Analysis of Carotenoid composition of mango fruit and its wine.
6.1 INTRODUCTION

Mango (*Mangifera indica* L.) fruit is commonly called as “King of fruits” ranking fifth in total production among major fruit crops world wide. The world production of mango is estimated to be over $23.4 \times 10^6$ MT per annum. India ranks first among world’s mango producing countries, accounting for 54.2% of the total mango produced world-wide (Tharanathan *et al.* 2006).

Mango is processed into various forms. Mango fruits during early stages of growth are commonly used for sweet or sour chutney (mango sauce) and a large number of products are prepared from ripe mango fruit, like frozen and canned slices, puree, jam, squash, juice, nectar, mango powder and mango toffee. Also, products derived from mango fruit are increasingly used in beverage, dairy, and confectionery industries, where fruit purees and concentrate are the major intermediates. Generally, modern industrial year-round production of mango juice, that is, nectar, is mostly from such puree intermediates, produced during peak harvest seasons. Production of wine from mango is one of the alternative ways to use and convert the surplus production into a valuable product (Reddy and Reddy 2005, Kumar *et al.*, 2009).

Carotenoids have attracted the interest of researchers from diverse fields including chemistry, biochemistry, food science and technology, medicine, pharmacy and nutrition for more than a century and these fascinating compounds continue to be intensely investigated. In nature, carotenoids are mainly responsible for the red, yellow and orange colors. However, in green plant tissues, the color of carotenoids is masked by the more dominant pigment, chlorophyll and becomes evident only during the degradation of chlorophyll. This phenomenon can be seen especially during the ripening of fruits as well as in autumn leaves. In food, in addition to their function as the natural pigments and pro-vitamin A carotenoids, these compounds can be used as food additives for coloring (European Parliament and Council Directive, 1994). These are naturally occurring colored compounds that are abundant as pigments in plant kingdom. About 600 specific carotenoids have been identified, of which only about 24 were commonly occurring in human foodstuffs and only about 50 have pro-vitamin A
activity. The principal carotenoids of foods are β-carotene, β-cryptoxanthin, lycopene, lutein and violaxanthin. Except for violaxanthin, these are also the principal carotenoids found in the human plasma and together with zeaxanthin, are the carotenoids most studied in terms of human health because of their diverse roles in photobiology, photochemistry and photomedicine. Also carotenoids have a diverse role in the biological functioning of living organisms, including pro-vitamin A activity, antioxidant activity, modulation of detoxifying enzymes, regulating gene expression, cell communication, immune function enhancement, U.V skin protection, and visible color (Clevidence et al. 2000).

6.1.1 Carotenoids of mango: Mango (*Mangifera indica* L.) can be considered a good source of dietary antioxidants, such as ascorbic acid, carotenoids and phenolic compounds. β-carotene is the most abundant carotenoids in several cultivars (Shieber et al., 2000). Among the carotenoid pigments widely distributed in plant tissues, β-carotene provides the highest vitamin A activity. Vitamin A and its metabolites are essential for vision, reproduction, and immune function, besides performing other important physiological functions, including the deactivation of reactive oxygen species (Chytil, 1999). It is a very popular carotenoid-rich fruit in many countries and carotenoids are responsible for the bright yellow colour of mango peel and pulp, which are important lipophilic radical scavengers found in many fruits and vegetables. Mango is regarded as a rich source of carotenoids. Mango carotenoids are synthesized in mango fruit and their concentrations increases towards ripening of the fruit (Fennema, 1996) and the carotenoids content in mango is cultivar dependent (Ornelas-Paz et al., 2007). Mango peel colour is an important component of fruit quality and as such, plays a major role in consumer acceptability. During ripening, most mango varieties change colour from green to yellow or orange, often showing a red flush. Colour development is associated with decrease in the concentration of chlorophylls and increase in the concentrations of carotenoids, a loss of texture, increasing sugar content, and decreasing acidity (Medlicott et al., 1986). In fruits, the green and yellow colouration is imparted by the lipid soluble chlorophylls and carotenoids present in the plastids, while the red
colour may be due to carotenoids or to the water soluble anthocyanins found in the vacuole.

Carotenoid pigments are fairly stable within intact plant cells, but they become much more labile when fruits are subjected to postharvest handling practices and processing methods. Carotenoids are sensitive to oxygen, peroxides, temperature, light, type of packaging (modified atmosphere packaging), and length of storage (Van de Berg et al., 2000). Exposure to any of these elements may cause undesirable alterations in structure and bioactivity of carotenoids (in terms of isomerization, oxidation, or degradation). These alterations may in turn alter the U.V-VIS properties of carotenoids, yet color appearance to the eye is mostly unaffected. In addition, antioxidant activity may be altered extensively (Fennema 1996). Many different ripening conditions, postharvest treatments and processing methods are used to prepare mangoes for marketing to consumers. Any of these processes can affect the synthesis of mango carotenoids during ripening (Fennema, 1996).

Carotenoids are comprised of two structural groups namely hydrocarbon carotenes and oxygenated xanthophylls. The basic carotenoid structural backbone consists of isoprene units linked covalently to create a symmetrical molecule. Mangos contain both provitamin A carotenoids (carotenes) such as α-carotene, β-carotene, and γ-carotene; and oxygenated carotenoids (xanthophylls) such as β-cryptoxanthin, lutein, zeaxanthin, violaxanthin, antheraxanthin, auroxanthin, and neoxanthin (Ben-Amotz and Fishler, 1998; Cano and Ancos, 1994). Godoy and Rodriguez-Amaya, (1989) reported that β-carotene is the dominant carotenoid in mango, comprising of 48-84% of the total carotenoid content and presences of 16 other carotenoids including neo-β-carotene, mono-epoxy-β-carotene, auroxanthin, zeaxanthan were also confirmed in the ripe mangoes (Klaui and Bauernfeind, 1981). Although carotenoids are naturally stabilised by the plant matrix, cutting or disrupting of fruit and vegetable tissues favours their exposure to oxygen and endogenous oxidative enzymes, thus provoking their oxidation leading to the formation of cis-isomers which possess different biological properties such as decreased provitamin A activity, and altered bioavailability and antioxidant capacity (Rodriguez-Amaya, 1999). In white grapes, color comes from the presence of
carotenoids, xanthophylls, and flavonols such as quercetin. Similar pigments occur in red grapes, but the predominant color comes from the production of anthocyanins. In red varieties, the concentration of both major (β-carotene and lutein) and minor (5,6-epoxylutein and neoxanthin) carotenoids falls markedly after veraison to maturity, but depend also on climatic factors, agricultural practices, grape cultivar and clone (Razungles et al., 1987, 1993) Carotenoids are mostly found in the skin, at levels two to three times higher than in the pulp, while they are absent from the juice (Razungles et al., 1988). In the same way, they are absent from wine, except in the case of fortified red wine, like Port wine, which contains more xanthophylls than carotene (Guedes de Pinho et al., 2001). Whereas in mangoes the carotenoids level significantly increases towards maturity both in peel and pulp. Medlicott et al. (1986) had shown breakdown of the chlorophyll and similarly the increase in peel carotenoids in ripe fruit of mango Cv. Tommy Atkins.

6.1.2 Carotenoids in grapes: Carotenoids in grapes has been extensively reported in the literature and the major (85% of the total) carotenoids were β-carotene and lutein in the range of mg/kg and the remaining fraction is represented at levels of µg/kg by other xanthophylls, including neochrome, neoxanthin, violaxanthin, luteoxanthin, flavoxanthin, lutein-5,6-epoxide and zeaxanthin, and cis isomers of lutein and β-carotene (Razungles et al., 1987, 1996; Marais et al., 1991; Mendes-Pinto et al., 2004). The carotenoids which were suggested to be directly involved in the aroma of wine are β-carotene and neoxanthin though lutein and violaxanthin can also be considered because they also undergo breakdown reactions that may produce norisoprenoid compounds (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998). Winterhalter and Rouseff, (2002) provided extensive information on norisoprenoids and other metabolite compounds derived from carotenoids which have aroma properties in nature. However both the qualitative and quantitative profiles of carotenoids in grapes were affected by several factors including plant variety, climatic conditions, stage of maturity, soil characteristics and viticulture practices. Most of the carotenoids are synthesized from the initial stages of fruit formation until veraison and then degrade till the end of maturity (Baumes et al., 2002). Numerous works on the evolution of
Carotenoids during the maturation of grapes have revealed that the levels of β-carotene, lutein, flavoxanthin and neoxanthin decrease drastically after veraison until maturation (Marais *et al.*, 1991; Razungles *et al.*, 1996; Bureau *et al.*, 1998) which was apparently related to chemical and enzymatic degradation of these compounds. Carotenoids are generally concentrated in the skin of mature grapes at levels 2-3 times higher than in the pulp (Razungles *et al.*, 1987, 1988, 1996; During, 1999; Guedes de Pinho *et al.*, 2001). It was showed that the genes encoding carotenoid biosynthesis (and catabolism) showed mostly skin-specific expression patterns (Grimplet *et al.*, 2007). The observed differences in carotenoid levels from the beginning till the end of maturation have been strongly suggested as an indication of the formation of $C_{13}$ norisoprenoids, i.e. the varietal aromas, responsible for the typical aromas of some grape varieties (Razungles *et al.*, 1988; Winterhalter and Rouseff, 2002; Lee *et al.*, 2007; Baumes *et al.*, 2002; Sefton *et al.*, 1993; Francis *et al.*, 1992; Marais *et al.*, 1992). In fact, the biogeneration of $C_{13}$ norisoprenoid compounds from carotenoids in grapes via a proposed biogenetic pathway was remarkably studied by Baumes *et al.*, (2002).

### 6.1.3 Carotenoids in musts and wines:

So far carotenoids have only been reported in must (macerated grapes with skin in contact with seeds and pulp) and wines of Port from the Douro Valley, northern Portugal (Guedes de Pinho *et al.*, 2001; Mendes-Pinto, 2003; Mendes-Pinto *et al.*, 2005). In industrial scale wine involves two different vinification conditions (stainless steel tank and foot-trodden "lagar") from the various cultivars of grapes, however β-carotene and lutein were present in the range of μg/L with a different behaviour according to the vinification conditions (Mendes-Pinto, 2003). It was shown that in Port wine, the qualitative profile of carotenoids was similar to that of its corresponding grapes but at lower concentrations; the highest values found for β-carotene and lutein were 358 and 106 μg/L, respectively. Carotenoid concentrations in the wine seems to be dependent on several factors and one amongst was the age of wines, with higher total carotenoid levels found in new than in aged Ports (Mendes-Pinto, 2003; Mendes-Pinto *et al.*, 2005). Carotenoids have not been reported in juice or wine from white grapes (pressed grapes without skin contact). The fact that carotenoid molecules exist in Port wines and not in red and white table wines is
probably related to the wine-making process. Port wine is naturally sweet wine produced by interrupting alcoholic fermentation prematurely by the addition of brandy externally and hence some compounds of the grape matrix remains intact in the respective wines. This is probably the main reason that carotenoids persist in Port wines. Moreover, the addition of brandy (up to 20% v/v ethanol) may facilitate the solubilization of these molecules.

6.1.4 Role of carotenoids as precursors of norisoprenoids: Carotenoids are unstable compounds because of the characteristic highly conjugated double-bond structure and thus undergo chemical and enzymatic reactions generating several compounds, some of which have powerful aroma properties called norisoprenoids (carotenoids are precursors of norisoprenoids) (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998; Lutz and Winterhalter, 1992). These breakdown products of carotenoids are carbonyl compounds with 13, 11, 10 or 9 carbon atoms, and retaining the terminal group of their carotenoid parent as illustrated in Figure 6.1.

![Diagram showing formation of norisoprenoid compounds from β-carotene](image)

Fig. 6.1. Formation of C_9, C_{10}, C_{11} and C_{13} norisoprenoid compounds from β-carotene
The C\textsubscript{13} compounds are the most abundant norisoprenoids in nature. They can be divided into: (i) compounds with the megastigmane structure, including the family of ionones and damascenes with oxygen at different positions e.g. with a keto group at C(9) as in β-ionone or at C(7) as in β-damascenone and (ii) compounds with the megastigmane structure but without oxygen in the lateral chain, e.g. (E,E)-megastigma-4,6,8-triene (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998) (Figure 6.2). Compounds such as 2,2,6-trimethylcyclohexen-1-one, β-cyclocitril and DHA1 (dihydroactinidiolide) are examples of C\textsubscript{9}, C\textsubscript{10}, C\textsubscript{11} norisoprenoids, respectively (Figure 6.1) (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998). As the breakdown products of carotenoids that participate in wine aroma, these are termed ‘norisoprenoids’.

6.1.5 Carotenoid degradation and formation of norisoprenoids: Carotenoids can be degraded by enzymatic or non-enzymatic reactions yielding norisoprenoids (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998; Baumes et al., 2002; Lutz and Winterhalter, 1992; Kanasawud and Crouzet 1990). However, these norisoprenoids can arise either by direct degradation of carotenoids or via glycosylated intermediates (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998; Strauss et al., 1987; Mathieu et al., 2005; Skouroumounis et al., 1992). Those that are in the nonglycosylated (free) fraction constitute the C\textsubscript{13} varietal aromas in grapes; the others that are glycoconjugated (bound fraction) are stored and can then release their volatile aglycone during the fermentation, via enzymatic and acid hydrolysis (Winterhalter and Rouseff, 2002; Wahlberg and Eklund, 1998). While for a long time the involvement of regiospecific enzymatic cleavage by 9',10'-carotenoid cleavage dioxygenase (CCD) in
the formation of \( C_{13} \) norisoprenoids in grapes was a hypothesis, albeit supported by negative correlations between carotenoids and \( C_{13} \) norisoprenoids levels during grape maturation, the recent discovery of the carotenoid cleavage dioxygenase gene in grapes by Mathieu et al., (2005, 2007) constitutes a significant step in elucidating the effective role of these enzymes on the biosynthesis of \( C_{13} \) norisoprenoids in grapes.

6.1.6 Proposed mechanisms for norisoprenoids formation: It was shown that \( \beta \)-damascenone was formed from the carotenoid neoxanthin and the general mechanism for the formation of norisoprenoids in plants requires three steps: (i) initial dioxygenase cleavage, (ii) enzymatic transformation of the primary cleavage products to give the non-aroma intermediate metabolites and (iii) acid catalysed conversion of these non-aroma intermediates into the aroma compounds.

6.1.6.1 Enzymatic reactions: Carotenoid biodegradation is assumed to be catalysed by a \( 9',10' \)-carotenoid cleavage dioxygenase (CCD) that is not specific for any particular carotenoid end group. The formation of \( \beta \)-ionone from \( \beta \)-carotene (in rose flowers) was shown by Eugster and Marki-Fischer, (1991) and a second molecule of \( \beta \)-ionone is proposed to be formed together with the \( C_{14} \)-diapocarotenoid rosafluene, by subsequent oxidative degradation of the \( C_{27} \) alcohol, \( 10'-apo-\beta \)-caroten-10'-ol. \( \beta \)-Ionone was considered to be the primary product of \( \beta \)-carotene by \( 9',10' \)-carotenoid cleavage dioxygenase activity (Wahlberg and Eklund, 1998). Hence the study of carotenoid cleavage enzymes in plants has been the focus of researchers for a long time.

6.1.6.2 Chemical reactions: (photo-oxygenation, thermal degradation in aqueous medium and acid hydrolysis of intermediate megastigma precursors) The oxidation of \( \beta \)-carotene under light exposure was also proposed by Isee et al. (1969) for the generation of a \( \beta \)-ionone and also dihydroactinidiolide (DHA). The extent of this photo-oxygenation reaction depends on the levels of oxygen and light intensity and is thus a reasonable proposal as a pathway that contributes to the presence of \( \beta \)-ionone in grapes \textit{in vivo}. Thermal degradation of \( \beta \)-carotene in aqueous medium leads to the formation of b-ionone and TCH, \( 2,2,6 \)-trimethylcyclohexen-1-one, \( \beta \)-cyclocitrал, DHA and other reaction products. The reaction at 97°C for 3 h involves initially epoxidation and
furanoid rearrangement. The extent of β-carotene degradation is strongly dependent on temperature and time of reaction (Winterhalter and Rouseff, 2002; Kanasawud and Crouzet, 1990).

6.1.7 Contributions of norisoprenoids to wine aroma: The overall contribution to the aroma of wine is by norisoprenoids that occur freely in the grape or those released by enzymatic or acid hydrolysis during fermentation and finally by the chemical reactions that can occur during wine aging and storage. The carotenoid breakdown reactions that occur during the maturation of grapes and the subsequent formation of norisoprenoids, as C13 varietal aromas, provide the contribution of the free fraction of norisoprenoids to the wine aroma (Winterhalter and Rouseff, 2002; Baumes et al., 2002; Lee et al., 2007). This contribution can be more or less relevant to the final aroma of wine depending on the viticulture conditions, which affect the carotenoid profile of the grape. It is worth mentioning though, that the several viticulture conditions or parameters should not be studied individually but as interacting effects, as is the case of light exposure and temperature (Lee et al., 2007). The release of glycosylated intermediates by acid hydrolyses occurs during fermentation and has been suggested to make a major contribution to the concentration of the aroma active norisoprenoids in wine (Wahlberg and Eklund, 1998; Winterhalter and Rouseff, 2002; Strauss et al., 1987). The study of glycol-conjugated norisoprenoids and the formation of the aroma compounds by glycosidase activity has been the focus of much work (Sefton et al., 1989, 1993; Strauss et al., 1987; Skouroumounis and Winterhalter, 1994). Some of this gives information about the glycol-conjugate composition of grapes of several red and white cultivars, and shows that levels of both glycol-conjugated and released compounds vary greatly among the different grapes (Sefton et al., 1993; Skouroumounis and Winterhalter, 1994; Lopez et al., 2004). Different fermentation processes are used according to the type of wines. Microvinification with different conditions of mild acid hydrolysis (temperature, maceration techniques, alcohol content and levels of sugar, yeast and bacteria activity) has been used as a small-scale wine-making process (Pogorzelski and Wilkowska, 2007).
In young wines, the aroma norisoprenoids composition comprises compounds arising directly from grapes and those released during the fermentation process, but the wine storage conditions, particularly temperature and oxygen, can also affect the development of aroma in wines. It has been observed that, in general, high oxygen intake and high temperatures stimulate the formation of norisoprenoids in wines. This is in agreement with the high levels of some norisoprenoids typically found in aged wines, in particular in aged Rieslings and in Ports (Mendes-Pinto, 2003; Ferreira and de Pinho, 2004). Port wine contains considerable levels of carotenoids and the important norisoprenoids identified in ports were 2,2,6-trimethylcyclohexanone (TCH), β-damascenone, β-ionone and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (Freitas et al., 1999; Ferreira and de Pinho, 2004), which have been proposed as carotenoid breakdown products might be because of chemical degradation and thus have a role in the evolution of aroma during the wine aging process. Moreover aging of Ports for long time (more than 4 years both for bottle-aged or barrel-aged) potentially also leads to chemical reactions with the norisoprenoid and other constituents of oak woods (Sefton et al., 1990).

6.2 REVIEW OF LITERATURE

6.2.1 Carotenoids present in the mango peel: Mango peel is a major by-product of mango processing industry and it constitutes about 15-20% of total weight of mango fruit. Peel has been found to be a good source of phytochemicals such as polyphenols, carotenoids, vitamin E and vitamin C (Ajila et al., 2007a, Ajila et al., 2010) and it exhibited good antioxidant property (Ajila et al., 2007b). In addition, mango peel has been reported to be a good source of dietary fiber, which plays an important role in many physiological processes and in the prevention of illnesses such as constipation, hypertension, liver cirrhosis, diabetes, and cancer (Ajila et al., 2008). Due to their antioxidant properties, these phytochemicals present in mango peel may exhibit protection against oxidative damage in cells by reactive oxygen species (ROS). Protective role of mango peel against hypothyroidism was reported by Parmar and Kar (2009) and Kim et al. (2010) showed antiproliferative activities of mango peel, which was correlating with its phenolic and flavonoid content. Further, Kim et al. (2012)
showed that ethanolic extract of mango peel are capable of exhibiting induction of apoptosis-mediated cell death in human cervical carcinoma, suggesting that it contains functional phytochemicals for preventing cervical cancer.

In addition to the presence of various polyphenolic compounds, carotenoids are the yellow coloured pigments which impart the colour to ripened mango peel. Ajila et al. (2010) reported the presence of carotenoids like violaxanthin, lutein and \( \beta \)-carotene by RP-HPLC in Indian mango cv. Badami.

6.2.2 Carotenoids present in the mango pulp: Carotenoids are a widespread group of naturally occurring fat-soluble pigments. They are especially abundant in dark green leafy vegetables, and yellow-orange fruits like carrots, passion fruit and mango. It was reported by Mercadante and Rodriguez-Amaya (1998) that the total carotenoids in mango increased with ripening of the fruit in two Brazilian cultivars and the total carotenoid rose from 12.3 to 38.0 \( \mu \)g/g in the cv. Keitt and from 17.0 to 51.2 \( \mu \)g/g in the cv. Tommy Atkins from the mature-green to the ripe stage and they noticed that the ripening alterations (increase) occurred principally in the major carotenoids violaxanthin and \( \beta \)-carotene. However Ribeiro et al. (2007) reported the increase in the total carotenoid concentration was from 1.91 to 2.63 mg/100g and the concentration of \( \beta \)-carotene was from 661.27 to 2,220 \( \mu \)g/100 g of mango pulp. Chen et al., (2004) reported the presence of about 25 carotenoids in Taiwanese mangoes and the principal carotenoids present were all-trans-\( \beta \)-carotene was present in largest amount (29.34), followed by cis isomers of \( \beta \)-carotene (9.86), violaxanthin and its cis isomers (6.40), neochrome (5.03), luteoxanthin (3.6), neoxanthin and its cis isomers (1.88), zeaxanthin (1.16) and 9- or 9'-cis-lutein (0.78). Mercadante et al., (1997) reported the presence of various carotenoids like \( \beta \)-Carotene (all-trans), \( \beta \)-cryptoxanthin (all-trans and cis), zeaxanthin (all-trans), luteoxanthin isomers, violaxanthin (all-trans and cis), and neoxanthin (all-trans and cis) in mango cv. Keitt by means of HPLC and found that the principal carotenoids present were all-trans-violaxanthin (21.1±2.9 \( \mu \)g/g), all-trans-\( \beta \)-carotene (15.1±1.5 \( \mu \)g/g), and a cis-violaxanthin (10.1±0.2 \( \mu \)g/g). Later, Ornelas-paz, (2007) also reported carotenoids content from seven Mexican mango cultivars and found that of all-trans-\( \beta \)-carotene ranged between 0.4 and 2.8 mg/100g, and Haden and
**Ataulfo** have the highest amounts. The results of this study indicate that *all-trans*-β-carotene, *all-trans*-violaxanthin, and 9-cis-violaxanthin were the most abundant carotenoids. Veda *et al.* (2007) reported the β-carotene contents from six Indian mango cultivars namely, **Badami, Raspuri, Mallika, Malgoa, Totapuri, and Neelam**, and found that β-Carotene was ranged from 0.55 ± 0.03 mg/100 g in **Malgoa** to 3.21 ± 0.25 mg/100 g in **Badami** cultivar.

### 6.2.3 Carotenoids present in the mango wine:

It was reported by Olle *et al.*, (1997) that various flavor compounds from ripe mango puree prepared by using cross-flow microfiltration on microporous alumina membrane, subsequent concentration by reverse osmosis and found that the terpene hydrocarbons, the main character impact compounds, and most oxygenated terpene derivatives were retained. However C<sub>13</sub> norisoprenoids and phenols increased, likely by chemical degradation of carotenoids and phenolic acids, respectively which could be due to heat treatment during the preparation process. Pino (2012), analyzed different volatile compounds from mango, **cv. Corazon** by using simultaneous distillation-extraction combined with GC-FID and GC-MS and estimated the most odour-active compounds in the fruit 128 were positively identified out of 16. Eighteen odorants amongst them were considered as the most odour-active compounds namely *(E)-*β-damascenone, ethyl butanoate, *(E,Z)-*nonadienal, ethyl 2-methylpropanoate, *(E)-*2-nonenal, *(E)-*β-ionone, terpinolene, δ-3-carene, β-caryophyllene, ethyl 2-methylbutanoate, limonene, myrcene, linalool, γ-octalactone, nonanal, methyl benzoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone and hexanal.

Further, Li *et al.*, (2012) reported the presence of both damascenone and β-ionone, the degraded compounds derived from carotenoids in wine from three mango cultivars but not from mango juice co-fermented with *Williopsis saturnus* and *S. cerevisiae*.

Carotenoids in mango wine were derived from its pulp similar to the origin of carotenoids in grape wine from its skin. However till date perhaps no report was available in the literature regarding the occurrence of carotenoids or their quantification. Hence this present chapter is aimed to track the changes (stability) and occurrence of carotenoids in mango pulp to wine during wine fermentation.
6.3 MATERIALS AND METHODS

6.3.1 Chemicals and sample processing: All chemicals used were of analytical grade. Carotenoids standards, neoxanthin (95%), violaxanthin (98%), lutein (99%), β-apo-8'-carotenal and all-trans-β-carotene (98%), were kindly donated by Dr. V. Baskaran, Department of Biochemistry and Nutrition, CFTRI, Mysore, India. Butylated hydroxy toluene (BHT) were purchased from Sigma-Aldrich (USA). All the seven mango cultivars of mangoes mentioned in Section 2.3.1 were used for screening and their processing was done according to section 2.3.2.

6.3.2. Wine yeast and preparation of inoculums: The Saccharomyces bayanus (wine yeast strain) was kindly donated by Prof. Roberto Ambrosoli; University of Turin, Italy was used in the experiments. The culture was maintained on MPYD slants containing Malt extract 3, Peptone 5, Yeast extract 3, Dextrose 10 and agar 20 (g/L) at 4°C.

The inoculum was prepared by inoculating a two-day old slant culture into 50 mL of the sterile MPYD liquid medium taken in 100 mL flask and growing it on a rotary shaker (3000 g) for 48 h. This inoculum (3×10^6 cells/mL) was used in the fermentation of mango must (juice) to mango wine.

6.3.3 Fermentation: The batch fermentation was carried out typically by inoculating the actively growing yeast, prepared as above into 1000 mL mango must contained in a amber coloured conical flask under stationary conditions and the inoculated flasks were incubated at 25°C for a period of 15 days. All of the glassware was covered with black cloth to prevent exposure to light. The samples were collected by separation of the cells by centrifugation at 5000 g for 10 min. The clear supernatant samples were kept at 20°C for a few weeks till the physicochemical and sensory analyses were completed and finally the wines were stabilized with the addition of 20 mg SO2/L and preserved.

6.3.4. Extraction of carotenoids from puree and wine: Carotenoids were extracted according to the procedure described by Raju et al. (2007). In brief, 250 g of puree was taken in a 1000 mL amber coloured volumetric flask, spiked with 100 µL of internal standard (β-apo-8'-carotenal; 100 mg/L) and stabilized with 2mM α-tocopherol in
methanol (100 μL/g). The mixture was stirred mechanically for 30 min with hexane/petroleum ether (50:50 v/v) and the resulting upper organic layer was separated. The extraction process was repeated thrice for the lower phase using 40 mL of the same solvent until the samples became colourless. The extract was dried over anhydrous sodium sulphate (20 g) and filtered under suction. The filtrate was evaporated to dryness in a rotary vacuum evaporator (Buchi, Switzerland) at 30-35°C and redissolved in a known volume of mobile phase, and an aliquot (20 μL) was used for HPLC analysis. Sample handling, homogenization and extraction were carried out at 4°C, under dim yellow light to minimize photo-isomerization and oxidation of carotenoids.

Carotenoids were extracted according to the procedure described as in the case of puree. The carotenoid content of wines was analysed by using HPLC conditions as described above. The λ_max values of these compounds were confirmed by their characteristic spectrum recorded with PDA detector. They were quantified from their peak areas in relation to respective reference standards.

6.3.5. Evaluation of colour: Colour measurements were made with a Hunter colorimeter (LabScan XE, Hunter Associate Laboratories, Inc., VA). The sample was placed in a 1 cm path length optical glass cell in the total transmission mode, using illuminant C and 2° observer angle. Five colour parameters were recorded: Hunter CIE lightness (L*), a* value, b* value, chroma (saturation, C*), and hue angle (h°). The tristimulus color coordinate system, the L* value is a measure of lightness and varies from 0 (black) to 100 (white); the a* value varies from −100 (green) to +100 (red); and the b* value varies from −100 (blue) to +100 (yellow). As the values of a* and b* rise, the color becomes more saturated or chromatic, but these values approach zero for neutral colors (white, grey or black) the instrument was calibrated with white standard calibration plate provided by manufacture (L* = 97.29; a* = −1.06; b* = 0.68).

6.3.6. Statistical analysis: This was performed by using SPSS, version 12.0, as described in section 2.3.13.
6.4 RESULTS AND DISCUSSION

6.4.1 Carotenoid composition of mango peel: Mango peel is a major by-product of mango processing industry and it constitutes about 15-20% of total weight of mango fruit. Peel has been found to be a good source of phytochemicals such as polyphenols, carotenoids, vitamin E and vitamin C (Ajila et al., 2007a) and it exhibited good antioxidant property (Ajila et al., 2007b). Polyphenol content of peel was reported to be more than that of pulp (Lakshminarayana et al., 1979). Due to their antioxidant properties, these phytochemicals present in mango peel may exhibit protection against oxidative damage in cells by ROS. Kim et al., (2010) reported that the mango peel contained more polyphenols and flavonoids than flesh and exhibited good antioxidant activity by effectively scavenging various free radicals, such as DPPH radicals, hydroxyl radicals and alkyl radicals. Further, it has been confirmed that the mango peel is a potential antiproliferative agent and concluded that the antioxidant and antiproliferative activities of mango peel might be due to the synergistic actions of bioactive compounds present in them. Carotenoids are widely distributed in nature and are lipophilic antioxidants and Medlicott et al., (1986) reported various colour pigments in mango peel during raw and ripe stage. The breakdown of the chloroplast thylakoid system during the initial stages of ripening can be correlated with the loss of chlorophyll. Similarly increase in peel carotenoids were shown in ripe fruit. The development of red blush from anthocyanins shown in this variety was enhanced by chlorophyll breakdown and carotenoid development, and not anthocyanin synthesis. In the present study, the total carotenoids concentration (mg/100g) in different cultivars of mango peel are presented in Table 6.1.
Table 6.1. Total carotenoids and total anthocyanins contents (mg/g) in mango peel of different cultivars

<table>
<thead>
<tr>
<th>Mango cultivar</th>
<th>Total carotenoids (mg/100g)</th>
<th>Total anthocyanins (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphonso</td>
<td>8.62±0.52</td>
<td>3.65±0.05</td>
</tr>
<tr>
<td>Banginapalli</td>
<td>2.56±0.22</td>
<td>Nd</td>
</tr>
<tr>
<td>Neelam</td>
<td>1.69±0.31</td>
<td>Nd</td>
</tr>
<tr>
<td>Raspuri</td>
<td>1.48±0.15</td>
<td>Nd</td>
</tr>
<tr>
<td>Rumani</td>
<td>1.91±0.12</td>
<td>2.13±0.13</td>
</tr>
<tr>
<td>Sindhura</td>
<td>6.38±0.92</td>
<td>6.23±0.07</td>
</tr>
<tr>
<td>Totapuri</td>
<td>2.02±0.64</td>
<td>Nd</td>
</tr>
</tbody>
</table>

Values were mean of triplicate analyses ± S.D; Nd= Not detected

The highest total carotenoids were present in the peel of Alphonso (8.62±0.52) followed by Sindhura (6.38±0.92 mg/100g) and the lowest was found in Raspuri (1.48±0.15 mg/100g). However the highest anthocyanins were found in Sindhura (6.23±0.07) followed by Alphonso (3.65±0.05) and Rumani (2.13±0.13 mg/100g). It is well known that during ripening of mango fruits, carotenoids raise with degradation of chlorophylls; also mango fruits will be exposed to different environments during transport or exposed to sunlight before being brought to the laboratory for analysis; which could be the reason for the degraded or bio-trasnformed carotenoids (Lakshminarayana et al., 2008). Ajila and Rao, (2008) also reported that total carotenoid content was more in acetone extract of ripe peel of Badami and Raspuri as 194±0.26 and 436±0.22 µg/g. However, they observed the presence of anthocyanins in acetone extracts of both the cultivars Badami and Raspuri but in the present study, anthocyanins were not observed in both of them.

6.4.2 Carotenoid composition in puree and wine: It was demonstrated that β-carotene was the major carotenoid in the mango pulp and Ornelas-Paz et al. (2007) demonstrated by HPLC-MS that β-carotene was the main provitamin A carotenoid in Ataulfo mango; however other carotenoids were also present in the mango pulp, but had no pro-vitamin A activity. In the present study, HPLC profile of carotenoids in the mango puree and
mango wine (Figure 6.3 and 6.4) has two classes of pigments in the order of their elution through a C\textsubscript{18} column. These are xanthophylls, and hydrocarbon carotenoids, these pigments are separated within 24 min. The detectable xanthophylls in mango puree were comprised of neoxanthin (peak 1), violaxanthin (peak 2), lutein (peak 3), and the hydrocarbon carotenoid \(\beta\)-carotene (peak 4). In case of mango wine the detected carotenoids were neoxanthin (peak 1), violaxanthin (peak 2), lutein (peak 3), internal standard (peak 4) and \(\beta\)-carotene (peak 5). These carotenoids were eluted under isocratic conditions and confirmed by their retention times and the absorption spectra of the respective reference standards. The results for neoxanthin, violaxanthin, lutein, \(\beta\)-carotene content are presented in Table 6.2. The total carotenoids were estimated as a sum of neoxanthin, violaxanthin, lutein, and \(\beta\)-carotene per cultivar. Among the carotenoids analysed in puree, \(\beta\)-carotene was the major carotenoid ranging from 65.4 to 94.1\%, followed by lutein (4.5-29.4\%), violaxanthin (0.6-3.8\%) and neoxanthin (0.7-3.1\%). The relative highest total carotenoid levels in puree were found in Sindhura (5810 \(\mu\)g/100g), followed by Alphonso (5720), Rumani (3970), Banginapalli (3955), Totapuri (1920), Raspuri (1080) and Neelam (980 \(\mu\)g/100g) is the poor source of carotenoids with respect to the mango purees studied (Table 6.2).

Total carotenoid concentrations in the mango pulp are usually in the range of 900-9200 \(\mu\)g/100 g (Litz, 1997). For ripe mango cv. Tommy Atkins, average amounts of 1920 \(\mu\)g of carotenoids per 100 g of mango puree were reported (Godoy and Rodriguez-Amaya, 1989). Even higher contents were found for mango cv. Keitt containing 5500 \(\mu\)g of total carotenoids per 100 g of mango (Mercadante et al. 1997).where as the Indian cultivar Alphonso showed exceptionally high values up to 11000 \(\mu\)g/100g (Padmini and Prabha, 1997). Veda et al. (2007) has reported 3210 \(\mu\)g/100g of \(\beta\)-carotene for mango cv. Badami, 1270 \(\mu\)g for cv. Totapuri, 1830 \(\mu\)g for cv. Raspuri and 1450 \(\mu\)g/100g for Neelam cultivars from South India. However, the \(\beta\)-carotene content was found to be different when compared to the present study (Table 6.2).
Fig. 6.3. HPLC chromatogram of carotenoids in mango puree (1, Neoxanthin; 2, Violaxanthin; 3, Lutein; 4, β-Carotene) in cultivars Alphonso, Banginapalli, Neelam, Raspuri, Rumani, Sindhura and Totapuri respectively.
Environmental differences such as temperature, soil and solar intensity could influence the carotenoid content in the same cultivar. Mercadante and Rodriguez-Amaya (1998) reported higher β-carotene contents in mangoes from the Northeast region than those from the Southeast region. The results from Veda et al., (2007) also indicated that the β-carotene content of mangoes was influenced by geographical location. The carotenoid profile in all the cultivars of mango wine had the same major carotenoids as those of their purees in different concentrations. The results for individual carotenoid content are presented in Table 6.2. The total carotenoids in the mango wines were in the range of 578-4330 μg/100g and the highest amount of total carotenoids from mango wine was in Alphonso (4330), followed by Sindhura (4101), Banginapalli (2943), Rumani (2857), Totapuri (690), Raspuri (634), and Neelam (578 μg/100g).

Results indicate that the xanthophylls (oxygenated carotenoids) were degraded more than β-carotene (hydrocarbon carotenoid). Among xanthophylls, lutein was degraded more ranging from 78.7 to 93.9%, followed by neoxanthin (26.8-83.3%) and violaxanthin (50-74.3%). This could be due to combined effect of acidic conditions of wine with temperature responsible for the significant degradation of xanthophylls compared to β-carotene (17.9-60.7%), which might be related to the presence of hydroxyl groups of xanthophylls.

These results support the higher ratio of β-carotene/xanthophylls concentrations in all the mango wine and suggest that xanthophylls are degraded more quickly than β-carotene during wine aging. The degradation of β-carotene during carrot juice fermentation was reported by Kun et al. (2008) by using Bifidobacterium bifidum B3.2 strain and similarly, the losses of β-carotene and zeaxanthin in spontaneous (non-inoculated) fermentation was also reported by Li et al. (2007) during preparation of the fermented porridge from maize.
Figure 6.4. HPLC chromatogram of carotenoids in mango wine (1, Neoxanthin; 2, Violaxanthin; 3, Lutein; 4, Internal standard (β-apo-8'-carotenal); 5, β-Carotene) in cultivars Alphonso, Banginapalli, Neelam, Raspuri, Rumani, Sindhura and Totapuri respectively.
<table>
<thead>
<tr>
<th>Mango Cultivar</th>
<th>Neoxanthin Puree μg/100g</th>
<th>Neoxanthin Wine μg/100g</th>
<th>Violaxanthin Puree μg/100g</th>
<th>Violaxanthin Wine μg/100g</th>
<th>Lutein Puree μg/100g</th>
<th>Lutein Wine μg/100g</th>
<th>β-Carotene Puree μg/100g</th>
<th>β-Carotene Wine μg/100g</th>
<th>Total Carotenoids Puree μg/100g</th>
<th>Total Carotenoids Wine μg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alphonso</td>
<td>41±2.42</td>
<td>12±1.4***</td>
<td>35±2.1</td>
<td>9±0.9***</td>
<td>258±15</td>
<td>55±7.8***</td>
<td>5385±142</td>
<td>4251±138**</td>
<td>5720±240</td>
<td>4330±176*</td>
</tr>
<tr>
<td>Banginapolli</td>
<td>56±3.02</td>
<td>12±1.3***</td>
<td>42±1.54</td>
<td>13±0.97***</td>
<td>326±24</td>
<td>20±5.5***</td>
<td>3531±92</td>
<td>2898±101*</td>
<td>3955±151</td>
<td>2943±141**</td>
</tr>
<tr>
<td>Neelam</td>
<td>14±3.42</td>
<td>4±0.95*</td>
<td>37±1.21</td>
<td>12±1.08***</td>
<td>288±13</td>
<td>19±6.4***</td>
<td>641±159</td>
<td>543±53</td>
<td>980±152</td>
<td>578±101</td>
</tr>
<tr>
<td>Raspuri</td>
<td>12±0.9</td>
<td>2±0.55***</td>
<td>16±2.06</td>
<td>8±0.31*</td>
<td>212±19</td>
<td>42±1.5***</td>
<td>840±114</td>
<td>617±59</td>
<td>1080±132</td>
<td>634±113</td>
</tr>
<tr>
<td>Ramani</td>
<td>123±1.7</td>
<td>96±1.8***</td>
<td>30±1.14</td>
<td>12±0.67***</td>
<td>270±15</td>
<td>28±6.6***</td>
<td>3550±121</td>
<td>2753±91**</td>
<td>3970±151</td>
<td>2857±104**</td>
</tr>
<tr>
<td>Sindhiura</td>
<td>102±6.6</td>
<td>31±0.84***</td>
<td>62±1.43</td>
<td>21±0.51***</td>
<td>452±21</td>
<td>37±4.1***</td>
<td>5192±109</td>
<td>4012±163**</td>
<td>5810±216</td>
<td>4101±163**</td>
</tr>
<tr>
<td>Totapuri</td>
<td>52±2.94</td>
<td>25±0.51***</td>
<td>15±1.01</td>
<td>7±0.49***</td>
<td>213±12</td>
<td>13±5.4***</td>
<td>3642±97</td>
<td>645±74**</td>
<td>1920±147</td>
<td>690±125**</td>
</tr>
</tbody>
</table>

***, **, * significantly varied when compared with the carotenoids in wine to puree at p ≤ 0.0001, 0.001 and 0.01 respectively.
Carotenoids were regarded as part of the aroma potential of grape and known as precursors of norisoprenoids, responsible for the typical aroma of same grape varieties. It was reported that norisoprenoids could originate from direct degradation of carotenoid molecules such as β-carotene, lutein, neoxanthin and violaxanthin involving molecular oxygen and also achieved via non-enzymatic or enzymatic mechanisms (Winterhalter and Rouseff 2002). Guedes de Pinho et al. (2001) reported that the levels of β-carotene and lutein found in fortified wines (118 and 29 µg/L) were lower than those found in musts (454 and 119 µg/L), other xanthophylls, such as neoxanthin, violaxanthin, and luteoxanthin exist in appreciable amounts in young ports. Further Mendes-Pinto et al. (2005) reported that in forced-aging study the lutein was more sensitive to temperature than β-carotene. Ferreira et al. (2008) reported that both lutein and β-carotene could contribute to the aroma of port wines, as they could be degraded into small molecules like norisoprenoid compounds. It is suggestive from the above that the degraded products of carotenoids might contribute to the wine aroma.

Very recently Li et al. (2012) reported the presence of both damascenone and β-ionone, the degraded compounds derived from carotenoids in wine from three mango cultivars but not from mango juice co-fermented with Williopsis saturnus and S. cerevisiae are in agreement with the present study. However further research need to be done to assess the possible relationship between the degradation of carotenoids in mango wines and their conversion into aroma compounds that can have sensorial impact in wines.

6.4.3. Hunter colour parameters: The colors, or pigments, in fruits and vegetables reflect the presence of certain biologically active phytochemical compounds and antioxidants that have been reported to promote good health and are important quality indexes in fresh fruits and their products. Positive values of a* and b* are attributed to carotenoids or anthocyanins present. The determination of the coordinates L*, a*, b* characterizes the color of any product. At this scale, The L* and b* values are indicators of lightness and yellow color in fruits respectively. L* measures luminosity that varies from zero (black) to 100 (pure white); a* and b* values represent the levels of tonality
and saturation, with +a (indicating red), -a (indicating green), +b (indicating yellow) and -b (indicating blue). The Hunter colour values in mango puree were presented in table 6.3. In the present study, generally the L* values in the puree were significantly higher than those of wine. These values varied among the cultivars and were in the range from 56.79 to 39.85; the highest value was observed in the cultivar Sindhura and the lowest in Raspuri. The highest a* value was observed in Rumani (13.1) and the lowest in Raspuri (2.27) and the highest b* value was observed in Neelam and the lowest in Raspuri (20.33). Among the wines, the wine from Banginapalli variety was the darkest (L* value of 28.11), while the wine from Totapuri was the lightest (L* value of 16.72) in colour (Table 6.3). However, all the wine were lighter when compared to that of their respective puree which could be due to filtration of puree to remove insoluble pectic substances.

Though all the wines are in yellow visually in terms of colour, there were little differences among the wines in b* parameter (yellowness). Positive values of a* and b*, as observed in this work, attributed to the carotenoids present in the wine. C* (Chroma) is a parameter that indicates the contribution of a* (redness) and b* (yellowness) and Chroma of the wines were ranging from 10.33 to 16.05 (Table 6.3). Alphonso wine was with larger chroma (16.05) than Totapuri wine (10.33) and Hue angle (colour, h°) was ranging from 77.6 to 82.69°. Overall, wine from Alphonso, Sindhura, Banginapalli and Rumani (mean hue angle value = 81.35°) were slightly more orange than the wines from other cultivars and were in comparison with the white wines, reported by Lee and Rennaker, (2007).

The color changes cannot be explained by changes in carotenoid content alone nor isomerization to cis-carotenoid (Purcell et al., 1969). Any change in physical state of the carotenoid probably is responsible for heat-caused color changes. The color shift during thermal processing was attributed to degradation of chromoplasts and solution of carotenes in other cellular lipids.
Table 6.3. Hunter color values of mango puree and its wine.

<table>
<thead>
<tr>
<th>Variety</th>
<th>L* value</th>
<th>a* value</th>
<th>b* value</th>
<th>Chroma (C)</th>
<th>Hue angle (h°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puree</td>
<td>Wine</td>
<td>Puree</td>
<td>Wine</td>
<td>Puree</td>
</tr>
<tr>
<td>Alphonso</td>
<td>55.46</td>
<td>25.54</td>
<td>5.79</td>
<td>2.04</td>
<td>34.26</td>
</tr>
<tr>
<td>Banginapalli</td>
<td>53.28</td>
<td>28.11</td>
<td>5.75</td>
<td>2.09</td>
<td>33.50</td>
</tr>
<tr>
<td>Neelam</td>
<td>52.64</td>
<td>22.79</td>
<td>4.54</td>
<td>1.88</td>
<td>35.00</td>
</tr>
<tr>
<td>Sindura</td>
<td>56.79</td>
<td>22.05</td>
<td>10.74</td>
<td>1.75</td>
<td>30.80</td>
</tr>
<tr>
<td>Raspuri</td>
<td>39.85</td>
<td>18.37</td>
<td>2.27</td>
<td>2.34</td>
<td>20.33</td>
</tr>
<tr>
<td>Rumani</td>
<td>51.26</td>
<td>20.50</td>
<td>13.10</td>
<td>1.93</td>
<td>31.94</td>
</tr>
<tr>
<td>Totapuri</td>
<td>52.73</td>
<td>16.72</td>
<td>5.56</td>
<td>2.22</td>
<td>33.68</td>
</tr>
</tbody>
</table>

Values presented are mean of duplicate analysis.

Chroma (C) = \([a^*]^2 + [b^*]^2]^{1/2}\) and Hue angle (h°) = \(\text{arctan}(b^*/a^*)\)
Furthermore, Genovese et al. (1997) speculated that since the color of juice was reflected by suspended pulp particles (juice sacs), changes in suspended pulp particles after thermal pasteurization probably would also affect color changes in juices. When compared to puree, the L*, a* and b* values of wine decreased drastically even though the puree was not pasteurized before using for wine fermentation. Particularly, L* value has been used in fresh-cut mango as a good indicator of browning surface in response to peeling or slicing, which facilitates the contact of oxygen and substrates with browning enzymes (Soliva-Fortuny and Martin-Belloso 2003). Fang et al. (1986) observed that heat treated passion fruit juice showed L* values higher (lighter color) than the control juice, and that those values decreased to values near to that of the control during storage. This increase in luminosity could be due to the destruction of the carotenoid structure giving a paler color, along the time, other compounds, resulting mainly from the non-enzymatic browning reactions, like Maillard’s and oxidation of ascorbic acid or precipitation of the pigments, contributed to reduction of the luminosity, giving a darker appearance to the juice. However the intensity of the red color (a* value) was lower in the heat treated juices than in the control. According to Fang et al. (1986), β-carotene partially lost its red color after heat treatment, probably because it changed to the cis form (Chen et al., 1995). The increase in the intensity of the dark colors, such as the red one decreased the yellow intensity, contributing negatively to the maintenance of the characteristic color of the juice; which might be due to the softening of fruit tissues and degradation of β-carotene.

6.5 CONCLUSIONS

The present study has shown that good amounts of carotenoids are present in mango puree and in mango wine. The data suggest that both xanthophylls and β-carotene are degraded during wine fermentation irrespective of the mango cultivar used, however the rate of degradation was more in xanthophylls. The hunter colour values were varied among puree and its wine. A significant reduction of hunter values was observed in wine, which could be due to the filtration of puree before allowing it for wine fermentation.