*., 2007). In their work, Schieber *et al*. (2000) found 6.9 mg/kg of gallic acid and 4.4 mg/kg of mangiferin in mango pulp. In a polyphenol screening of 20 mango varieties, Saleh and El-Ansari (1975) reported the co-occurrence of mangiferin, isomangiferin, and homomangiferin in mango fruit pulp. Mangiferin has been shown elsewhere to be the main compound of leaves and stem bark with great medicinal values. It has been reported that phenolic compounds, such as gallic acid, and their associated antioxidant capacity decrease as fruit ripens (Kim *et al*., 2007). Gallotannins represent the major components of unripe fruits and seeds. According to Prabha and

Patwardhan (1986) gallic acid is the substrate of polyphenol oxidase in the fruit pulp, whereas ellagic acid is the predominant substrate in mango peel. The report on qualitative presence of various (individual) polyphenolic compounds present in Indian cultivars was not much available. For the first time, Kumar *et al*. (2012) reported various polyphenolic compounds in mango wine (Table 1.4).

1.3.4 Terpenoids: These are compounds belonging to the prenyl lipids class and represent probably the most widespread group of natural products. Monoterpenes and diterpenes, the main components of essential oils, can act as allelopathic agents, as attractants in plant-plant or plant- pathogen/herbivore interactions, or as repellents. In addition to carotenoids, which are tetraterpenoids, mangoes contain mono-, di- and triterpenoids, including ocimene, myrcene or limonene, terpinolene, and carene (Andrade *et al*., 2000; John *et al*., 2003). Several factors can affect the biosynthesis of aroma volatile compounds in mango (Pino *et al*., 2005). Terpenoids in mangoes have biological activity, contributing to raise the functional potential of the fruit, and the need for further studies to investigate their nutraceutical effects.

1.3.5 Fiber: Studies on mango pulp showed that a high proportion of the fiber fraction consists of pectin and its content in the peel is also quite high (Berardini *et al*., 2005; Sirisakulwat *et al*., 2008). Pectin is not hydrolyzed in humans by endogenous digestive enzymes but is fermented by the colon microflora, thus showing a prebiotic effect. Its biological activities have attracted interest in the last decades, because of their postulated positive effects on health such as cholesterol-lowering, cancer-preventing, and blood glucose-regulating properties (Kim, 2005).

The presence of pectin in mango pulp adds to it a functional attribute, and this point to the need for further investigation of fiber in mango pulp from different varieties. Fiber extracted from fruit agro-industrial residues can be used in the industry as a food ingredient. Although the pectin extracted from mango peel had net yield similar to apple pectin, its low content of anhydro-galacturonic acid leads to a low jellifying capacity (Sirisakulwat *et al*., 2008).

Table 1.4 Phenolic compounds identified in *Alphonso* mango wine by LC-MS.

Compound name	**Molecular weight**	***m/z*** **(M-H)**
p-Hydroxy benzoic acid	138	137 (93)
Caffeic acid	175	173 (135)
Vanillic acid	169	167 (123)
Ferulic acid	193	192 (134, 149, 179)
Syringic acid	197	198 (182, 153)
Protocatechuic acid	155	153 (109)
Leutolin	281	280 (217, 241, 175)
Sinapic acid	223	224 (208, 179, 149)
Quinaldinic acid	173	171 (165, 156)
Phenylphosphoramidic acid	325	324 (190)

Source: Kumar *et al*. (2012)

1.3.6 Antioxidant minerals: In the area of human nutrition, selenium, copper, zinc, iron, and manganese are included in the group of antioxidant minerals and their deficiency in the body affects the activity of enzymes involved in protection against oxidative stress. Thus, these elements have been considered essential minerals for the optimization of the antioxidant enzyme response. Compared with other foods, mango flesh contains lower levels of antioxidant minerals such as copper, iron, manganese, and zinc (Leterme *et al*., 2006). Nevertheless, the mineral content should not be neglected because mango consumption is associated with the intake of numerous antioxidants acting synergistically.

1.4 Total antioxidant capacity of mango fruit: Bioactive compounds can protect against diseases via several mechanisms, but it is believed that the antioxidant activity is extremely important for protection against diseases related to oxidative stress (Halliwell and Gutteridge, 1999). Mango contains at least three classes of compounds, i.e. ascorbic acid, carotenoids, and phenolic components that can support the antioxidant defense in humans. Despite the low content of the minerals copper, zinc, manganese, and iron in mango flesh, their importance should not be disregarded, as the fruit intake provides a set of antioxidants that may offer protection to the organism in a synergistic way.

The *in vivo* action of antioxidants demonstrates the synergism phenomenon, which is a cooperative action among several substances with antioxidant properties to protect oxidation targets (Zhou *et al*., 2005). Synergism occurs by co-antioxidant effect, involves more than one antioxidant with different reduction potentials and polarities participating in redox reactions in a system under pro-oxidant conditions, until a nonreactive product is formed, stabilizing the medium. Considering that mango contains this group of compounds, it can be assumed that the mango is a fruit with high antioxidant potential. Evidence suggests that a single antioxidant cannot replace a combination of antioxidants. Thus, a powerful antioxidant defense can be achieved in the biological media through mango consumption.

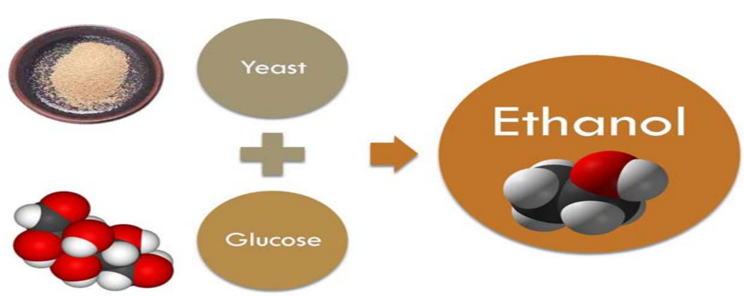
A research group performed an experiment supplementing the animals' diet with lyophilized mango flesh at 3%, which is a concentration equivalent to human consumption level. After having induced the oxidative stress, the animals were fed the diet containing mango at 3% in the subsequent 24 hours. The animals were then euthanized, and blood and liver were collected for analysis. A hepatoprotective effect was demonstrated with reduction of serum aminotransferases, mediated by antioxidant mechanism with decreased lipid peroxidation in liver homogenates. This finding confirmed that at concentrations similar to usual human consumption, mangoes provided protection to hepatic tissues against induced oxidative injury. These studies have demonstrated the potential bioactivity of compounds in mango flesh involving redox mechanisms.

1.5. Fruit wastage and value destruction: A staggering 50% of production of fruits and vegetables in India is lost due to wastage and value destruction. India ranks first few positions globally in the production of fruits and vegetables. It accounts for 8% of the world's production of fruits and leads in mango, banana, cauliflower and green peas. But this premier position in production is not reflected in processing, value addition and exports. Value added processed food in India accounts for only 2% by volume and 10% by value of the total production. Only 0.5-1% of the fruit and vegetable raw materials are processed in India compared to countries like Brazil and U.S. with 70% utilization. Our idea is to divert locally produced mangoes for juice and wine production.

1.6 Wine: It is an alcoholic beverage made from the juice of grapes or other fruits such as pears, apples, berries, and even flowers such as dandelions. During fermentation by yeast, sugars are metabolized to alcohol and CO_2. Wine contains about 85-89% water, 10-14% alcohol, less than 1% fruit acids, and hundreds of aroma and flavor components in very small amounts. The character of a wine is derived from factors in the fruits themselves used for wine-making and from the yeast species and strain used for fermentation.

Wine-making is one of the most ancient of man's technologies and is now one of the most commercially prosperous biotechnological processes. Advances in the second half of the 20^{th} century have clearly shown that fermentation of grape-must and the production of quality wines is not a simple process. Considerable progress has been made over the last decade in understanding the biochemistry and interactions of yeast, lactic acid bacteria, and other microorganisms during the wine-making process. The biological process of wine-making is the result of a series of biochemical transformations brought about by the action of several enzymes from different microorganisms, especially yeasts, which are responsible for the principal part of the process, alcoholic fermentation. Lactic acid bacteria are responsible for a secondary process, known as malolactic fermentation. The grape-must is also exposed to numerous enzymes originating from sources other than the yeasts that produce the alcoholic fermentation or the bacteria that produce malolactic fermentation.

1.6.1 Alcoholic fermentation: It is the anaerobic transformation of sugars, mainly glucose and fructose, into ethanol and carbon dioxide. This process, which is carried out by yeast and also by some bacteria such as *Zymomonas mobilis*, can be summarised by this overall reaction.



$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

1.6.1.1 Yeasts: These are single cell microorganisms classified in the kingdom Fungi. Regarding the morphology, yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 µm in diameter, with some being even larger (Barnett *et al*., 2000). Yeasts can grow over wide ranges of acid pH and with specific treatment they can produce alcohol up to 21% and grow in the presence of 55-60% sucrose (Thomas and Ingledew, 1992). Yeast colonies show colours from creamy, to pink, to red are produced by yeasts (Kurtzman and Fell, 2006). Approximately 1,500 (Kurtzman, 1998) and 678 species (Barnett *et al*., 2000) of yeasts have been described, most of which reproduce by budding, although in a few cases by binary fission. Of these, *S. cerevisiae* is widely accepted as the principal wine yeast due to its ethanol tolerance.

1.6.1.2 *Saccharomyces* yeasts: These are having a unicellular, globose, and ellipsoid to elongate shape. Multilateral (multipolar) budding is typical for vegetative reproduction (Vaughan-Matini and Martini, 1995). The two primary species found in wines are *S. cerevisiae* and *S. bayanus. Saccharomyces* can be detected, but is present on grape surfaces at very low levels (Prakitchaiwattana *et al*., 2004) and has been undetectable in some studies (Raspor *et al*., 2006). *Saccharomyces* is more commonly isolated from damaged grapes (Mortimer and Polsinelli, 1999). In general, the number of yeasts present on grapes increases with ripening. Seasonal variation has also been observed with warmer and dryer years yielding increased yeast populations (Rementeria *et al*., 2003). *S. cerevisiae* is the most important yeast for wine production and is responsible for the metabolism of grape sugar to alcohol and CO_2 (Pretorius, 2000). It has an equally important role to play in the formation of secondary metabolites of importance to wine (Pretorius *et al*., 2003), as well as in the conversion of grape aroma precursors to varietal aromas in wine (Ribereau-Gayon *et al*., 2000). For these reasons *S. cerevisiae* is often simply referred to as "the wine yeast".

1.6.1.3 Non-*Saccharomyces* yeasts: The presence of non-*Saccharomyces* species becomes more important in wine-making than in the past, although *S. cerevisiae* is principally responsible for the alcoholic fermentation. At different stages of the spontaneous fermentation, different phenotypes of the non-*Saccharomyces* yeasts are represented (Romano *et al*., 1997). The early stages of the spontaneous fermentation are characterized by the growth of *Kloeckera apiculata, Hanseniaspora uvarum, Hansenula anomala, Candida stellata, Candida pulcherrima* and several other species (Heard and Fleet, 1986). Low numbers of yeasts (10-10^3 CFU/g) are found on unripe grapes, but as the grapes ripen the numbers increase to 10^4-10^6 cfu/g (Fleet, 2003). They produce the metabolites which can contribute to the final taste and flavour of wines (Rainieri and Pretorius, 2000). Therefore, in recent years, wine researchers have realized that non-*Saccharomyces* yeasts can improve quality of wine more than previously thought of (Sommer *et al*., 2007). Numerous enological researches associated with non-*Saccharomyces* yeasts were conducted to study about their production of metabolites. A dominant characteristic of non-*Saccharomyces* yeasts is to produce great amount of components like esters, higher alcohols, acetic acid, acetoin thus volatile metabolites of these yeasts have been mainly focused. These components can make a contribution to the desirable fermentation bouquet of wine and on the other hand, they can also be considered detrimental to the wine quality (Heard and Fleet, 1986).

1.6.2 Malolactic fermentation (MLF): MLF in wine is a secondary fermentation that usually occurs at the end of alcoholic fermentation by yeasts, although it sometimes occurs earlier. It is practically a biological process of wine deacidification in which the dicarboxylic L-malic acid (malate) is converted to the monocarboxylic L-lactic acid (lactate) and carbon dioxide (Fig. 1.1). This process is normally carried out by lactic acid bacteria (LAB) isolated from wine, including *Oenococcus oeni* (formerly *Leuconostoc oenos*), *Lactobacillus* spp. and *Pediococcus* spp.

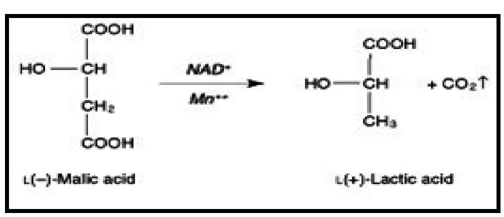


Fig. 1.1 Malolactic conversion.

1.6.2.1 *Oenococcus oeni*: It is described as a Gram-positive non-mobile coccus and frequently occurs in pairs and chains of different sizes. *Oenococcus* is a facultative acidophilic anaerobe and grows at pH 4.8 with temperatures between 18 °C and 30

°C. It requires a rich medium supplemented with tomato juice or grape juice, and its growth is not inhibited in the presence of 10% ethanol. Glucose is fermented in lactic acid, carbon dioxide, acetic acid and ethanol (it is a heterofermenter). It converts malate into lactate and CO2 in the presence of fermentable carbohydrate.

1.6.2.2 *Lactobacillus*: *Lactobacillus* represents a highly diverse group of Gram-positive, microaerophilic bacteria; its cells are non-mobile and they have long rod-like forms or short rods and can appear as single cells, in pairs or in chains of different sizes. Bacteria belonging to this genus are facultative anaerobes and require a rich medium containing fermentable sugar. Several species of *Lactobacillus* have been isolated from grapes and wines worldwide, including *L. brevis*, *L. buchneri*, *L. casei*, *L. paracasei*, *L. cellobiosus*, *L. curvatus*, *L. delbrueckii*, *L. diolivorans*, *L. fructivorans*, *L. heterohiochii*, *L. hilgardii*, *L. jensenii, L. kunkeei*, *L. leichmanni*, *L. lindneri, L. mali*, *L. nagelli*, *L. paracasei, L. plantarum*, *L. trichodes, L. vermiforme*, *L. vini*, *L. yamanashiensis* and *L. zeae* (Moreno-Arribas and Polo, 2008).

1.6.2.3 *Pediococcus*: Cells are non-mobile and have a spherical shape; these are the only LAB that separate into two planes, which results in the formation of pairs, tetrads or large clumps of spherical cells. Bacteria belonging to these genera are facultative anaerobes and require a rich medium containing growth factor and fermentable sugar for their development. Their optimum temperature is 25–30 °C with a pH value of 6. They are homofermentative, which means that all the glucose is metabolized into lactic acid and they do not ferment pentose. Among the approved species of *Pediococcus*, only four have been isolated from wines: *P. damnosus*, *P. parvulus*, *P. inopinatus* and *P. pentosaceus* (Edwards and Jensen 1992); *P. pentosaceus* and *P. parvulus* are the most common species in this medium.

1.7 Non-grape fruit wine: The word '*wine*' has more than one definition and means different things in different parts of the world. The most restrictive definition, and probably the original and most widely accepted one, is 'the fermented juice of freshly crushed and pressed grapes.' This demonstrates the dominance of the grape over other sources (fruits particularly) in the production of alcoholic beverages. In many countries alcoholic beverages made from fruits other than grapes enjoy a significant economic standing and a good reputation, although they often have to contend with deep seated prejudices against them, in favor of grape wine. Alcoholic beverages can be made from any source of fermentable carbohydrates: fruits, vegetables, cereals, roots, honey, sap, leaves and flowers, with or without the use of adjuncts. In English speaking countries, these beverages are often known generally as ‘country wines’ and those made from fruits are often simply called ‘fruit wines’.

Wines have been consumed since human settlement in the Tigris-Euphrates River basin, when they were used as therapeutic agents. The *Rig Veda*, one of the sacred scriptures of Hinduism composed between 1500 and 900 B.C., also mentioned the medicinal value of wine. The actual birthplace of wine is unknown, although it is known that wines were prepared by the Assyrians by 3500 B.C. Cider (or sicera) fermentation has been practiced for over 2,000 years and was a common drink at the time of Roman invasion of the British Isles in 55 B.C. Essentially, wine is a transformation of juice by yeast during fermentation, involving a series of biochemical reactions.

In many cases, the driving force behind the production of non-grape alcoholic beverages is their conversion to spirits by distillation. This includes the following conversions: ‘grain or cereal wines’ to Akvavit (Aquavit), gin, vodka, whiskey or whisky, ‘rice wine’ to soju or shochu, fruit wine to fruit spirit and Schnaps, potato and other ‘wines’ to aquavit or vodka, and sugar cane or molasses ‘wine’ to rum. However Wines are preferable to distilled liquors for their stimulatory and healthful properties. Moderate consumption of low levels of alcohol has been associated with lowered mortality from not only all coronary heart diseases (CHD) but also other diseases. These beverages are regarded as an important adjunct to the human diet.

1.8. Factors affecting quality of fruit wines: The quality of wine is determined primarily by the composition and quality of the raw material used and the wine-making practices followed. These factors include the specific fruit variety; stage of harvest and maturity of the fruit; total sugar, acid, phenols, pigments, nitrogenous compounds, etc., in the fruit or its juice; additives such as nitrogenous compounds, pectinase, sulfur dioxide, or other preservatives; fruits yielding pulp or juice; yeast strains and other wine-making practices; post-fermentation operations (especially the length of maturation); and the method of preservation employed. Sugar is the second-most important raw material dictating the cost of production of these beverages. Almost all the non-grape fruits, their juices are low in sugar but have high acidity and must be ameliorated to produce a table wine (Table 1.5). Even when the sugar level is satisfactory, the high acidity demands dilution, consequently requiring addition of sugar to the juice. Despite these deficiencies, some fruit wines (as well as cider and perry) are produced commercially from their fruit juices without any or with only little adjustment. However, Reddy and Reddy (2005) found that the sugars in the matured mango are adequate to produce a high quality wine without ameliorating with external sugars, and also have low acidity when compared to other fruits.

Yeast growth in wine fermentation is influenced by a number of factors including composition of juice, clarification of juice, addition of SO_2 and or other preservatives, temperature of fermentation, inoculation of juice or pulp, and interaction with other microorganisms. Although *S. cerevisiae* can grow over a wide temperature range (0 to 45ºC), the favorable temperature is between 22 and 27ºC. The fermentation temperature greatly influences the production of aroma-active compounds, because at higher temperatures more loss of volatile compounds results, while at lower temperatures more entrainment of alcohol by CO_2 takes place. Different microorganisms belonging to molds, yeast, and bacteria are involved in wine making. In addition to damaging the fruits, molds can affect the flavor of wine by their growth on the cooperage. However, molds, being aerobic, cannot grow in wine due to the inhibitory effect of ethanol and the prevailing anaerobic conditions. Yeasts are important microorganisms associated not only with the production but also the spoilage of wine. Although the yeast strains of *S. cerevisiae* predominantly carry out the alcoholic fermentation of fruit juices, others, known as wild yeasts, may spoil the wine. Some of these wild yeasts called “killer yeasts” secrete toxins affecting the fermentation. Slow or incomplete alcoholic fermentation is a chronic problem for the wine industry, and the premature termination of alcoholic fermentation is known as stuck fermentation. Several factors have been held responsible for impacting fermentation rate and leading to stuck and sluggish fermentation, including nutrient limitation, ethanol toxicity, organic and fatty acid toxicity, presence of killer factors or other microbially produced toxins, cation imbalance, temperature extremes, pesticide/ fungicide residue, microbial competition, poor enological practices, excessive SO_2 use, excessive must clarification, and lack of agitation. The wine making process could be seen as the product of enzymatic transformation of fruit juices. Enzymes could be contributed by the juice or yeast or could be added exogenously. A number of enzymes, such as pectinases, oxidoreductase, proteases, glycosidase, and lipoxygenase, play a significant role in grape wine-making.

A typical wine contains ethyl alcohol, sugars, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, and fatty acids in addition to minor constituents such as methanol and flavoring compounds. Quantitatively, ethyl alcohol is the most important component present in all alcoholic beverages and is associated with the stimulating and intoxicating properties of these beverages.

Alcoholic beverages are distinguished based on their quantity of ethyl alcohol. Dry wines have very low or almost negligible sugar, while sweet/dessert wines may contain considerably higher quantities. Acids are significant in maintaining pH, balancing the sugar level, and helping flavor development. Grapes and apples contain tartaric and malic acid, respectively, as major acids, but in wine acetic acid is responsible for acidity. Fruit wines have pigments derived from the fruits themselves. Methanol is also found in alcoholic beverages, and its quantity is influenced by the type of fermenting agent, the temperature, and the raw materials used. Naturally fermented alcoholic beverages contain abnormally high levels of methanol, which arises from hydrolysis of pectins by the action of pectinases.

Among the minerals, K, Ca, Na, and Mg are the major elements present in wines that play an important role in alcoholic fermentation, being used by yeast for its metabolism. Trace elements (Fe, Cu, Mn, and Zn) are significant for the normal alcoholic fermentation and growth of yeast, by taking part in the oxidation reduction system. The zinc content of wines (which normally occurs as the result of contact with galvanized iron utensils) should not exceed 5 mg/L (legal limit and threshold for astringent taste). Normal levels of Cu, Mn, and Zn in wines are not toxic.

Biochemically, higher alcohols constitute as important components of wine. The color and taste of wines depend upon the total amount and properties of the phenolics of wine. Using the biochemical characteristics, quality-defining values, called the “RSK value,” have been developed for wine. Esters are another indispensable component influencing wine quality, imparting the fruity flavor to wines. Normally, the amount of esters increases with enhancement in maturation periods.

Table 1.5 Sugar and acid content of crushed fruit used to make fruit wines. These are typical values in % w/w of fresh fruit, assuming fully but not overripe fruit (Source: Buglass, 2011)

Fruit	**Apple**	**Apricot**	**Banana**	**Bilberry/ blueberry**	**Blackberry**	**Blackcurrant**	**Cherry**
Sugar content	14	14	17	11/10	8	10	14
Acid content	1.0	1.5	0.3	0.8/0.3	1.2	3	1.0

Fruit	**Crab apple**	**Damson**	**Gooseberry**	**Grape (wine)**	**Grape (table)**	**Loganberry**	**Mulberry**
Sugar content	16	14	9	20	14	11	10
Acid content	2.0	2.0	1.5	0.5	0.3	2.0	1.0

Fruit	**Orange**	**Pear**	**Plum**	**Raspberry**	**Strawberry**
Sugar content	11	16	15	11	8
Acid content	1.0	0.6	1.2	1.5	1.2

1.9. Mango wine – an alternative process to avoid fruit wastage: Wine manufacture is challenging, in the sense of obtaining a product which is acceptable in the market, however, the processes involved in its production are relatively straightforward (Vine *et al*., 1997). Grapes have been used as the main raw material in the production of wine from past many centuries. However the temperature during cultivation plays a major role and all grapes are not suitable for wine-making, and are not abundantly available throughout the world. Hence wine from non-grape fruits were tried by many number of researchers and found other suitable fruits for wine production. Over the years, fruit wine has been prepared from several different fruits, such as caja, banana, pupunha, mango, acerola and cocoa (Duarte *et al*., 2009). The fruits commonly used for making wine are: apple, pear, peach, plum, cherry, strawberry, blackberry, raspberry and blueberry. The choice of fruit depends upon several factors including market demand, availability of raw material, production facilities, and sound economic reasons (Dharmadhikari, 1996). Highly acceptable wines can be made practically from all fruits. There are some soft fruits from both temperate and tropical regions whose pigment stability and flavour profiles match those of any wine from grapes, but suffer from the lack of intensive research and development that is given to grape wine (Cheirslip and Umsakul, 2008). Among those fruits mango (*Mangifera indica*, L.) is the most popular and the choicest fruit of India. It is highly perishable and seasonal fruit, and one of the methods for processing and preserving mango is to ferment the juice, which has high carbohydrate content into wine. The research on mango wine lacked intensive drive till recently although it started from 1960's. For the first time Czyhrinciwk, (1966) reported the technology involved in mango wine production. Later Onkarayya and Singh, (1984) screened twenty varieties of mangoes that are available in India for wine production. Obisanya *et al*. (1987) studied the fermentation of mango juice into wine using locally isolated *Saccharomyces cerevisiae* and *Schizosaccharomyces* species of palm wine and they concluded that *Schizosaccharomyces* yeasts were suitable for the production of sweet, table mango wine and *Saccharomyces* yeasts were suitable for the production of dry mango wine with a higher ethanol level.

From the physicochemical characteristics of the mango wine produced, it was observed that aromatic components were comparable in concentration with those of wine from grapes (Reddy and Reddy, 2005). In view of the differences noticed in some mango wine with different varieties of mangoes necessitates the screening and selection of good quality of mango fruit, which is an important step to get good quality product. Reddy and Reddy, (2005) screened six varieties of mangoes that are locally available in Chittoor district of South Andhra Pradesh and reported that *Banginapalli* and *Alphonso* varieties are most suitable for mango wine production. Later Reddy and Reddy, (2009) studied the effect of enzymatic maceration on synthesis of higher alcohols during mango wine fermentation and found that higher amounts of volatiles were observed in wine produced from *Totapuri* cultivar than wine from the cultivar *Banginapalli*. Also, treatment of mango pulp with the enzyme pectinase increased the synthesis of iso-amylalcohol, 2-phenyl ethanol, n-propanol, n-butanol and methanol during fermentation. Sensory evaluation scores of wine correlated to the sum of higher alcohols.

Kumar *et al*. (2009) had optimized condition for mango wine-making using statistical software, response surface methodology (RSM) by using *S. bayanus*. The predicted values for optimization process conditions were shown to have good agreement with experimental data. The results showed that a satisfactory production of ethanol from the mango juice could be achieved reaching up to 10%, and showed that overall acceptance of mango wine with good quality. The volatile aroma composition of mango wine from the two cultivars *Banginapalli* and *Alphonso* were reported by Reddy *et al*. (2010) by gas chromatography coupled with mass spectrometry (GC-MS) and showed thirty-two volatile compounds in wines and concluded that the wine prepared from the cultivar *Banginapalli* had better aroma composition and taste than that from the *Alphonso*. Carotenoid composition of seven cultivars of mango wine and its antioxidant activity were reported by Varakumar *et al*. (2011) and concluded that the percentage decrease in xanthophylls levels in mango wine were in the range of 69.3–89.7%, and >80% degradation was noted in *Banginapalli*, *Neelam*, *Sindhura* and *Totapuri* and 15.3–26.5% for β-carotene. However, significant degradation of β-carotene was observed in only *Totapuri* wine, indicating that lutein was more sensitive to degradation than β-carotene during fermentation. They also concluded that mango wine is capable of preventing lipid peroxidation through scavenging free radicals and the antioxidant activities of mango wine could be due to the synergic effect of carotenoids, glutathione and polyphenols.

Reddy and Reddy, (2011) reported the effect of fermentation conditions (temperature, pH, SO_2 and aeration) on wine fermentation and evaluated yeast growth, duration, fermentation rate and volatile composition and concluded that the temperature (25ºC), pH (5), SO_2 (100 ppm) for low alcoholic fermentation temperatures in wine making. Li *et al*. (2011) compared the chemical and volatile composition of mango wines fermented with 3 yeast stains and measured various volatile compounds like fatty acids, alcohols and esters and concluded that it may be possible to produce mango wines with differential characteristics using different *S. cerevisiae* strains. Pino and Queris, (2011) reported the volatile compounds of mango wine by using GC-FID and GC-MS. A total of 102 volatile constituents were identified in the mango wine and these account for about 9 mg/L, including 40 esters, 15 alcohols, 12 terpenes, 8 acids, 6 aldehydes and ketones, 4 lactones, 2 phenols, 2 furans, and 13 miscellaneous compounds. This study concluded that the compounds potentially most important to mango wine were ethyl butanoate and decanal.

Varakumar *et al*. (2012a) prepared the mango wine using a new yeast-mango-peel immobilized biocatalyst system and concluded that it is a good and effective system for wine fermentation at both low and room temperatures, as the wines produced by this procedure had a potentially better aroma than those obtained by free-cell fermentation. The biocatalyst is economical, food grade, and does not need special pretreatment before use. Mango peels, which otherwise may pollute the environment can be beneficially used as an alternative cell immobilization support. Again from the same research group based in India (Varakumar *et al*., 2012b) reported that the aroma and sensory properties of mango wine were improved by co-fermentation with *S. cerevisiae* and *Torulaspora delbrueckii* or *Metschnikowia pulcherrima* and concluded the evaluation of non-*Saccharomyces* yeasts used in the present study might be of great value for the mango wine-making industry.

Kumar *et al*. (2012) reported phenolic composition (LC-MS method), antioxidant and sensory properties of mango wine and concluded 11 phenolic compounds were identified in mango wine (*Alphonso* cultivar) and from the antioxidant assays, it was found that mango wines possess a substantial antioxidant capacity. They also concluded that wines made from *Alphonso* and *Banginapalli* cultivars have been found to have better sensory properties. Li *et al*. (2012a) reported the volatile composition of mango wine fermented with two *Williopsis* yeast strains and reported various classes of volatile compounds like terpenoids, esters, alcohols, acids, aldehydes and ketones, ethers, phenols and sulphur compounds. This study concluded that unlike mango wine fermented with *S. cerevisiae*; most terpenoids derived from mango juice were retained in the resultant mango wine fermented with the two *Williopsis* yeast strains, suggesting that the mango wine could retain the aromatic hints of fresh mango. Again from the same research group based in Singapore (Li *et al*., 2012b) reported the behavior and fermentation performance of mixed yeasts using *S. cerevisiae* and *Williopsis saturnus* in juices of three mango varieties. This study concluded that mango wine from cultivar *Nam Doc Mai* possessed the highest aroma intensity with winey, yeasty, fruity and floral notes attributed to higher amounts of alcohols, acetate esters and ethyl esters. Varakumar *et al*. (2013) reported role of malolactic fermentation on the quality of mango wine and concluded that wine yeast using (S.C., UCD 522 and UCD 595) the simultaneous inoculation method with yeast and *O. oeni* at the beginning of alcoholic fermentation resulted in an increased consumption of malic acid and imparted better sensorial contributions when compared to sequential method.

Li *et al*., (2013a) reported the impact of pulp on the chemical profile of mango wine and concluded macerated wine contained more terpenes, terpenols, higher alcohols and fruity acetate esters than the non-macerated wine and the non-macerated wine possessed a higher concentration of medium-chain fatty acids and corresponding ethyl esters. The inclusion of pulp in mango wine fermentation, which is similar to the maceration process in red grape wine fermentation, was beneficial for retaining the original terpenic character of mango, and also improved other mango wine aroma characters (i.e. complexity) such as fruitiness. Again from the same research group based in Singapore (Li *et al*., 2013b) reported influence of pulp maceration and β-glucosidase on mango wine physico-chemical properties and volatile profiles and concluded that pulp contact could enhance monoterpene hydrocarbons, higher alcohols & acetate esters and the addition of β-glucosidase could accelerate the release of odour-active volatiles such as terpenols, acetate esters, benzene derivatives and C_{13}-norisoprenoids. Furthermore, both β-glucosidase addition and pulp contact could mitigate production of excess fatty acids and their ethyl esters (‘yeasty’ note in sensory test). This application of β-glucosidase with mango pulp contact was effective in intensification, diversification and also balancing of mango wine aroma profile. However, no study was reported regarding quality improvement of mango juice and its wine by using gamma irradiation and *in vivo* antioxidant activity of mango juice and wine. Hence studies are needed to look in to these quality matters.

1.10 Food irradiation: Food irradiation is the process of exposing food to ionizing radiation to destroy microorganisms, bacteria, viruses, or insects that might be present in the food. Further applications include sprout inhibition, delay of ripening, increase of juice yield, and improvement of re-hydration. Irradiation is a more general term of deliberate exposure of materials to radiation to achieve a technical goal (in this context "ionizing radiation" is implied). As such it is also used on non-food items, such as medical hardware, plastics, tubes for gas-pipelines, hoses for floor-heating, shrink-foils for food packaging, automobile parts, wires and cables, tires, and even gemstones. Compared to the amount of food irradiated, the volume of those every-day applications is huge but not noticed by the consumer.

The specialty of processing food by ionizing radiation is that the energy density per atomic transition is very high; it can cleave molecules and induce ionization (hence the name), which is not achieved by here heating. This is the reason for both new effects and new concerns. The treatment of solid food by ionizing radiation can provide an effect similar to heat pasteurization of liquids, such as milk. However, the use of the term "cold pasteurization" to describe irradiated foods is controversial, since pasteurization and irradiation are fundamentally different processes. Food irradiation is currently permitted by over 40 countries, and the volume of food so treated is estimated to exceed 500,000 metric tonns annually worldwide.

By irradiating food, depending on the dose, some or all of the harmful bacteria and other pathogens present are killed. This prolongs the shelf-life of the food in cases where microbial spoilage is the limiting factor. Some foods, e.g., herbs and spices, are irradiated at sufficient doses (five kilograys or more) to reduce the microbial counts by several orders of magnitude; such ingredients will not carry over spoilage or pathogen microorganisms into the final product. It has also been shown that irradiation can delay the ripening of fruits or the sprouting of vegetables. Furthermore, insect pests can be sterilized (be made incapable of proliferation) using irradiation at relatively low doses. In consequence, the United States Department of Agriculture (USDA) has approved the use of low-level irradiation as an alternative treatment to pesticides for fruits and vegetables that are considered hosts to a number of insect pests, including fruit flies and seed weevils; the U.S. Food and Drug Administration (FDA) has cleared among a number of other applications the treatment of hamburger patties to eliminate the residual risk of a contamination by a virulent *E. coli*. The United Nations Food and

Agricultural Organization (FAO) have passed a motion to commit Member States to implement irradiation technology for their national phytosanitary programs; the general assembly of the International Atomic Energy Agency (IAEA) has urged to make wider use of the irradiation technology. Additionally, the USDA has made a number of bi-lateral agreements with developing countries to facilitate the imports of exotic fruits and to simplify the quarantine procedures.

The European Union has regulated processing of food by ionizing radiation in specific directives since 1999; the relevant documents and reports are accessible online. The "implementing" directive contains a "positive list" permitting irradiation of only dried aromatic herbs, spices, and vegetable seasonings. However, any Member State is permitted to maintain previously granted clearances or to add new clearance as granted in other Member States, in the case the EC's Scientific Committee on Food (SCF) has given a positive vote for the respective application. Presently, six Member States (Belgium, France, Italy, Netherlands, Poland, and United Kingdom) have adopted such provisions.

Because of the "Single Market" of the EC, any food - even if irradiated - must be allowed to be marketed in any other Member State even if a general ban of food irradiation prevails, under the condition that the food has been irradiated legally in the state of origin. Furthermore, imports into the EC are possible from third countries if the irradiation facility had been inspected and licensed by the EC and the treatment is legal within the EC or some Member State. The SCF of the EC has given a positive vote on eight categories of food to be irradiated. However, in a compromise between the European Parliament and the European Commission, only dried aromatic herbs, spices and vegetable seasonings can be found in the positive list. The European Commission was due to provide a final draft for the positive list by the end of 2000; however, this failed because of a veto from Germany and a few other Member States. In 1992 and in 1998 the SCF voted "positive" on a number of irradiation applications that had been allowed in some Member States before the EC Directives came into force, to enable those member states to maintain their national authorizations.

In 2003, when Codex Alimentarius was about to remove any upper dose limit for food irradiation, the SCF adopted a "revised opinion", which in fact was a re-confirmation and endorsement of the 1986 opinion. The opinion denied cancellation of the upper dose limit, and required that before the actual list of individual items or food classes can be expanded, new individual studies into the toxicology of each of such food and for each of the proposed dose ranges are requested. The SCF has subsequently been replaced by the new European Food Safety Authority (EFSA), which has not yet ruled on the processing of food by ionizing radiation. Other countries, including New Zealand, Australia, Thailand, India, and Mexico, have permitted the irradiation of fresh fruits for fruit fly quarantine purposes, amongst others. Such countries as Pakistan and Brazil have adopted the Codex Alimentarius Standard on irradiated food without any reservation or restriction: i.e., any food may be irradiated to any dose (Kyle Wilson *et al*., 2003).

1.10.1 Unit of measure for irradiation dose: The dose of radiation is measured in the SI unit known as Gray (Gy). One Gy dose of radiation is equal to 1 joule of energy absorbed per kg of food material. In radiation processing of foods, the doses are generally measured in kGy (1,000 Gy).

1.10.2 Applications: On the basis of the irradiation dose, applications are generally divided into three main categories. They are:

- Low dose applications (up to 1kGy)
- Medium dose applications (1 kGy to 10 kGy)
- High dose applications (above 10kGy)

1.10.2.1 Low dose applications (up to 1 kGy)

- Sprout inhibition in bulbs and tubers (0.03-0.15 kGy).
- Delay in fruit ripening (0.25-0.75 kGy).
- Insect disinfestations including quarantine treatment and elimination of food borne parasites (0.07-1.0 kGy).

1.10.2.2 Medium dose applications (1 kGy to 10 kGy)

- Reduction of spoilage microbes to prolong shelf-life of meat, poultry and sea foods under refrigeration (1.5–3.0 kGy).

- Reduction of pathogenic microbes in fresh and frozen meat, poultry and sea foods (3.0–7.0 kGy).
- Reducing the number of microorganisms in spices to improve hygienic quality (10.0 kGy).

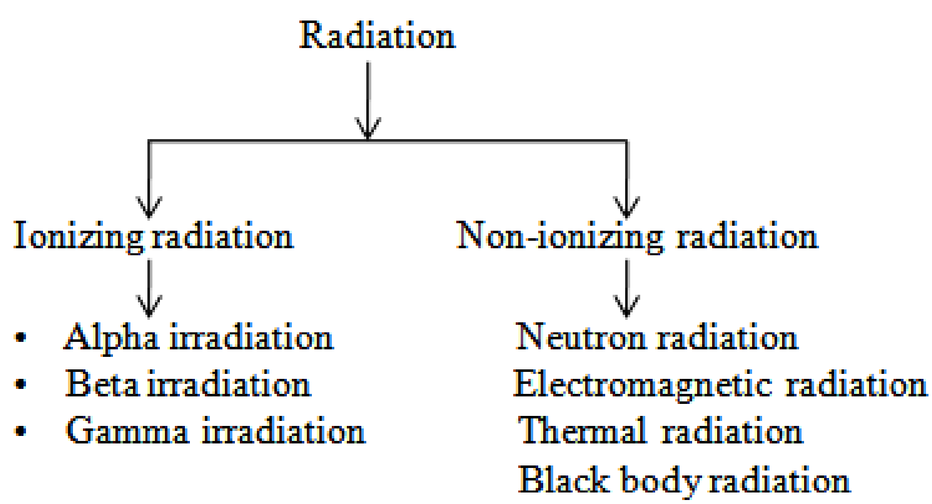
1.10.2.3 High dose applications (above 10 kGy)

- Sterilization of packaged meat, poultry and their products which are shelf stable without refrigeration. (25.0-70.0 kGy).
- Sterilization of Hospital diets (25.0-70.0 kGy).

1.10.3 Types of radiation: There are two types of radiation. They are

1. Ionizing radiation
2. Non-ionizing radiation

These radiations are again classified in to different types. They are:



1.10.4 Gamma irradiation: It is a radiation of photons in the electromagnetic spectrum. The radiation is obtained through the use of radioisotopes, generally cobalt-60 or caesium-137. Cobalt-60 is intentionally bred from cobalt-59 using specifically designed nuclear reactors. Caesium-137 is recovered during the refinement of “spent nuclear fuel”, formerly referred to as “nuclear waste”. Because this technology- except for military applications- is not commercially available, insufficient quantities of it are available on the global isotope markets for use in large scale, commercial irradiators. Presently, Caesium-137 is used only in small hospital units to treat blood before transfusion to prevent Graft-versus-host disease.

Food irradiation using Cobalt-60 is the preferred method by most processors, because of its deeper penetration (Fig. 1.2) enables administering treatment to entire industrial pallets or totes, reducing the need for material handling. A pallet or tote is typically exposed for several minutes to hours depending on dose. Radioactive material must be monitored and carefully stored to shield workers and the environment from its gamma rays. During operation this is achieved by substantial concrete shields. With most designs the radioisotope can be lowered into a water filled source storage pool to allow maintenance personnel to enter the radiation shield. In this mode the water in the pool absorbs the radiation. Other uncommonly used designs feature dry storage by providing movable shields that reduce radiation levels in areas of the irradiation chamber. One variant of gamma irradiators keeps the Cobalt-60 under water at all times and lowers the product to be irradiated under water in hermetic bells. No further shielding is required for such designs.

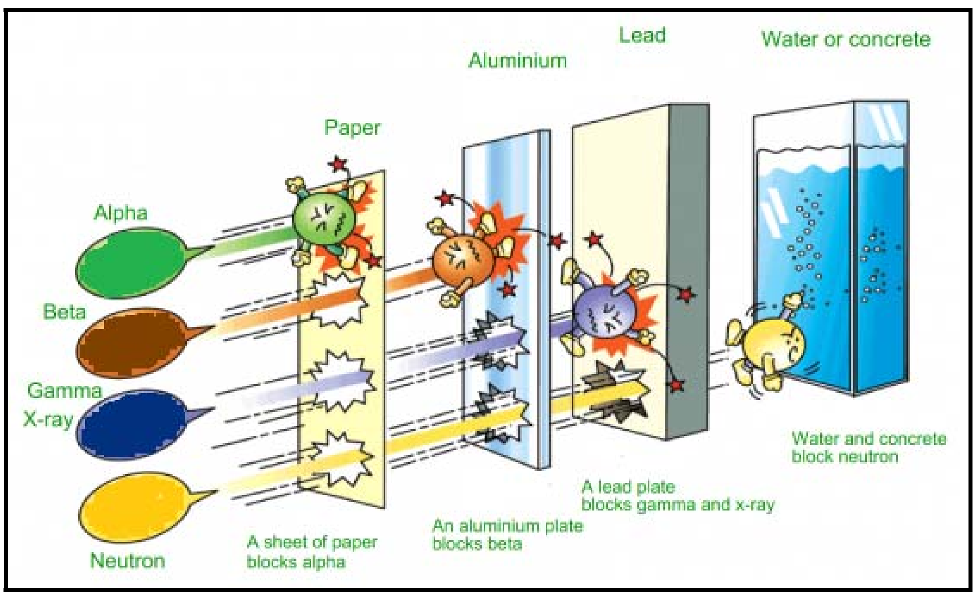


Fig. 1.2 The penetration of various types of radiation

1.10.5 Rationale: In BARC, a research team (Dharkar and Sreenivasan, 1966) initiated studies on the evaluation of effects of gamma radiation on tropical fruits, in the early sixties including mango. Initial experiments were carried out using a small irradiation equipment Gammacell-220. The delay in ripening of about a week, observed in mangoes exposed to low dose of 250 Gy and stored at ambient temperature of 25-30ºC, was quite remarkable and had potential commercial application in India. These studies were published in well-known scientific journals. A few years later, the efficacy of irradiation for disinfestation of fruits, killing of fruit flies and stone weevils, for overcoming quarantine barriers with similar doses was also demonstrated. It was found that the best results were obtained, when mangoes were irradiated at a hard mature stage (80% maturity), during the pre-climacteric phase.

Ionizing irradiation has long been practical to extend the shelf-life of fresh fruits. The process can be applied in juice manufacture by either irradiating the fruit prior to extraction or treating the juice. Although juice enzymes are quite resistant to irradiation, elimination of pathogens is accomplished by reasonably low doses in the order of 0.2 to 0.5 kGy (Thayer and Rajkowski, 1999). Interestingly, irradiation long scorned by technophobes with little understanding of radiation chemistry, is an effective way of safely maintaining fresh juice quality under refrigeration. Although the products must be identified as having been irradiated through the display of the international symbol the Radura, it should be popular as a safe alternative to thermal processing. Irradiation may have the more immediate use as a whole fruit surface pasteurization step prior to juicing. As an insect disinfestation process in whole fruit, it is unparalleled.

Wine aging is one of the key steps for improving the overall quality of wines. Reported methods for wine aging include those of a chemical nature and of physical nature. While the latter attracted less attention than the former, it is of practical importance since the aging period can be shortened considerably. Like chemical aging methods, physical aging methods can have a profound influence on the properties of wine. This is because treatments such as irradiation and ultrasonic waves may trigger complicated biochemical reactions, thereby leading to compositions that are significantly different from those without treatments, which implies that a comprehensive study of the influence of a treatment on the nature of wine is complex.

Some applications of gamma irradiation in wine and/or wine-related fields were found such as application in wine making materials (Ayed et *al*., 1999; Bachir, 1999). Caldwell and Spayed (1989) studied the effects of gamma irradiation on chemical and sensory evaluation of

Cabernet Sauvignon wine. In their study they found gamma irradiation of red wine did increase the chemical colour age of the Cabernet Sauvignon wines. Sensory evaluations found no perceivable difference between the doses (600, 1200, and 2400 Gy) used. Also the gamma irradiation did not decrease the astringency of the wines studied. They also reported that the use of higher dose rates to rapidly age Cabernet Sauvignon wines does not appear to be feasible. This paper did not have any information on the use of lower dose rates to rapidly age Cabernet Sauvignon wines.

Hua *et al.,* (1989) studied the acceleration of yellow wine mellowness by using cobalt-60 gamma rays; it was written in Chinese and not published internationally. They conducted the study by using 10–60 krad gamma ray irradiations. Their results showed that the ester compounds were increased after gamma irradiation. When comparing yellow wine irradiated with 10–60 krad gamma rays to wine conventionally aged for half a year, a year, and one and a half years, they found that the irradiated yellow wine quality reached that of the one and a half year conventionally aged yellow wine. The flavour, taste and mellowness of the irradiated yellow wine improved. They also did hygienic and safety experiments and showed that the irradiated wine had no influence on human health and that the hygienic quota satisfied their national standard. In their study, they performed toxicity tests on rats. Rats fed 10g/kg by weight were found to be safe. Also, an experiment on micro-nuclear cells showed negative results in rats; however, many important things were not described or not described in detail, plus the language was not English, nor was the publication available to the general public.

Chang and Chen (2002) reported the effects of numbers of treatments of 20 kHz ultrasonic waves on the properties of rice and maize wines. Their analysis was extended by Chang (2004) in a recent study, where the effects of various accelerating techniques on maize wine maturation were investigated. Twenty kHz and 1.6 mHz ultrasonic and gamma-irradiation treatments were applied, and the titratable acidity and the concentrations of the key components of maize wine in the final product were measured and compared with those of a one year conventionally matured wine. Most of the key components were found to correlate with the number of treatments/dosage and, based on a regression analysis, it was concluded that they are linearly dependent, that is, a simple linear model is applicable.

Rice wine was fermented with *Saccharomyces sake* and was matured using gamma irradiation as an accelerated physical maturation method. Tests were conducted on the accelerated matured rice wine for appearance, titratable acidity, presence of gamma irradiation residues, alcohol content, gas chromatographic measurements and sensory evaluation. The relationship of the content levels of titratable acidity, alcohol, ester, volatile acids, and aldehyde to sensory evaluations was also studied, as well as the time of maturation. Results showed that the gamma irradiation, in a suitable dosage, appeared to be a suitable method for improving some rice wine defects and producing a higher taste quality in the rice wine, without the presence of irradiation residues (Chang, 2003).

Statistical analysis was conducted to interpret the recently observed effects of various accelerating techniques on maize wine maturation (Chang, 2004). Instead of the previously reported simple linear relationship between the concentrations of the key components of maize wine in the final product and the number of treatments or the dosage, various types of non-linear behaviours were observed. A general polynomial regression model is used to describe these behaviours, and the adjustable parameters were estimated from the experimental data. The performance of the proposed regression model, which plays a key role in the design of an efficient accelerating process, was satisfactory. Some specific variations of the key components of maize wine, as a function of the number of treatments or the dosage, were observed.

The work carried out in this thesis is focused on the mango juice and its wine because mango wine is a popular product and gaining importance all over the world. Moreover, most scientists have already focussed on fruit wines, so a study of mango wine would contribute to the completeness of the field. The aim of this work is to evaluate the effects of gamma irradiation treatment, at different irradiation doses, as a cold sterilization technique for mango juice and wine, effects of gamma irradiation on total polyphenols, flavanoids and antioxidant potential of mango juice and wine, effects of gamma irradiation on mango wine maturation in terms of appearance, titratable acidity, presence of gamma irradiation residues, alcohol content, volatile composition, sensory evaluation, the relationship of the contents of titratable acidity, alcohol, ester, volatile acids, and aldehydes to sensory evaluations, the time of wine maturation and finally *in vivo* antioxidant potential of mango juice and wine.

Keeping in view of the facts about harmless effects of radiation on food and wine the following objectives have been drawn to investigate.

Objectives:

1. Effect of γ-irradiation on physico-chemical and microbiological properties of mango juice and wine.
2. Impact of γ-irradiation on total polyphenols, total flavanoids and antioxidant potential of mango juice and wine.
3. Effect of γ-irradiation on volatile composition and organoleptic properties of mango juice and wine.
4. *In vivo* antioxidant potential of mango juice and wine.