Effect of radiation processing on flavor precursors in grapes and potential technological applications

By

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I, hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree / diploma at this or any other Institution / University.

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List of publications arising from thesis

Publications

1. Application of mass spectrometry based electronic nose and chemometrics for fingerprinting radiation treatment. Sumit Gupta, Prasad Variyar, Arun Sharma. Radiation Physics and Chemistry, Volume 106, January 2015, Pages 348-354

2. Influence of radiation processing of grapes on wine quality. Sumit Gupta, Rupali Padole, Prasad Variyar, Arun Sharma. Radiation Physics and chemistry, Volume 111, June 2015, Pages 46-56

Poster presentations

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2. Gupta, S., Padole, R., Prasad, S. V., Sharma, A. K. "Radiation processing of grapes (Vitis Vinefera) for improved wine quality" at LSS, 17-19 December, 2012 held at TSH-BARC, Mumbai.

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4. Presented poster entitled "Total mass chromatogram and chemometrics as a tool for fingerprinting radiation treatment". XXI IFST, AFSTI. 20-21 January, Pune. (Best Poster award)

Dedicated to my family.....

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1. **Name of the Student:** Mr. Sumit Gupta
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SYNOPSIS

Aroma is one of the main quality attributes of any food product which decides its quality and consumer acceptability. Volatile constituents present in any food product contribute towards aroma quality while non- volatile compounds account for taste. Besides, these there exists a class of glycosidically bound aroma compounds occurring mainly as O-β-D-glucosides and O-diglycosides. Although, odorless they are able to release free aroma compounds by enzymatic or chemical hydrolysis during processing and storage. These precursors in many cases have been shown to be more abundant than free form to the extent of 70-90%. In view of importance of glycosidic precursors as potential source of aroma compounds, there is increased interest in their occurrence, chemical composition and role in imparting aroma quality to food products.

Presence of glucoconjugated forms of aroma was first demonstrated in rose petals in 1969 (1). Since then, their presence in over 150 plant species distributed in over 50 families has been reported. Glycoconjugates are present not only in green aerial parts and fruits of plants but also in roots, rhizomes, petals and seeds.

Chemically aroma glycosides identified in plants are mainly O-β-D-glucosides or O-diglucosides. In few cases, trisaccharide glycoconjugates are also present. The aglycone moiety is always linked to β-D-glucopyranose. In diglycosides, the glucose moiety is further substituted with one of the following four monosaccharides in grapes: β-D-glucopyranose, α-L-arabinofuranose, α-L-rhamnopyranose and β-D-apiofuranose (2). The aglycones of plant glycosides are structurally complex and highly diverse group of compounds. More than 200 different aglycones have been identified in different plant species till date. The aglycone moieties present are alkanols, alkenols, monoterpenes, C13-norisoprenoids, C15-sesquiterpenoids and benzene derivatives. Released aglycones may already be odorous such as linalool, geraniol and nerol etc. or may lead to formation of potent flavor molecules such as vitispirane, β-damascenone and theaspirane by further enzymatic and chemical transformations during food processing (3).

Grape (*Vitis vinifera*), is one of the earliest fruit crops known to man, and is widely cultivated all over the world. It is believed that grape cultivation started during Neolithic period (~6000 BC) in Near east (4). They are non-climacteric type of fruits that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange and pink. Commercially cultivated grapes are usually classified as either table or wine grapes. Table grape cultivars are large, seedless/seeded berries with relatively thick skin in comparison to small, seeded and juice grapes used for wine making. Globally grapes are mainly used for wine and raisin making followed by consumption as fresh. Most important product prepared from grapes is wine which is produced by fermentation of grape musts.

Grape wine can also be defined as the fermented juice of grapes, made in many varieties, such as red, white, sweet, dry, still, and sparkling, for use as a beverage, in cooking, in religious rites, etc., and usually having an alcoholic content of 14 percent or less. Wine as a drink has a history of over 7000 years. The wine production process starts in the vineyard, continues through fermentation and maturation, and concludes at packaging. Consumer acceptability of wine mainly depends on its aroma quality. More than 800 compounds have been recognized in wine thus far, of which 160 are esters. Wines generally contain 0.8–1.2 g of aromatic compounds per liter, of which the most common are fusel alcohols, volatile acids, and fatty acid esters. Complex interactions among these volatile constituents decide final aroma quality of wine. Another important group of compounds present in wine are phenolic constituents. Phenolic compounds contribute to wine organoleptic characteristics such as color, astringency, bitterness, and aroma. Moderate wine consumption is related to several beneficial physiological effects. Phenolic compounds induce endothelial nitric-oxide-dependent vasorelaxation and inhibit oxidation of human low density lipoproteins and platelet aggregation. All these effects are associated with lower incidence of cardiovascular diseases. The anticarcinogenic activities of wine phenolic compounds have also been demonstrated.

Food irradiation is the treatment of food by ionizing radiation. The process involves exposing food, either packaged or in bulk, to carefully controlled amounts of ionizing radiation for a specific time to achieve certain desirable objectives. It is one of the most extensive and thoroughly studied methods of food preservation. Expert group constituted by WHO/FAO/IAEA in 1998 affirmed the safety of food irradiated to doses above 10 kGy. Food irradiation is generally used for sprout inhibition in tubers, delayed ripening in fruits and microbial decontamination of various food products.

There are several reports on the impact of radiation processing on glycosides in various food products. Radiation induced degradation of glycosides have been reported in saffron, monsoon coffee, nutmeg, fenugreek and papaya. Radiation induced degradation of glycosides and corresponding increase in aglycones resulted in either enhanced color as in saffron or increased

aroma in coffee, nutmeg, papaya and fenugreek (5). Radiation processing is also reported to have significant influence on phenolic content of food products. Increased antioxidant and phenolic contents due to radiation processing in several other products such as brazilian mushrooms, carrot and kale juice, fresh cut mangoes, almond skins, rosemary, niger seeds and soybean is reported (6). The enhanced antioxidant capacity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonia-lyase and peroxidase activity) or to the increased extractability from the tissues. One of the main uses of radiation processing is control of microbial spoilage of food products. Gamma radiation being an ionizing radiation causes radiolysis of water thereby producing reactive hydroxyl radical. Hydroxyl radicals are extremely reactive and attack and damage cellular components, especially DNA. Due to damage to genetic material there is inhibition in microbial growth. Radiation processing has successfully been used for microbial decontamination of spices, juices, meat products, fresh cut fruits and vegetables and many other products (7). Thus, it could be concluded that radiation processing can enhanced aroma by hydrolyzing aroma glycosides, increase total phenolics and antioxidant capacity, and bring about microbial decontamination of food products. However, studies on impact of radiation processing on grapes and grape products such as wine are far and few in between. The present thesis thus aims at understanding impact of gamma radiation on aroma constituents in grapes. Based on changes in volatile constituents during radiation processing a rapid electronic nose based method for detecting radiation treatment has been developed. Improvement in wine organoleptic quality using radiation processing of grapes is attempted. Finally, use of radiation processing for inactivation of wine spoilage microorganisms is demonstrated.

Chapter 1 of the thesis introduces the subject of aroma glycosides with special emphasis on grapes. Importance of aroma glycosides in wine making is described. This chapter also describes the scientific literature related to the present work. Based on the review of available literature, it was found that radiation processing could hydrolyze aroma glycosides thereby resulting in enhanced

aroma. It can also lead to increased phenolic content and microbial decontamination. However, there are no reports dealing with the impact of radiation processing on aroma glycosides in grapes. The present thesis deals with the effect of gamma irradiation on aroma quality of grapes and its potential application for improving wine organoleptic quality.

Chapter 2 of the thesis describes the materials and experimental methods. Grape samples of different varieties were obtained from local growers in Nashik, Maharashtra. Irradiation was carried out using a food package irradiator (AEC, Canada) at BARC, Mumbai.

Aroma glycosides were extracted using solid phase extraction using C-18 reverse phase cartridges. Solid phase microextraction technique was used for isolation of aroma compounds. Further analysis was done by GC/MS. Volatile head space of grapes as extracted by SPME was also analyzed by directly infusing in mass spectrometer to obtain total mass spectrum. Total mass spectrum thus obtained was further processed using chemometrics (PCA and LDA) for identification of radiation treatment.

Wines were prepared using yeast strain SC-101 and maturation was performed for period of four months. Total antioxidant analysis of wine was performed using DPPH assay while total phenolics were analyzed using Folin's method. Color analysis of wines was performed by directly measuring the absorbance on a spectrophotometer. Wines were also analyzed for alcohol percentage, reducing sugars and pH using standard methods. Phenolic constituents in wines were analyzed using HPLC while volatile composition were analyzed using GC/MS. Sensory analysis of wines were carried out using quantitative descriptive analysis. Inactivation of wine spoilage microorganisms using radiation processing was also attempted. D10 values of three wine spoilage microorganisms *Dekkera bruxellensis*, *Acetobacter aceti* and *Pediococcus acidilactici* were calculated.

Chapter 3 deals with the results obtained. It has been divided into following subsections.

3.1 Effect of radiation processing on free and bound flavor precursors in grapes: Methodology for extraction and identification of aroma glycosides was optimized. For isolation of glycosides,

extraction of grape juice using C-18 reverse phase cartridges provided best results among various methods tested. Hydrolysis of isolated glycosides using β-glycosidase, pectinase and mineral acid was attempted. Best results in terms of number of aglycones identified were obtained after pectinase hydrolysis. Effect of radiation processing (0.5–2 kGy) on free and bound aroma precursors of three varieties i.e. Chenin Blanc, Cabernet Sauvignon and Shiraz were studied. Significant ($p<0.05$) effect of radiation processing on free and bound aroma precursors was observed. In Chenin Blanc variety, a radiation induced decrease in content of various terpenes such as α-pinene, β-pinene, myrcene, limonene and α-terpineol could be clearly observed. However, no significant changes were observed in other compounds such as alcohols and aldehydes. Interestingly, for Cabernet Sauvignon variety in case of terpenes, a decrease in content of bound precursors of only α-terpineol was observed. No changes were however observed for other terpenes such as α-pinene, β-pinene and limonene. Furthermore, a radiation induced decreased content of glycosidic precursors of various alcohols such as hexanol, heptanol, 1-octen-3-ol, 2-ethyl hexanol, benzyl alcohol and 1-decanol was observed. Similar results were obtained for Shiraz variety where decrease in alcohols such as hexanol and benzyl alcohol and terpenes such as geraniol and isogeraniol was observed. Surprisingly, an increase in content of bound precursor of methyl salicylate was observed in case of Shiraz and Cabernet Sauvignon variety.

A corresponding increase in content of free forms of these compounds was observed. Furthermore, an 80% increase in content of hexanal and *trans*-hex-2-enal was observed in the free volatiles of Chenin Blanc variety. Chenin Blanc is a green wine making variety and increase in these compounds might be due to lipid radiolysis and a consequent increased availability of free linolenic acid for the enzymes of lipo-oxygenase pathway.

3.2 Influence of radiation processing of grapes on wine quality: Grapes were subjected to radiation processing (up to 2 kGy) and wines were prepared and matured (4 months, 15°C). The wines were analyzed for chromatic characteristics, total anthocyanin (TA), phenolic (TP) and total antioxidant

(TAC) content. Aroma of wines was analyzed by GC/MS and sensory analysis was carried out using descriptive analysis. With regard to red wine making varieties, TA, TP and TAC were 77, 31 and 37 percent higher for irradiated (1.5 kGy) Cabernet Sauvignon wines, while irradiated Shiraz wines demonstrated 47, 18 and 19 percent higher TA, TP and TAC, respectively. HPLC-DAD analysis revealed that radiation processing of grapes resulted in increased extraction of phenolic constituents in wine with no qualitative changes. No major radiation induced qualitative changes were observed in aroma constituents of wine. Sensory analysis revealed that 1.5 kGy irradiated samples had higher fruity and berry notes. In case of white wine making variety (Chenin Blanc), radiation processing resulted in improved antioxidant and phenolic content, however, wines prepared were significantly brown in appearance. Thus the results clearly demonstrate that radiation processing of grapes resulted in wines with improved organoleptic and antioxidant properties in case of red varieties but the process is not suitable for white wine making varieties.

3.3 Cold pasteurization of wine using radiation processing: Shiraz wines were subjected to radiation processing (0.5 -2 kGy). No significant changes were observed in color, phenolics and antioxidants up to a dose of 1.5 kGy. However, higher doses resulted in significant ($p<0.05$) decrease in these parameters. Sensory analysis also revealed that there is no significant ($p<0.05$) change in wine organoleptic quality up to a dose of 1.5 kGy. Therefore, these results suggest possibility of using radiation processing for cold pasteurization of wines. Three different wine spoilage microorganisms i.e. yeast (*Dekkera bruxellensis*), lactobacillus bacteria (*Pediococcus acidilactici*) and acetic acid bacteria (*Acetobacter aceti*) were chosen for present study. D10 values for these microorganisms for irradiation in both saline and wine medium was estimated. D10 values for these microorganisms were 900, 833 and 220 Gy in saline, respectively. Interestingly, when irradiated in wine D10 values reduced to 500, 490 and 170 Gy, respectively. Presence of high concentration of alcohol in wine might have synergistically acted with radiation thereby resulting in reduction in D10 values. These

results suggest possibility of using radiation processing for cold pasteurization of wines and also for using lesser amount of SO_2.

3.4 SPME-HS in combination with chemometrics for fingerprinting radiation treatment: Volatile head space extracted from grapes using SPME was directly injected in mass spectrum and total mass spectrum was obtained. Mass spectrum thus obtained was analyzed using principal component analysis and linear discriminant analysis. PCA resulted in complete segregation of control and irradiated samples, thus suggesting variability in data set that can be used for analysis by supervised technique such as LDA. LDA models were subsequently built and previously unused data was analyzed for testing suitability of model generated. Complete classification of control and irradiated samples at each dose was obtained. Developed methodology was also successfully demonstrated on two varieties of apples (Royal Gala and Red Delicious). Thus, total mass spectrum in combination with chemometrics was successfully demonstrated for rapid screening of radiation processed samples.

Chapter 4 is the concluding chapter of the thesis. This chapter would focus on discussions on the possible future directions that can be explored further.

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Chapter 1: *Introduction*

Aroma is one of the main attribute of any food product which decides its quality and consumer acceptability. Volatile constituents present in any food product contribute towards aroma quality while non- volatile compounds account for taste. Besides, these there exists a class of glycosidically bound aroma compounds occurring mainly as *O*-β-D-glucosides and *O*-diglycosides. Although, odourless they are able to release free aroma compounds by enzymatic or chemical hydrolysis during processing and storage. These precursors in many cases have been shown to be more abundant than free form to the extent of 70-90% (1). In view of importance of glycosidic precursors as potential source of aroma compounds, there is increased interest in their occurrence, chemical composition and role in imparting aroma quality to food products.

1.1 Occurrence and chemical composition of glycosidic aroma precursors

Presence of glucoconjugated forms of aroma was first demonstrated in rose petals in 1969 by Francis and Allock (2). Since then, their presence in over 150 plant species distributed in over 50 families has been reported. Glycoconjugates are present not only in green aerial parts and fruits of plants but also in roots, rhizomes, petals and seeds.

Chemically aroma glycosides identified in plants are mainly *O*-β-D-glucosides or *O*-diglucosides. In few cases, trisaccharide glycoconjugates are also present (3). The aglycone moiety is always linked to β-D-glucopyranose. In diglycosides, the glucose moiety is further substituted with one of the following six monosaccharides: α-L-arabinofuranose, α-L-arabinopyranose, α-L-rhamnopyranose, β-D-glucopyranose, β-D-apiofuranose and β-D-xylopyranose (Figure. 1). However, in grapes glucose moiety is substituted with only four monosaccharides which are: β-D-glucopyranose, α-L-arabinofuranose, α-L-rhamnopyranose and β-D-apiofuranose (3).

Figure 1. Structrue of glycosidic portion of aroma glycosides

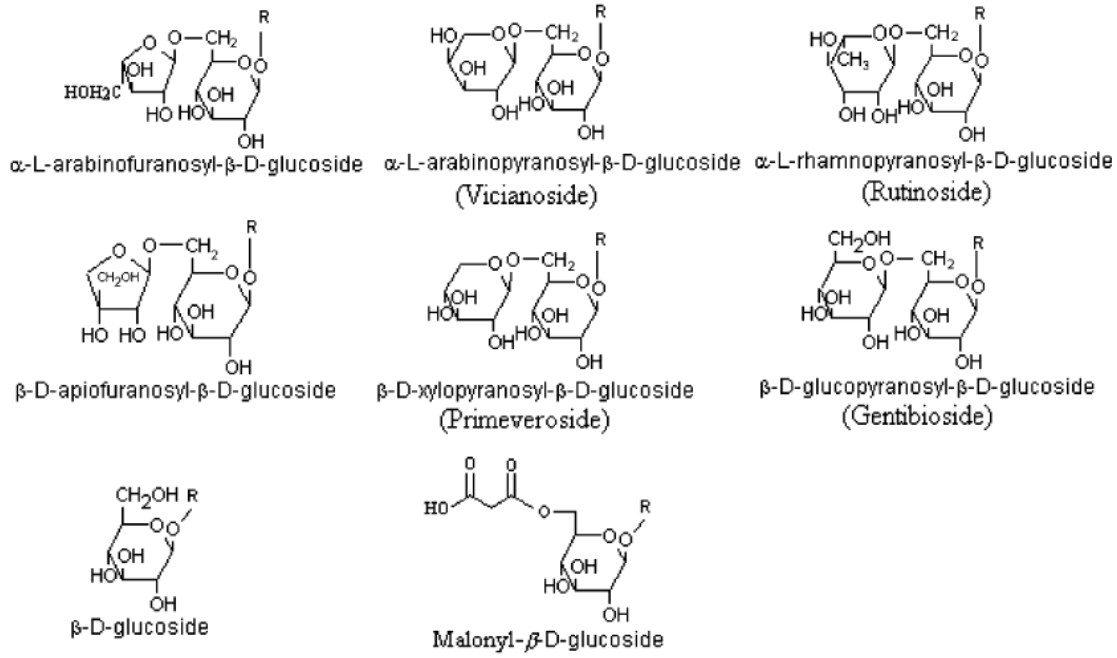


Figure adapted from Sarry and Gunata (2004) (3)

Figure 2. Structures of major aglycones present in plants

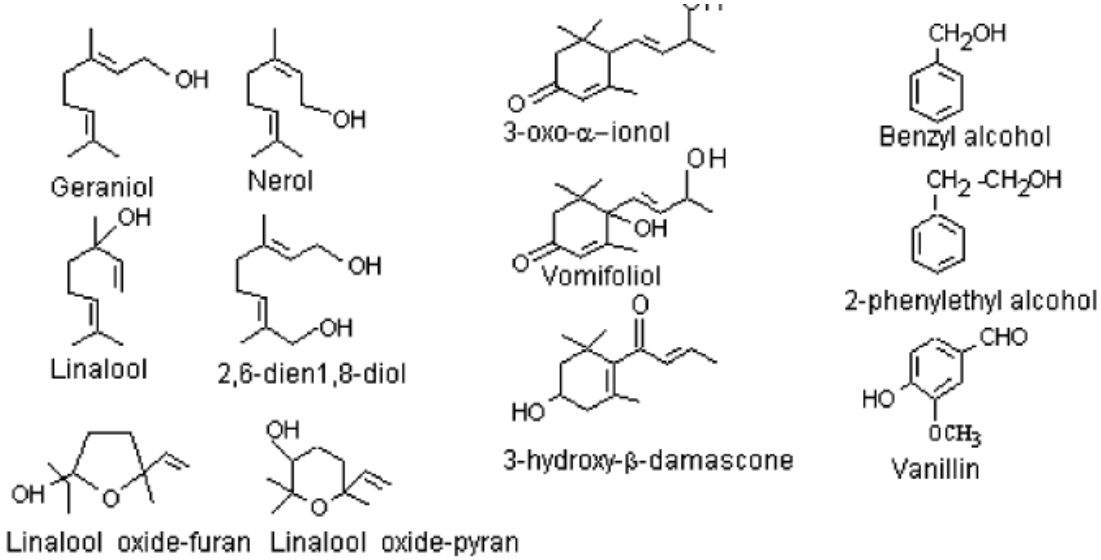


Figure adapted from Sarry and Gunata (2004) (3)

The aglycones of plant glycosides are structurally complex and highly diverse group of compounds. More than 200 different aglycones have been identified in different plant species till date. The aglycone moieties present are alkanols, alkenols, monoterpenes, C_{13}-norisoprenoids, C_{15}-sesquiterpenoids and benzene derivatives (Figure. 2). Released aglycones may either be odorous such as linalool, geraniol and nerol etc. or may lead to the formation of potent flavour molecules such as vitispirane, β-damascenone and theaspirane by further enzymatic and chemical transformations during food processing (1).

1.2 Role of glycosidically bound volatiles in plants

Presence of vast number of aroma glycosides and their widespread distribution in the plant kingdom indicates that these compounds are involved in different processes. Glycosides are important for accumulation, storage and transport of hydrophobic substances. In various fruits and vegetables, the amount of bound volatiles is considerably higher as compared to free forms. Generally, amount of bound flavours was found to exceed the free aroma in ratio of 2:1 to 5:1.

Two distinct chemical properties of glucoconjugates as compared to their corresponding aglycones are their enhanced water solubility and decreased chemical reactivity. This explains the reason for accumulation of glucoconjugates rather than free forms in plant kingdom. Glycoconjugation allows for a better storage in cell vacuole and also protects plant cells from toxicity exhibited by the free aglycone. High concentrations of aglycones such as lipophilic phenols or alcohols are likely to cause damage to membrane structures of plant cell. Protective mechanism of glycosides was clearly demonstrated by Berger and Drawert (4) in their studies on plant cell cultures. Different plant cell lines were

grown in media having excess of lipophilic substrates. Rosemary and grape cell lines stopped growing. Pea and peppermint cell lines that converted (conversion rate > 40%) and accumulated high amount of lipophilic substrates to glycoconjugates continued growing. Besides these storage and protective functions glycoconjugates serve another important function in transport of aroma volatiles in plants. Aroma volatiles, biosynthesized in the leaves are glycosylated and transported via the phloem to the flowers. Glycosides accumulate as flavourless precursors in the flower buds and during flower opening the precursors are enzymatically hydrolysed into volatile compounds. Glycoconjugates thus also play an important role in contributing to flower fragrance formation and in attracting pollinating insects and bees. However, extent of release of accumulated aroma compounds *in vivo* and possible physiological role remain subject for future research.

1.3 Methods of analysis of aroma precursors

Aroma glycosides are generally extracted from aqueous extracts of various plant parts. The technique mainly used for isolation of glycosides from aqueous extracts involves selective retention of glycosides on hydrophobic adsorbents such as C_{18}-reversed phase, Amberlite XAD-2 and Amberlite XAD-16. Glycosides were then recovered using desorption by polar solvents such as ethyl acetate and methanol. Glucoconjugates thus obtained can be hydrolysed by either enzymatic or acid hydrolysis and the aglycones thus released are subsequently identified using gas chromatography mass spectrometry (GC-MS). In order to obtain information regarding chemical structure of the individual glucoconjugates in the glycosidic extracts, sophisticated chromatography techniques such as HPLC, size exclusion chromatography and countercurrent chromatography (CCC) are

generally used. Alternatively, complex glycosidic mixture obtained can be derivatized (acetylation, methylation or silylation) to their more volatile form which could then be analysed using online coupled techniques such as GC-MS or GC-FTIR. Rapid methods to obtain total aroma glycosides present in fruits have also been proposed. Williams et al., 1995 (5) reported a method based on hydrolysis of glycosidic extract and determination of released glucose by rapid enzyme assay. This rapid method allows identifying aroma potential of grape varieties for wine making.

1.4 Grapes (*Vitis vinifera*)

Grapes are fruiting berry of the deciduous woody vines belonging to botanical genus *Vitis*. They are non-climacteric type of fruits that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange and pink. "White" grapes are actually green in colour, and are evolutionarily derived from the purple grape. Mutations in two regulatory genes responsible for production of anthocyanins result in white grapes. Grapes are typically of an ellipsoid shape resembling a prolate spheroid. They can be eaten raw or can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grape seed oil.

1.4.1 History

Grape (*Vitis vinifera*), is one of the earliest fruit crops known to man, and is widely cultivated all over the world. It is believed that grape cultivation started during Neolithic period (~6000 BC) in Near east (6). Seeds of domesticated grapes dated from ~6000 BC are found in archaeological sites of Georgia and Turkey. Nevertheless, seeds dating to Neolithic times have also been discovered in sites from western Europe and bronze age sites of France, suggesting domestication of grapes at these places around 6000 BC. From

these regions grape cultivation gradually spread to Egypt and Mediterranean region around 3000 BC. Grape cultivation reached Japan and China around 1200 BC and 200 AD, respectively. After 16th century grape cultivation was introduced in newer regions of world where it was not indigenous. It was introduced in America, Australia, New Zealand and South Africa by end of nineteenth century. However, at the end of nineteenth century disease causing agents such as mildew and Phylloxera resulted in destruction of many European vineyards thus reducing diversity of this species. Moreover, in last 50 years globalization of wine companies has led to emergence of worldwide grown cultivars such as Chardonnay, Cabernet Sauvignon, Syrah (Shiraz) and Merlot, and the disappearance of old local cultivars or landraces, thus further reducing diversity of species. Thus, human history has considerably shaped grape cultivation around the world.

1.4.2 Present distribution around globe

According to Food and Agricultural Organization, approximately 7,586,600 ha of total area are under grape cultivation. Spain has highest area (943,000 ha) in world under grape cultivation followed by France, Italy and China with 760805, 696756 and 600000 ha of area under cultivation, respectively. USA, Turkey, Iran and Argentina are other major countries having large amount of area under grape cultivation. India ranks fifteenth in world with an area under cultivation of 112000 ha.

China leads world production with an annual production of 9600000 MT followed by USA, Italy and France. India ranks 14th in world with an annual production of 1240000 MT. Approximately 71 percent area under cultivation is used for growing wine making varieties and rest is used for table grapes. In wine making varieties largest area under

cultivation is covered by Cabernet Sauvignon followed by Merlot and Airen (7). While in table grapes Thomson Seedless is most widely grown variety worldwide.

1.4.3 Table grapes and wine grapes

Commercially cultivated grapes are usually classified as either table or wine grapes. Table grapes are generally eaten raw whereas wine grapes are used to make wine. Although, table and wine grapes belong to same species i.e. *Vitis vinifera,* they have significant differences morphologically. Table grape cultivars are large, seedless fruit with relatively thin skin whereas wine grapes are smaller, usually seeded, and have relatively thick skins. Wine grapes are also very sweet and are harvested at the time when their juice is approximately 24% sugar by weight while table grapes have sugar content of around 15% in their juice.

1.4.4 Products prepared from grapes.

Grapes can be processed into many different products such as raisins, juice, jam, jellies, marmalade, vinegar, brandy, and wine. Grape pomace is a waste product of wine industry and is produced in huge quantities. It contains grape skins and seeds. Skins still contain large quantities of anthocyanins and their extracts are used as natural food colorants. Unsaturated oil is extracted by pressing grape seeds and is used for cooking. Most important product prepared from grapes is wine which is produced by fermentation of grape musts.

1.4.4.1 Wine

Wine is an alcoholic beverage prepared by fermentation of grapes or other fruits. Worldwide wine is produced mainly from grapes. Grape wine can also be defined as the fermented juice of grapes, made in many varieties, such as red, white, sweet, dry, still,

and sparkling, for use as a beverage, in cooking, in religious rites, etc., and usually having an alcoholic content of 14 percent or less (8). Wine as a drink has a history of over 7000 years. The earliest evidence of wine production was found in Iran at the Hajji Firuz Tepe site in the northern zagros mountains approximately 5400–5000 BC (6). However, modern wine production started around 17 century with widespread use of sulphur which allowed a better control of wine making process resulting in high quality wines (9).

Wine is the product of a complex biological and biochemical interaction between grape juice and different microorganisms such as fungi, yeasts, lactic acid bacteria and acetic acid bacteria (10). The wine production process starts in the vineyard, continues through fermentation and maturation, and concludes at packaging. It is affected by the various viticultural and oenological practices available to the grape-grower and winemaker, respectively. Commercial wine manufacturing consists of 6 basic steps:

Step 1- Harvesting

This is the most critical stage of process. The grapes must be harvested when the sugars, organic acids, phenols and aroma compounds are optimized for the style of wine desired. Generally grapes are harvested when total soluble solids content reaches 24 °Brix indicating a sugar content of 24 percent in juice.

Step 2- Crushing and Destemming

The grapes are removed from the stems and gently crushed to break the skins. Sulphur dioxide is added to the grapes at this stage to prevent oxidation of polyphenols and inhibit microbial activity. Pectinolytic enzymes may also be added to break down the cell walls and aid the release of juice.

Step 3- Pressing

The juice extraction process depends on the types of wines to be used. In case of white wines immediately after destemming and crushing berries are squeezed and juice is released. After pressing, the juice is allowed to stand to separate the solids. If necessary the juice may be clarified by filtration or centrifugation. However, in case of red wines juice is not pressed and skins and seeds are allowed to remain in contact with juice till completion of fermentation. This allows extraction of polyphenols from skin into wines.

Step 4- Fermentation

The juice is inoculated with *Saccharomyces cerevisiae*, which carries out the fermentation reaction:

$$C_6H_{12}O_6\,(\text{Sugar}) \longrightarrow 2C_2H_5OH\,(\text{Ethanol}) + 2CO_2$$

This reaction occurs through many intermediary biochemical steps. The process is carried out anaerobically. In the presence of oxygen (O_2) the phenols are oxidized and the sugars and ethanol are converted to CO_2 and H_2O.

Step 5- Maturation

The term wine 'maturation' refers to changes in wine after fermentation and before bottling. During this period, the wine is subjected to various treatments, such as malolactic fermentation, clarification, stabilization, and bulk storage. The important feature of this phase is that the wine is periodically exposed to air where many oxidative reactions influence the changes in wine composition.

Step 6- Purification, bottling and ageing

Unwanted solids, salts and microorganisms are removed through a variety of physical processes such as filtration, clarification by using bentonite or diatomaceous earth etc.

and then the wine is bottled and commercialized. The term ageing describes changes in wine composition after bottling. After bottling, once the oxygen present at the time of bottling is consumed, the wine is in the absence of oxygen resulting in a reductive atmosphere. Many reactions occur during this phase to contribute to the final wine aroma.

1.4.4.1.1 Chemical composition of wine

Knowledge of the chemical composition of wine has advanced greatly in the last 30–40 years. Development of gas chromatography (GC), high pressure liquid chromatography (HPLC), thin layer chromatography (TLC), droplet counter-current chromatography (DCCC), infrared spectroscopy (IRS), and nuclear magnetic resonance (NMR) has aided in identifying large number of compounds in wine. Hyphenated techniques such as GCMS and HPLCMS have been especially important in identifying unknown compounds. More than 800 compounds have been recognized in wine thus far, of which 160 are esters. The concentrations of the majority of compounds range between 10^{-1} and 10^{-6} mg/L. At these levels the individual compounds play very little or no role in the human organoleptic (taste) perception, but collectively they may be very significant. Majority of compounds responsible for aroma and taste of wine are metabolic by-products of yeast metabolism during fermentation. Wines generally contain 0.8–1.2 g of aromatic compounds per litre, of which the most common are fusel alcohols, volatile acids, and fatty acid esters. Fusel alcohols often constitute 50% of all volatile substances in individual wines. Carbonyls, phenols, lactones, terpenes, acetals, hydrocarbons, sulphur, and nitrogen compounds, although present in very low concentrations, play an important role in providing specific sensory characteristics to wine. The taste and mouth-

feel sensations are primarily due to compounds such as water, ethanol, organic acids, sugars, and glycerol. Major chemical constituents of wine are:

a) Water

Water is the predominant chemical constituent of wine. It is an essential component in many of the chemical reactions involved during fermentation and in wine ageing. Compounds insoluble or only slightly soluble in water rarely play a significant role in wine.

b) Sugars

The principal grape sugars are glucose and fructose, and they occur in roughly equal proportions at maturity, whereas overmature grapes often have a higher proportion of fructose. Sucrose and other sugars are found in insignificant amounts in *Vitis vinifera* grapes. The primary wine yeast, *Saccharomyces cerevisiae*, derives most of its metabolic energy from glucose and fructose and has limited ability to ferment other substances. Residual sugars in dry wines, generally below 1.5 g/L, consist mainly of pentoses such as arabinose, rhamnose, and xylose. Their levels may increase slightly during maturation in oak cooperage via the breakdown of glucosides in the wood, as well as from their synthesis and release by yeast cells. In addition to being absolutely essential for fermentation and production of ethanol, sugars are metabolized to higher alcohols, fatty acid esters, and aldehydes, which give different wines their individual aromatic character. High sugar concentrations can also increase the volatility of aromatic compounds.

C) Polysaccharides

Polysaccharides are generally present in low amounts in finished wines. They are partially water soluble and are extracted into the juice during crushing and pressing, but

during fermentation polysaccharides form complex colloids in the presence of alcohol and tend to precipitate. The addition of pectolytic enzymes following crushing also significantly reduces the pectin content in wine.

D) Alcohols

The most important and abundant alcohol in wine is ethanol. Under standard fermentation conditions, ethanol can accumulate to ~14–15%, but generally ethanol concentrations in wine range between 10–13%. Ethanol production is mainly governed by sugars, temperature during fermentation and yeast strain. Ethanol plays an important role in stability, ageing, and sensory properties of wine. As its content increases during fermentation, it limits the growth of most microorganisms, allowing *Saccharyomyces cerevisiae* to dominate the fermentation process. The inhibitory activity of ethanol, combined with the acidity of the wine and the added potassium metabisulfite, allows wine to remain stable for years in the absence of air. During production of red wine, ethanol acts as an important solvent in the extraction of pigments and tannins. It also influences the types and amounts of aromatic compounds produced by affecting the metabolic activity of yeasts. During ageing, along with other alcohols, it slowly reacts with organic acids to produce esters. Moreover, it also reacts slowly with aldehydes to produce acetals. Furthermore, ethanol acts as an essential reactant in the formation of volatile compounds produced during fermentation and those formed during ageing in wood cooperage.

Methanol is a minor constituent of wine (0.1–0.2g/L) and has no direct sensory effect. It is predominantly generated from the enzymatic breakdown of pectins. Methanol is released on degradation of methyl groups associated with pectins. Pectolytic enzymes

added to juice or wine to aid clarification inadvertently increase the methanol content of wine. Wine has the lowest concentration of methanol of all fermented beverages. Other potentially significant higher alcohols in wine are the straight-chain alcohols: 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol. The formation of higher alcohols occurs as a by-product of yeast fermentation and is markedly influenced by vinification practices such as temperature, presence of oxygen, suspended solids, and yeast strain. Higher alcohols mainly originate from grape-derived aldehydes and by deamination or reductive denitrification of amino acids.

E) Acids

In wine, acids are divided into two categories: volatile and fixed. Volatile acids can be readily removed by distillation, whereas fixed acids refer to the carboxylic acids. The most common volatile acid in wine is acetic acid. Carboxylic acids such as tartaric, malic, lactic, succinic, oxalic, fumaric, and citric acids control the pH of wine. They also play an important role in providing taste to wine.

F) Phenolic constituents

Chemically, phenols are cyclic benzene compounds possessing one or more hydroxyl groups associated directly with the ring structure. Phenolic compounds constitute one of the most important quality parameters of wines, since they contribute to wine organoleptic characteristics such as colour, astringency, bitterness, and aroma. Moderate wine consumption is related to several beneficial physiological effects. Consumption of red wine is associated with lowering risk of cardiovascular diseases and heart failure. This phenomenon is also known as “French paradox” which indicates comparatively low incidence of coronary heart disease in France despite relatively high levels of saturated

fat in the traditional French diet. Due to their antioxidant and anti-inflammatory properties phenolic compounds are associated with useful physiological effects that are derived from moderate wine consumption. They induce endothelial nitric-oxide-dependent vasorelaxation and inhibit oxidation of human low density lipoproteins and platelet aggregation. All these effects are associated with lower incidence of cardiovascular diseases. The anticarcinogenic activities of wine phenolic compounds have also been demonstrated. Source of phenolic compounds in wine are fruits (skins and seeds), vine stems, yeast metabolism and wood cooperage. Primarily two different types of phenolic compounds are found in wine i.e. flavonoids and non-flavonoids.

1) Flavonoids: Flavonoids are molecules having two phenol rings joined by a pyran (oxygen-containing) carbon ring structure. The most common flavonoids in wine are flavonols, catechins (flavan-3-ols), and, in red wines, anthocyanins. Flavonoids are found both as free form or polymerized to other flavonoids, sugars, non-flavonoids, or a combination of these compounds. Those esterified to sugars or non-flavonoids are called glycosides and acyl derivatives, respectively.

Flavonols are yellow pigments mainly located in the vacuoles of the epidermal tissues. In *Vitis vinifera* grapes, they exist as the 3-O-glycosides of four main aglycones: myricetin, quercetin, kaempferol and isorhamnetin. However, in wines free forms of these compounds are also found which are formed due to hydrolysis of glycosidic forms during wine formation. Flavan-3-ols or flavanols are found in the solid parts of the berry (seed, skin and stem) in monomeric, oligomeric, or polymeric forms; the latter two forms are also known as proanthocyanidins or condensed tannins. The flavan-3-ol monomeric units found in *Vitis vinifera* grapes and wines are (+)-catechin, (−)-epicatechin, (+)-

gallocatechin, and (−)-epigallocatechin. Their polymeric forms i.e. proanthocyanidins are largely responsible for the astringency and bitterness of the wine. They participate in chemical and enzymatic oxidative browning reactions, in haze formation and in interactions with proteins, as well as in numerous condensation reactions during wine maturation and ageing. Anthocyanins are mainly located in the grape skins and are largely responsible for the colour of red wines. The anthocyanins identified in grape skins and in wine from *Vitis vinifera* are the 3-O-monoglucosides and the 3-O-acylated monoglucosides of five main anthocyanidins: delphinidin, cyanidin, petunidin, peonidin, and malvidin. During the winemaking process, anthocyanins are involved in oxidation, hydrolysis, and condensation reactions that are responsible for important colour changes in wine.

2) Non-flavonoids: Main non-flavonoids compounds present in grapes and in wine are phenolic acids (hydroxybenzoic and hydroxycinnamic acids) and other phenolic derivatives such as stilbenes. Gallic acid is the only hydroxybenzoic acid that has been identified in native state in grapes, found in the solid parts of the berry, either in free form or in the form of flavanol ester (i.e., epicatechin-3-O-gallate). However, other major hydroxybenzoic found in wines are p-hydroxybenzoic, protocatechuic, vanillic, syringic, and gentisic acids. Apart from native state methyl esters and glucoside derivatives of these hydroxybenzoic acids are also found.

The hydroxycinnamic acids are located in the vacuoles of the skin and pulp cells in the form of tartaric esters. Caffeoyltartaric (caftaric), p-coumaroyltartaric (cutaric), and feruloyltartaric (fertaric), p-coumaric and ferulic acid are major hydroxycinnamic acids present in wine. Stilbenes are third category of non-flavonoids present in wine. They are

phytoalexins synthesized especially in skins, roots and leaves of plants in response to fungal infections and ultraviolet (UV) light. *Trans* and *cis* resveratrol and their glucosides are major stilbenes reported in wines. Grapes and their products are main dietary source of stilbenes.

1.4.4.1.2 Present status of production and consumption of wine around world

According to International organization of vine and wine (11), production of wine around world is more or less stable at around 270 million hectolitres (Mhl). In years from 2000 to 2011 wine production varied between low of 257 Mhl in 2002 to high of 296 Mhl in 2004. Production of wine stood at 265 Mhl in year 2011. France is largest producer of wine in world with a production of 50 Mhl followed by Italy and Spain having a production of 41 and 33 Mhl respectively. India is relatively a very small player in world wine production with a production of only 0.062 Mhl annually.

In contrast, global wine consumption is increasing steadily. Wine consumption globally was 225 Mhl in 2002 which steadily rose to 244 Mhl in 2011. Largest consumption of wine is in France followed by USA and Italy. Per capita consumption of wine in France is about 50 L annually while in comparison in India it is only 0.07 L annually. Poor storage and transport facilities are the main problems of wine marketing in the country. Other constraints are the lack of promotional activities for wine consumption in the country and unfavourable rules for domestic marketing of wines except in few states.

1.5 Food Irradiation

Food irradiation is the treatment of food by ionizing radiation. The process involves exposing food, either packaged or in bulk, to carefully controlled amounts of ionizing radiation for a specific time to achieve certain desirable objectives. It is one of the most

extensive and thoroughly studied methods of food preservation. There are various methods used to irradiate food and non-food materials such as exposing the materials to be irradiated to electron beams, X-rays or γ-rays. They differ in their penetration power, frequency, wavelength and effects on biological systems. As per the Codex Alimentarius Commission 1984, the irradiation sources permitted for use in radiation processing of foods are gamma rays from Cobalt-60 or Cesium-137, machine generated electron beams (maximum energy 10 MeV) and X-rays (maximum energy 5MeV). In 1980, Joint Expert Committee of Food and Agriculture Organization / International Atomic Energy Agency / World Health Organization on Food Irradiation FAO/IAEA/WHO, 1981 concluded "The irradiation treatment of any food commodity up to an overall average dose of 10 kGy present no radiological, microbiological or toxicological hazard". Another group of experts was constituted by world health organization (WHO) in 1994 and the committee again reviewed the wholesomeness data available till then and validated the earlier conclusion. In 1998, one more expert group constituted by WHO/FAO/IAEA affirmed the safety of food irradiated to doses above 10 kGy. In view of these recommendations, the Codex Committee on Food Standards of the Codex Alimentarius Commission has also revised in 2003 the Codex General Standard for Irradiated Foods that sets standards for process foods world-wide. Codex Alimentarius standard set upper limit for radiation dose for food irradiation at 10 kGy, except in cases where higher doses are required to achieve a legitimate technical purpose. As a result toxicological testing of foods so treated is no longer required. Food irradiation is now legally accepted in many countries.

Food irradiation applications are divided based on radiation dose required to achieve desired objective. Dose is measured as energy deposited in food product by ionizing

radiation. The dose of radiation is measured in the SI unit known as Gray (Gy). One Gy of radiation is equal to one joule of energy absorbed per kilogram of food material. In radiation processing of foods the doses are generally measured in kGy (1000Gy). The ranges of dose commonly employed in various food irradiation applications to achieve different objectives can be classified into three groups i.e. low dose applications (10 Gy to 1 kGy), medium dose applications (1 – 10 kGy) and high dose applications (10 – 100 kGy). Table 1 briefly describes applications of all three groups of food irradiation.

1.5.1 Approval of food irradiation in India

In 1994 Government of India amended Prevention of Food Adulteration Act (1954) Rules and approved irradiation of onion, potato and spices for domestic market. Additional items were approved in April, 1998 and in May 2001 (Table 2). In 2004, the government amended plant protection and quarantine measures. Laws and regulations enacted under the Atomic Energy Act enforced by the Atomic Energy Regulatory Board, an independent body, govern operations of irradiators used to process non-food products, such as medical supplies as well as food. Many medical product irradiators are operating in India and around the world. The plants that must be approved by the government before construction and operation are subject to regular inspection, safety audits, and other reviews to ensure that they are safely and properly operated. Only those foods approved under the Prevention of Food Adulteration (PFA) Act rules can be irradiated and sold in domestic market.

Table 1. Various applications of food irradiation

SL. No.	Food applications of various radiation doses	Dosage (kGy)
1.	**Low Dose Applications**	
	• Sprout inhibition in bulbs and tubers.	0.03-0.15
	• Delay in fruity ripening.	0.25-0.75
	• Insect disinfestations and elimination of food borne parasites.	0.07-1.00
2.	**Medium Dose Applications**	
	• Reduction of spoilage microbes to improve shelf-life of meat, poultry and sea foods under refrigeration.	1.50-3.00
	• Elimination of pathogenic microbes in fresh and frozen animal foods.	3.00- 7.00
	• Reducing number of microorganisms in spices to improve hygienic quality.	7.00- 10.00
3.	**High Dose Applications**	
	• Sterilization of packaged meat, poultry and their products which are shelf stable without refrigeration.	25.00- 70.00
	• Sterilization of hospital diets.	25.00- 70.00
	• Product improvement as increased juice yield or improved rehydration.	25.00- 70.00

Table 2. Food items approved for radiation processing in India under PFA rules

Name of Food	Purpose	Dose (kGy)	
		Min	**Max**
Onion	Sprout inhibition	0.03	0.09
Potato		0.06	0.15
Ginger, garlic		0.03	0.15
Shallots (Small onion)		0.03	0.15
Mango	Disinfestation (Quarantine)	0.25	0.75
Rice, semolina (rawa), whole wheat flour (*atta*) and maida	Insect disinfestation	0.25	1.00
Raisins, figs and dried dates		0.25	0.75
Pulses		0.25	1.00
Dried seafoods		0.25	1.00
Meat and meat products including chicken	Shelf-life extension and pathogen control	2.50	4.00
Fresh seafood	Shelf-life extension under refrigeration	1.00	3.00
Frozen seafood	Pathogen control	4.00	6.00
Spices	Microbial decontamination	6.00	14.00

1.6 Review of literature and Aims and Objectives

This section focuses on literature about the effect of various processing methods on aroma glycosides. Use of various enzyme treatments to improve aroma quality of various food products is also discussed. Furthermore, present literature about use of various physical treatments for improving wine quality is also presented. Interest on studying composition and role of bound aroma precursors in plants is increasing worldwide. Nature, occurrence and role of aroma glycosides in plants were reviewed in detail by Winterhalter and Skouroumounis (1) and are briefly described in section 1.1. It is now well established that the glycosidically bound fraction forms a reserve of aroma which can be exploited for enhancement in aroma of various food products. The quantity of glycosidically bound compounds can differ based on particular plant variety, climate, soil, ripeness or kind of tissue. In several fruits, volatiles originating from glycosides have been detected at concentrations several-fold greater than their free counterparts.

Most of research work on glycosides has been directed towards grapes and its products such as juices and wines. Flavour enhancement of juices and wine through the hydrolysis of the glycoside aroma precursors has attracted much attention. Therefore, glycosidases from grapes, filamentous fungi and yeast have been the subjects of extensive research for their possible involvement in the hydrolysis of glycosides.

β-glucosidase is the most abundant glucosidase activity in grapes, occurring together with other endogenous grape glycosidases, e.g. α-arabinosidase, α-rhamnosidase and possibly β-apiosidase (12). However, unfortunately the endogenous β-glucosidases in grapes show low stability under juice processing and winemaking conditions. The optimum pH activities of grape glucosidases are generally in the range 4.0–6.0. In the low pH of fruit

juices and wines, only 5–15% of the maximum activity of most glycosidases was observed (13). Therefore, inherent grape glycosides did not play much important role in hydrolysis of glycosides for possible aroma enhancement in commercial processes for wine and juice preparation.

Apart from indigenous grape glucosides, fermenting yeasts (*Saccharomyces cerevisiae*) also contribute towards β-glycosidase activity in wine preparation. Principal wine yeast, *S. cerevisiae*, displays low levels of α-arabinofuranosidase, α-rhamnosidase and β-glucosidase activity under fermentation conditions (14). However, β-glucosidase in Saccharomyces strains is associated with the cell wall and is found in the insoluble fraction obtained from lysed yeast cells, which is the main drawback for its technological application (15). Although, Saccharomyces β-glucosidase is generally glucose-independent, it is inhibited by approximately 50% by 5% ethanol in the medium. Therefore, the use of this enzyme is restricted to the first stages in the wine-making process.

Due to the limited effect of glycosidases from grapes and *S. cerevisiae* in winemaking, a large proportion of glycosides are still present in young wines. Therefore, attention has been focused on the use of exogenous glycosidases from yeasts and filamentous fungi to enhance wine aroma. Extracellular enzymes from the genera *Trichoderma, Penicyllium* and *Aspergillus* have been found to possess interesting properties for practical use. The most common enzyme preparations used industrially are from *Aspergillus niger*, which is recognized as a safe microorganism (Generally Regarded as Safe (GRAS)). The exoglycosidases and β-glucosidase from *Aspergillus niger* have good stability at the acidic pH of fruit juices, contrary to the enzymes from *Saccharomyces cerevisiae.*

Furthermore, glycosidases from fungi are more heat resistant than those from plants and yeasts (16). There are several reports demonstrating use of enzymes for flavour enhancement in juices and wine. β-glucosidase from *Candida molishiana* significantly increased the level of free aglycones in peach, cherry, strawberry, passion fruit, papaya, orange and apple juices (17). Successful enhancement in passion fruit aroma was demonstrated by use of immobilized β-glucosidase from *Aspergillus niger* (18). The use of enzyme preparations for aroma enhancement in wines is also reported. There are several reports demonstrating use of pectinase enzyme preparations for enhancing wine aroma. The enzyme-treated wines were highly significantly different from the control wines and were preferred (19-21). Masino et al., 2008 (22) reported use of β-glucanase to produce wines with higher concentration of many volatile compounds. Use of glycosidases for enhancing fresh and fruity characteristics of Albillo wines was successfully demonstrated by Sanchez-Palamo et al., 2007 (23). The use of glycosidic enzymes allows the levels of monoterpenes, C13-norisoprenoids and benzene derivatives to be increased. The effect of enzyme treatment is quickly perceived, since the liberated volatile aglycones are odorous and occur at concentrations exceeding their threshold levels. Among the most important aroma compounds released due to enzymatic hydrolysis are linalool, nerol, geraniol and citronellol which are most-flavour active, due to their low sensory threshold. Some liberated aglycones, such as β-damascenone, vitispirane and theaspirane, give rise to potent flavour after further enzymatic or chemical transformations during wine processing.

However, according to Tamborra et al., 1999 (24), glycosidase activity is often accompanied by collateral activities, such as esterase, oxidase, hydrolase, etc. Such

activities can annul the effect of glycosidases and produce a negative impact on the aroma and colour of juice or wine. Presence of oxidase activity results in higher content of oxidized monoterpenols (e.g. geranial, neral) in wines. Furthermore, glycosidase-rich pectinase/cellulase preparations could result in hydrolysis of glucosylated anthocyanidins leading to colour loss in red wines. Therefore, caution must be observed while choosing enzyme preparation for quality improvement in wines.

Apart from use of commercial pectinase and β-glucosidase enzymes there are several reports demonstrating that high temperature storage of wine results in hydrolysis of aroma glycosides and subsequent enhancement in free volatiles. Zoecklein et al., 1999 (25) demonstrated in their studies on white Riesling wines that thermal storage at 45°C for 20 days resulted in 33 percent decrease in total glycosides and increase in content of benzyl alcohol. Similarly, thermal storage of Chardonnay wines resulted in decrease in total glycosides. Wines stored at elevated temperature for 30 days had higher honey, rubber, tea/tobacco, butter/vanilla and oak notes with decreased fruity aroma (26). Losco et al., 2010 (27) also found that most of aromatic compounds from grape flavour precursors increased significantly in the first week when the wines were heated to 50°C to mimic wine ageing in bottle. Although, high temperature storage could possibly hydrolyse aroma glycosides and release free volatiles it could also sharply reduce the contents of aromatic compounds in wine (25, 28) and accelerate the process of browning of white wine (29).

Thus, enzyme treatments and thermal storage could hydrolyse aroma glycosides and enhance free aroma but both techniques have certain drawbacks as discussed above.

Another important group of substances influencing various organoleptic properties such as colour, astringency and bitterness of wine are the phenolic compounds (30). Phenolic compounds also have antioxidant properties and are responsible for health promoting properties of wine (31). As a consequence of the large influence of phenolic compounds on quality of red wine, a great effort has been devoted in recent years to develop different techniques to enhance their extraction during wine making process. Various techniques proposed to enhance extraction of these compounds are increasing fermentation temperature, extending maceration time, heating grape berries for short time, freezing grape berries before fermentation and pulsed electric fields (32, 33). However, using these techniques could lead to wines with poor and unstable colour characteristics (34). Therefore, there is a need for use of technique which could lead to improved aroma and phenolic contents with lesser deleterious effects on final wine quality.

SO_2 is widely used in wine making as it assists in colour extraction and preservation. It is also an antimicrobial and prevents wine from spoilage organisms such as yeasts and bacteria. Despite the fact that SO_2 is a versatile and efficient additive due to its antiseptic and antioxidant properties, it has been related to intolerance or even allergic reactions in some consumers (35). Due to the health related problems that have been associated with SO_2 use, the International Organization of Vine and Wine (OIV) has progressively reduced the maximum concentration permitted in wines, to currently 200 mg/L (36). Moreover, traditional techniques for microbiologically stabilizing wines, such as filtration, pasteurization and flash pasteurization at 60–70 °C all have a negative effect on their sensorial quality and reduce their polyphenolic compound, pigment and volatile compound contents. Due to these reasons there is an increasing interest in developing non

thermal processing techniques for wine preservation. In this regard use of high hydrostatic pressure (HHP), pulsed electric fields, UV irradiation, microwave processing, high power ultrasonics and addition of plant phenolics for microbial decontamination of wine has been successfully demonstrated. HHP is most widely reported technique for cold pasteurization of wine. Use of HHP for controlling wine spoilage yeast *Dekkera bruxellensis* was successfully demonstrated by Morata et al., 2012 (37). These authors also report that HHP does not significantly alter volatile composition and colour characteristics of wine. Up to 5 log cycle reduction in total yeast and bacterial count was observed using HHP treatment of wine with no were no differences in the aroma, taste, mouth-feel, and overall sensory quality (38). However, HHP treatment of white wine resulted in wines with brownish colour, cooked aroma and lower phenolic content as compared to control (39). Use of pulsed electric field for inactivation of wine and grape juice spoilage yeasts and lactic acid bacteria were successfully demonstrated by Puértolas et al., 2009 (34) and Marsellés-Fontanet et al., 2009 (40), respectively. However, no studies on the effect of PEF treatment on wine sensory quality were performed by these workers. Recently, efficacy of high power ultrasonics (HPU) for controlling several wine spoilage yeasts and bacterial species has been demonstrated. However, HPU also led to significant sensory changes in wine quality (41). UV irradiation was also reported to cause average 4 log cycle reduction in various wine spoilage microorganisms such as *Brettanomyces bruxellensis*, *Lactobacillus plantarum, Acetobacter aceti* and *Oenococcus oeni*. It was also reported that UV-C efficacy may be influenced by liquid properties such as colour and turbidity (42). In a study conducted on Pinot noir grapes it was demonstrated that microwave processing of grape musts resulted in rapid decrease in

grape associated yeast population. This led to shorter lag phase before onset of alcoholic fermentation. Furthermore, wines prepared with microwave treated musts had higher concentrations of total phenolics, anthocyanin, tannin and pigmented tannin in 18 months bottle age, compared with control wine (43). Apart from physical techniques mentioned above, González-Rompinelli et al., 2013 (44) reported use of phenolic extracts from almond skins and eucalyptus leaves for prevention of microbial spoilage during wine ageing. Wine added with phenolic extracts remained stable during ageing even in presence of lower amount of SO_2 (80 mg /L) as compared to control wines with 160 mg /L of SO_2. Although addition of plant phenolic extracts led to significant changes in volatile aroma profile and phenolic composition no significant changes in global sensory perception of wines could be noted.

1.6.1 Effect of radiation processing on aroma glycosides, phenolics and microbial quality of food products.

There are several reports describing impact of radiation processing on glycosides in various food products. Zareena et al., 2001 (45) reported gamma radiation induced enhanced colour in saffron. These authors attributed this increased colour to radiation degradation of carotene glucosides in saffron resulting in increased aglycone content. However, no changes in volatile profile of saffron were reported due to radiation. Gamma radiation induced enhanced rate of monsooning in monsooned Arabica coffee was reported by Variyar et al., 2003 (46). Increased rate of monsooning was due to higher content of 4-vinylguaiacol and isoeugenol in radiation processed samples. Release of these compounds from their corresponding glycosides due to radiation processing was also demonstrated. Similar results were also reported for radiation processing of nutmeg,

fenugreek and papaya. A 50 percent reduction in content of aroma glycosides in nutmeg was reported at a dose of 5 kGy (47). In case of radiation processing of fenugreek and papaya, an increased content of volatile phenol due to radiation processing was also reported (48, 49). Radiation dose dependent increase in volatile phenol was found to be due to degradation of phenol glycoside. Method of detection of radiation treatment in papaya and fenugreek based on detection of volatile phenol was also proposed. It was also reported that this release of volatile phenol does not adversely affect sensory quality of radiation processed papaya and fenugreek. However, radiation processing always does not lead to breakdown of aroma glycosides and consequently enhancement in free aglycones. Kumar et al., 2010 (50) while working on radiation processing of cured vanilla beans observed no radiation induced degradation of vanillin glucoside even up to dose of 30 kGy. By pulse radiolysis experiment it was observed that highly stable oxygen–carbon linkage between vanillin and glucose limits the possible enhancement of aroma quality of irradiated beans. Thus, from above mentioned studies it could be inferred that radiation processing could result in hydrolysis of aroma glycosides and a subsequent enhancement in free aroma volatiles. This phenomenon could be used for improving quality as in case of saffron, nutmeg and monsoon coffee or could be used for detecting radiation treatment as in case of fenugreek and papaya.

Radiation processing is also reported to have significant influence on phenolic content of food products. Irradiation can influence the levels of antioxidants and the capacity of a specific plant to produce them at different levels. It has been reported that under certain favourable conditions, the concentration of plant phytochemicals might be enhanced. This depends on the dose applied (usually low and medium doses have insignificant

effects on antioxidants), the sensitivity of the antioxidant or the phytochemicals towards irradiation, and the effect of irradiation itself on other food constituents that might be responsible for the production and/or the accumulation of antioxidants in the plant. There are several reports on radiation induced enhancement in phenolic content in plant produce. An increase in phenolic compounds due to radiation processing (2 kGy) in mushrooms (*Agaricus bisporus*) was reported by Beaulieu et al., 1999 (51). Gamma radiation induced accumulation of phenolic compounds was reported in clementines peel during storage period of 49 days (52). Similarly, Fan, 2005 (53) while working on three vegetables i.e. romaine, iceberg lettuce and endive observed enhanced total phenolic and antioxidant content due to radiation processing. Increased antioxidant and phenolic contents due to radiation processing in several other products such as Brazilian mushrooms, carrot and kale juice, fresh cut mangoes, almond skins, rosemary, niger seeds and soybean was previously reported (54). The enhanced antioxidant capacity of a plant after irradiation is mainly attributed either to increased enzyme activity (e.g., phenylalanine ammonia-lyase and peroxidase activity) or to the increased extractability from the tissues.

Fan et al., 2003 (55) while working on lettuce reported that the free radicals generated during irradiation might act as stress signals and may trigger stress responses in lettuce, resulting in an increased antioxidant synthesis. Similar improvement in antioxidant activity due to enhanced production of phenolics caused by radiation processing in three vegetables i.e. romaine, iceberg lettuce and endive was also reported (53). Improvement in antioxidant content due to increased extractability of anthocyanins by radiation processing was reported for grape pomace (56). Quantitative differences in the

constituents of nutmeg oil, as well as an increased amount of phenolic acids, were detected after γ-irradiation, which was attributed to the degradation of tannins and consequently higher extractability of phenolic acids (57). Variyar et al., 2004 (58), while working on soybeans, reported an enhancement in their antioxidant potential with dose of γ-irradiation, which was attributed to increased levels of genistein (an isoflavone) and to a lesser extent on the antioxidant activities of diadzein degradation products.

Apart from increase in antioxidant capacity there are several reports available wherein radiation treatments have been shown to reduce the antioxidant compounds. Radiation induced degradation of phenolic acids such as cinnamic, p-coumaric, gallic and hydroxybenzoic acid and flavonoids (catechin and kaempferol) were reported for strawberries. Similar reduction of phenolic acids has also been demonstrated for radiation processing of tomatoes, and spices such as cinnamon, ginger, nutmeg, anise, vanilla, mint and black pepper. Gamma radiation also resulted in decreased content of tocopherol in cashew nuts thereby reducing their antioxidative capacity (54). In general, decrease in antioxidant capacity due to radiation processing in attributed to formation of free radicals during radiation which could further lead to degradation of phytochemicals. Interestingly, no change in antioxidant capacity was observed for radiation processing of certain products such as turmeric, sweet basil and green tea (54). Thus, radiation processing could either increase or decrease or it may also result in no significant changes in antioxidant capacity based on product, dose given and post irradiation storage conditions.

One of the main uses of radiation processing is control of microbial spoilage of food products. Gamma radiation is an ionizing radiation, and it causes radiolysis of water producing reactive hydroxyl radical. Hydroxyl radicals are extremely reactive and attack

and damage cellular components, especially DNA. Due to damage to genetic material there is inhibition in microbial growth. Radiation processing has successfully been used for microbial decontamination of spices, juices, meat products, fresh cut fruits and vegetables and many other products (59). Radiation processing has been widely used for cold pasteurization of various fruit juices such as pomegranate (60), apple and orange (61), fresh carrot (62), ashitaba and kale (63), sugarcane (64) and cantaloupe (65). No significant effects on sensory quality of these products were observed due to radiation processing. Interestingly in some cases, it was observed that radiation processed juices had better sensory characteristics as compared to control. However, no reports were available for wine or grape juice pasteurization using radiation processing.

Thus from above cited reports it could be concluded that radiation processing could hydrolyse aroma glycosides which could result in enhanced aroma in food products. Radiation processing could also be very helpful in increasing total phenolics and antioxidant capacity and for microbial decontamination of food products. However, studies on impact of radiation processing on grapes and grape products such as wine are scanty. Chang, 2003 and 2004 (66, 67), reported the use of gamma radiation for rapid maturation of rice and maize wine, respectively. Wines aged with gamma radiation were found to be similar with that of conventional aged wines in sensory analysis. However, to best of our knowledge there are no studies on impact of radiation processing on aroma glycosides in grapes, impact of radiation processing of grapes on final wine quality and possible cold pasteurization of wine using radiation processing. Thus, in view of above aims and objectives of present study are as follows.

Aims and Objectives

The major aims of this project are as follows:

1) Isolation and quantification of flavour glycosides from grapes.

2) Identification and characterization of these isolated flavour glycosides.

3) Effect of postharvest radiation processing on flavour glycosides.

4) Technological applications of aroma glycosides to enhance aroma quality of beverages such as wine.

5) Inactivation of wine spoilage microorganisms using radiation processing

Chapter 2: *Materials and Methods*

2.1 Materials

2.1.1 Grape samples and radiation processing

One table grape variety i.e. Sonaka and three wine making varieties i.e. Cabernet Sauvignon, Shiraz and Chenin Blanc were chosen for present study. Sonaka (table grape) is green and seedless while Chenin Blanc is seeded green variety for wine making. Cabernet Sauvignon and Shiraz are red seeded grapes for wine making purposes. Sonaka grapes were procured from local market while the three wine making varieties were harvested at optimum maturity (24 °Brix) from vineyards located at Narayangaon, Maharashtra. Samples were brought to laboratory within twelve hours of harvesting and were then packed (500 g) in perforated polyethylene bags.

2.1.2 Chemicals and materials

Amberlite XAD-2 resin was procured from Sigma-Aldrich, USA. Reverse phase (C18) solid phase extraction cartridges with 6 mL volume and 0.5 g active phase were procured from Supelco, USA. Solid phase micro extraction (SPME) fibres used were Polydimethoxysiloxane-divinylbenzene-carboxen (PDMS/DVB/CAR) with film thickness of 0.65 μM and 1 cm length, bought from Supleco, USA.

Methanol and dichloromethane was procured from Merck India Pvt. Ltd. Diethyl ether and n-butanol was purchased from S.D. fine chemicals, India. All solvents used were of analytical grade and were redistilled before use. HPLC grade solvents such as acetonitrile, glacial acetic acid and *o*-phosphoric acid were procured from Merck Ltd., Germany.

Potassium metabisulphite, Ferric chloride and DPPH was purchased from HiMedia Laboratories, India. Folins-ciocalteu reagent was purchased from Merck, India. Disodium

hydrogen-*o*-phosphate and sodium dihydrogen-*o*-phosphate was from Qualigens fine chemicals and Thomas baker Ltd., respectively. Trichloro acetic acid and trolox was procured from Sigma Aldrich, USA. Potassium ferricyanide and sodium bicarbonate was from BDH laboratory chemicals and chemco fine chemicals, respectively.

Various other standards used i.e. gallic acid, caffeic acid, catechin, epicatechin, quercitin and malvidin-3-glucoside were procured from Sigma Aldrich, USA.

2.2. Methodology

2.2.1 Radiation processing of samples

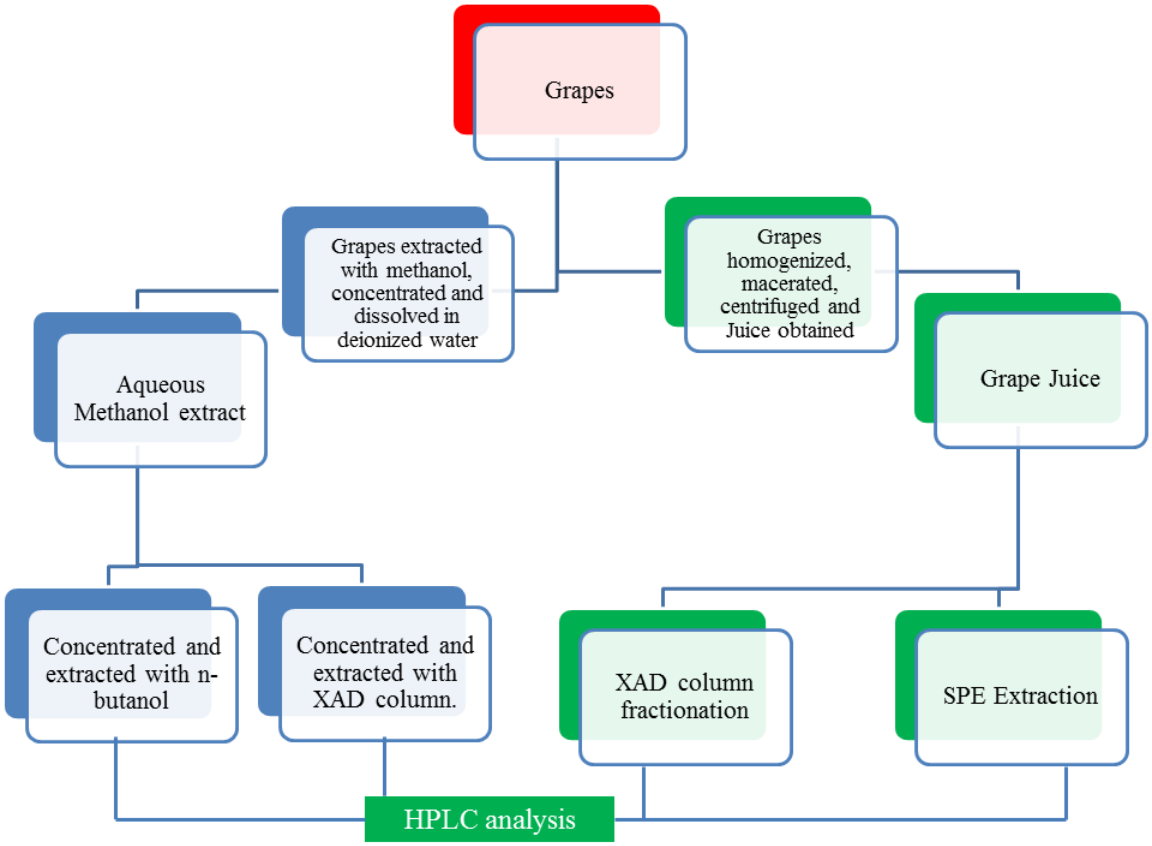
Packaged samples were then subjected to radiation processing (0.5, 1, 1.5 and 2 kGy) in Food Package Irradiator (AEC, Canada) having a source strength of 60 kCi. Radiation was carried out at room temperature (25 ± 2°C). Irradiator was calibrated using Fricke's dosimetry system before radiation processing. Dose uniformity ratio of this irradiator was 1.2. Packaged and irradiated samples were then stored at -30 °C till further analysis or processing.

2.2.2 Isolation and identification of bound aroma precursors

2.2.2.1 Isolation of bound aroma precursors

Optimization of procedures for isolation of bound aroma precursors was carried out using grapes of Sonaka variety. Aroma glycosides were isolated using XAD column, liquid phase extraction using n-butanol and solid phase extraction (SPE) using C18 cartridges. Extraction using XAD column and n-butanol was essentially performed as per procedure described earlier by Arul et al., 2006 (47) while procedure followed for SPE extraction was as per Solis et al., 2007 (68). A detailed diagram depicting different methodologies followed for extraction of bound flavour precursors is demonstrated in Figure. 3.

Figure 3. Schematic of extraction procedures followed for bound aroma precursors.



Different methods followed for extraction of bound flavour precursors is briefly described below:

2.2.2.1.1 Preparation of grape extracts

Prior to extraction of glycosides, using XAD, n-butanol or SPE, grape extracts were prepared using two different approaches.

A) Grapes berries (50 g) were homogenized with 200 mL of methanol using a high speed mixer (Omnimixer, Sorvall, USA) for three min at a speed corresponding to the position of the knob at position five. The resultant slurry was filtered through a Buchner funnel under suction. Residue obtained was then extracted twice more using same solvent. Extracts from all three extractions were pooled and evaporated to dryness under vacuum

(40 mbar, 40 °C) using a rotary evaporator (Buchi, Switzerland). Dried residue was then dissolved in 150 mL of deionized water. This was designated as aqueous methanol extract.

B) In second approach, 150 g of berries were homogenized using a high speed homogenizer (B400, Buchi, Switzerland). Resulting slurry was kept at 4 °C for 2 h for proper maceration and was then centrifuged (5810R, Eppendorf, Germany) at 12000 rpm for 15 min. Clear juice thus obtained was directly used for further extraction of aroma glycosides.

2.2.2.1.2 Extraction of aroma glycosides from grape extracts

Aqueous methanol extract was extracted using n-butanol extraction. In a separate experiment the aqueous methanol extract was also passed through a XAD column to isolate the aroma glycosides. The grape juice was also independently subjected to both XAD column and SPE cartridge extraction. Procedure followed for different extractions is as follows:

A) n-butanol extraction: Aqueous methanol extract (150 mL) was washed thrice with 50 mL of diethyl ether to remove lipophilic substances. Remaining extract was then extracted using n-butanol (3 × 50 mL). n-butanol fractions were pooled and concentrated to dryness using rotary evaporator. Residue obtained was dissolved in 10 mL of deionized water for further analysis.

B) XAD column: Both aqueous methanol and grape juice were subjected to XAD column to isolate aroma glycosides. XAD resin was packed in glass column of 2.5 cm I.D. up to a length of 16 cm using methanol. Column was then washed using 200 mL of methanol followed by 200 mL of diethyl ether. Finally, equilibration was done using 200 mL of

deionized water. 150 mL of the above grape extracts was then separately loaded on XAD column with a flow rate of 1.5 mL min^{-1}. Post loading, column was washed by 200 mL deionized water to remove polar impurities such as proteins and sugars. Subsequently, elution was performed using 200 mL of diethyl ether followed by 200 mL methanol. Ether fraction contained the free aroma volatiles while methanol fraction had bound aroma precursors or aroma glycosides. Methanol fraction was further evaporated to dryness using rotary evaporator and the residue obtained was finally dissolved in 10 mL of deionized water for further analysis.

C) SPE extraction: Grape juice was subjected to extraction on SPE cartridges. SPE cartridge was activated by passing 10 mL methanol at a flow rate of 1 mL min^{-1}. Cartridge was then equilibrated by eluting with 10 mL of deionized water. 10 mL sample was then loaded at flow rate of 1 mL min^{-1} on to the cartridge. Cartridge was subsequently, washed again with 10 mL of deionized water, free volatile forms were eluted with 10 mL of diethyl ether and bound flavour precursors were further eluted with 10 mL of methanol.

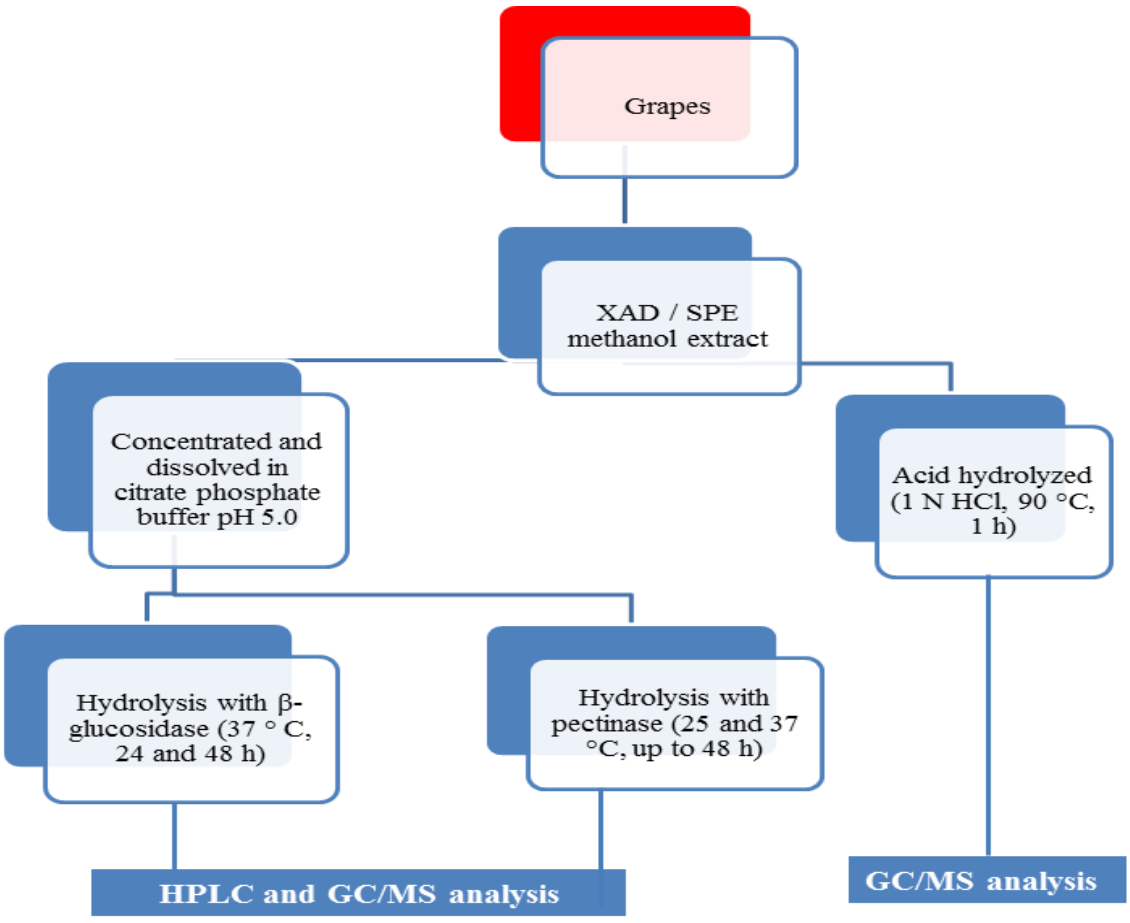
2.2.2.2 Analysis of bound aroma precursors

Various extracts obtained as described in section 2.2.2.1 were analysed using HPLC (Jasco, Japan) which was equipped with a UV-Vis detector, a binary gradient pump, C-18 reverse phase column (5 µm particle size, 4.6 mm I.D. × 250 mm L, Thermo Scientific, USA) with guard column and manual rheodyne injector. Solvent system used was water acetonitrile gradient (water 100% at t = 0 min, 90% at t = 20 min, 65% at t = 50 min, 0% at t = 60 min. Detection wavelength was set at 208 nm. 20 µL of 10 times diluted extracts of bound aroma precursors obtained were injected after filtering through 0.45 µm filter.

2.2.2.3 Identification and quantification of bound aroma precursors

Bound aroma extracts obtained using XAD and SPE extraction of aqueous extract of grapes were hydrolysed using either acid hydrolysis by 1 N HCl or enzymatic hydrolysis using pectinase or β-glucosidase. Conditions for optimum enzymatic hydrolysis were optimized. Figure. 4 shows a schematic diagram for procedures followed for identification and quantification of aroma glycosides. Detailed procedure followed is as follows:

Figure 4 Schematic diagram of procedure followed for identification and quantification of aroma glycosides



A) Acid hydrolysis: 10 mL of final aqueous solution obtained after extraction as described in section 2.2.2.1.2 was added with concentrated HCl to make a 1 N acid solution. This solution was then heated at 90 °C for 1 h in stoppered conical flask. Post hydrolysis solution was extracted thrice with 20 mL of diethyl ether for extraction of released free forms. Organic phase was pooled together and washed with deionized water (20 mL × 3) for removal of acid. This fraction was then dried over anhydrous sodium sulphate and concentrated to less than 1 mL of volume using Kuderna-Danish concentrator (Supelco, USA). Extract was finally concentrated to a volume of 100 µL using a gentle stream of nitrogen and injected (1 µL) in GC/MS for identification of free forms.

B) β-glucosidase hydrolysis: Methanol fraction containing aroma glycosides obtained as described in section 2.2.2.1.2 was concentrated to dryness using rotary evaporator and residue was dissolved in 15 mL of citrate phosphate buffer (0.1 M, pH 5). To this solution was added 100 IU of enzyme and mixture was kept at 37 °C for incubation. Two incubation times (24 and 48 h) were tried for hydrolysis using β-glucosidase. After completion of incubation, hydrolysed samples were analysed using HPLC with similar conditions as described in section 2.1.2.1 for analysing efficacy of hydrolysis procedure. 20 µL of hydrolysate after filtration from 0.45 µM filter was directly injected for HPLC analysis. Post hydrolysis samples were extracted using diethyl ether (3 × 10 mL) and organic phase was concentrated and analysed using GC/MS.

C) Pectinase hydrolysis: Methanol fraction containing aroma glycosides obtained as described in section 2.2.2.1.2 was concentrated to dryness using rotary evaporator and residue was dissolved in 15 mL of citrate phosphate buffer (0.1 M, pH 5). Pectinase

preparation (500 µL) was added into this solution and the samples were then kept for incubation. Two different temperatures of incubation i.e. 25 and 37 °C were chosen for optimization of hydrolysis using pectinase. At 25 °C samples were kept for 24 and 48 h while at 37 °C samples were kept for 3, 5, 24 and 48 h. Post incubation hydrolysed samples were analysed using HPLC with similar conditions as described in section 2.1.2.1 for analysing efficacy of hydrolysis procedure. 20 µL of hydrolysate after filtration from 0.45 µM filter was directly injected for HPLC analysis. Samples hydrolysed using pectinase were further analysed by two different methods for identification of free forms using GC/MS. In first approach, sample post hydrolysis was extracted thrice with 20 mL ether. Ether fractions were pooled together and concentrated to a volume of 100 µL using Kuderna-Danish concentrator followed by gentle stream of nitrogen. Ether concentrate thus obtained was injected in split mode (split ratio 5) in GC/MS. In second approach, sample post hydrolysis was taken into a 40 mL SPME vial and was then added with 4.5 g of NaCl. Sample was equilibrated at 30 °C for 45 min and then extracted with a preconditioned (270 °C, 10 min) SPME fibre (PDMS/DVB/CAR). Extraction was carried out by exposing SPME fibre in sample headspace for 20 min at 30 °C. Post extraction fibre was desorbed in split/splitless port of GC/MS kept at 270 °C and analysis was carried out in splitless mode. Before SPME extraction 10 µL of 10^4 diluted (0.824 µg) of 2-octanol was added as internal standard. Each analysis was carried out in triplicate for quantification.

2.2.2.3.1 GC/MS analysis of free forms released after hydrolysis of aroma glycosides

Samples were analysed on a GC/MS (QP5050A, Shimadzu, Japan) instrument equipped with RTX-5 column (5% diphenyl-dimethyl-polysiloxane, 0.25 µm I.D., 30 m length,

Restek corporation, USA). GC column temperature program was set as follows: Initial 60 °C for five min, which was then increased to 200 °C at rate of 4 °C min^{-1}. At 200 °C temperature was held for 5 min and then further increased to 280 °C at 10 °C min^{-1}. Column was held at final temperature for 10 min. Helium was used as a carrier gas. MS parameters were: ionization voltage 70 eV and electron multiplier voltage of 1 kV. Data was acquired in scan mode from m/z 40 to 350. Peaks were identified by comparing their mass fragmentation pattern and Kovat's indices with that of standard compounds as well as from the data available in the spectral (Wiley/NIST) libraries of the instrument. Quantification was performed by comparing peak areas of compounds with that of internal standard and results were obtained as µg kg^{-1}.

2.2.3 Isolation and identification of free volatile forms

2.2.3.1 Isolation of free volatiles

Free volatile aroma compounds were isolated from grapes using three different methods briefly described below:

A) XAD ether extract: 150 mL of grape juice was subjected to XAD column as described in section 2.2.2.1.2. Ether fraction obtained from XAD column contained free volatiles. 200 mL of ether fraction obtained was concentrated using Kuderna-Danish concentrator to volume of less than 1 mL which was further concentrated to 100 µL using gentle stream of nitrogen. This concentrate was injected (1 µL) in GC/MS (Section 2.2.2.3.1) in split mode (split ratio 5) for identification of free volatile compounds.

B) Steam distillation extraction: 150 g of grape berries were crushed and extracted by steam distillation extraction for 2 h using a Nickerson & Lickens apparatus. 100 mL

diethyl ether was used as an extracting solvent. Ether extract obtained after extraction was concentrated to a volume of 1 mL using Kuderna-Danish equipment and then to 100 µL using a gentle stream of nitrogen. This concentrate was injected (1 µL) in GC/MS (Section 2.2.2.3.1) in split mode (split ratio 5) for identification of free volatile compounds.

C) Solid phase micro-extraction (SPME): 15 mL of grape juice was taken in 40 mL SPME vial (Supelco, USA) and added with 4.5 g of NaCl. Sample was further added with 10 µL of 10^4 diluted 2-octanol (0.824 µg) as internal standard. Samples were equilibrated for 30 min at 30 °C with continuous shaking and headspace was subsequently extracted by preconditioned PDMS/DVB/CAR fibres (Supleco, USA) for 20 min at 30 °C. After extraction fibres were desorbed (270 °C) in injection port of GC/MS. Quantification was performed by comparing peak areas of compounds with that of internal standard and results were obtained as µg kg^{-1}.

2.2.4 Chemometric studies for rapid identification of radiation treatment

Various extracts obtained as described above in section 2.2.3 containing free volatile compounds were also analyzed using GC/MS to obtain total mass spectrum of entire sample. Data of total mass spectrum obtained was analyzed using chemometric techniques for rapid identification of radiation treatment. Methodology followed for obtaining total mass spectrum and subsequent chemometric analysis is briefly described below:

A) XAD ether extract as obtained, as described above (2.2.3.1) was subjected to GC/MS analysis and conditions were similar to as already described in section (2.2.2.3.1). Total mass chromatogram for every sample was obtained by integrating TIC spectrum in range

of 7 min to 55 min using software GCMS solution (Shimadzu Corporation, Kyoto, Japan). After integration mass table having data of various m/z fractions with their corresponding relative intensities was obtained for each sample. This data was then transferred to spreadsheet software (Excel 2010, Microsoft Corporation) and a matrix having all m/z with their corresponding relative intensities for all samples was obtained. m/z fractions having relative intensity of less than 0.5 % were ignored for analysis. Data thus obtained was subjected to principal component analysis (PCA) and linear discriminant analysis (LDA).

B) Steam distillation extract obtained as described above in section (2.2.3.1), was injected directly (0.1 µL) in mass spectrometer (QP5050A, Shimadzu, Japan) using a direct probe sampler. Direct probe after insertion into mass spectrometer was heated to 280 °C at rate of 40 °C min^{-1}. Hold time at final temperature was 5 min. Data was scanned in m/z range of 75 to 350. Lower m/z range was kept to 75 to eliminate all the ions originating from solvent i.e. diethyl ether. Single peak obtained in total ion current (TIC) chromatogram was integrated using GC/MS solution software (Shimadzu, Japan) to obtain total mass spectra of sample. Mass table having data of various m/z fractions with their corresponding relative intensity was obtained for each sample. This data was then transferred to spreadsheet software (Excel 2010, Microsoft Corporation) and a matrix having all m/z with their corresponding relative intensity for all samples was obtained. m/z fractions having relative intensity of less than 0.5 % were ignored for analysis. Data thus obtained was subjected to principal component analysis (PCA) and linear discriminant analysis (LDA).

C) SPME analysis: 15 mL of grape juice was taken in 40 mL SPME vial to which 4.5 g NaCl was added. After equilibration of 45 min at 30 °C with continuous stirring, sample headspace was extracted by exposing a preconditioned (270 °C, 10 min) PDMS/DVB/CAR fibre in vial headspace for 20 min. Post extraction SPME fibre was desorbed in GC/MS injection port at 270 °C for five min for analysis. GC/MS equipment was suitably modified to rapidly obtain total mass spectrum of sample. Instead of column, a transfer line without stationary phase (Gerstel GmbH & Co., Germany) was installed in GC. Specifications of transfer line used were (Length 85 cm and I.D. 0.15 µm). The operating conditions were: Column, injector and interface temperatures were kept at 280, 210 and 280°C, respectively. Helium (flow rate: 1.0 mL/min) was used as carrier gas. MS parameters were 70 eV ionization voltage and electron multiplier voltage of 1 kV. Mass scan range was from 35 to 300 m/z. Using this type of analysis a single peak of entire sample head space was obtained in TIC chromatogram. This peak was integrated to obtain total mass spectrum of sample. After integration mass table having data of various m/z fractions with their corresponding relative intensities was obtained for each sample. This data was then transferred to spreadsheet software (Excel 2010, Microsoft Corporation) and a matrix having all m/z with their corresponding relative intensity for all samples was obtained. m/z fractions having relative intensity of less than 0.5 % were ignored for analysis. Data thus obtained was subjected to principal component analysis (PCA) and linear discriminant analysis (LDA).

2.2.4.1 Chemometric analysis of data

Data obtained in terms of m/z fractions with their corresponding relative intensities was subjected to principal component analysis (PCA). PCA was carried out on relative

intensities of various m/z. Score plots were drawn and results were visually analyzed. Data obtained for principal components was further analyzed using ANOVA and means comparison was performed using Duncan's multiple range test. Total mass data was also analyzed using a supervised technique of linear discriminant analysis (LDA) with full cross validation of models. Statistical analysis was carried out using XLSTAT 2012 software (Addinsoft Inc. U.S.A.).

2.2.5 Effect of radiation processing of grapes on wine quality

Wines were prepared with control and irradiated (0.5, 1.0, 1.5 and 2.0 kGy) grapes from all three varieties and were analyzed for colour, total antioxidants, total phenolics, aroma quality, phenolic constituents using HPLC and sensory quality. Procedure followed for wine preparation and subsequent analysis is mentioned below:

2.2.5.1 Preparation of wine

Wine preparation was carried out essentially as per procedure detailed earlier (69). In brief, for red varieties (Cabernet Sauvignon and Shiraz) berries (2 kg) were crushed and resultant musts were adjusted to 24° Brix by adding required amount of glucose. pH of musts was also adjusted to 3.5 using tartaric acid. Musts were then added with 50 ppm potassium metabisulfite. After 2 h musts were inoculated with 1% yeast (*Saccharomyces cerevisiae*) (strain SC-101, CFTRI, India) inoculum and fermentation was carried out at 25 ± 1 °C. Yeast inoculum was prepared by inoculating yeasts cells in autoclaved potato dextrose broth (PDA). 1 mL of this overnight grown culture was then added to autoclaved grape juice. Inoculated grape juice was then kept at orbital shaker (150 rpm, 25 °C) for 24 h. This culture was then used as final inoculum for wine preparation.

During fermentation flasks were shaken twice a day and cap was punched. After completion of fermentation (200 h) seeds and skins were separated from wine by straining through muslin cloth and wines were raked twice. Wines were then added with another 25 ppm potassium metabisulfite and stored in amber coloured bottles at 15 °C for a period of 4 months for maturation.

For white variety (Chenin Blanc) berries (2 kGy g) were crushed along with 50 ppm potassium metabisulfite to inhibit activity of poly phenol oxidase and prevent browning of juice. Resulting musts was then pressed to separate skin and seeds from juice. Total soluble solids (TSS) of extracted juice was adjusted to 24 °Brix using glucose and pH was adjusted to 3.5 using tartaric acid. Juice was then added with 1 percent yeast inoculum which was prepared as described above and fermentation was carried out at 15 ± 1 °C without shaking to prevent oxidation of phenolics which could result in browning. Fermentation was carried out for 15 days and after that wines were raked twice and added with 25 ppm of potassium metabisulfite. Wines were then kept for maturation at 15 °C for four months.

2.2.5.2 Chemical analysis of wine

2.2.5.2.1 Reducing sugars, ethanol content and pH analysis of wine

Reducing sugar estimation was carried out by the Dinitrosalicylic acid (DNS) method described earlier (70). Glucose was used as standard for reducing sugars. DNS reagent was prepared by dissolving 1 g DNS, 200 mg crystalline phenol and 50 mg sodium sulphite to 100 mL of 1 percent NaOH. Rochelle salt solution was prepared by dissolving 10 g potassium sodium tartarate ($NaKC_4H_4O_6$) in 25 mL distilled water. Wines were decolourized by incubating with activated charcoal for 2 h at 40 °C. Samples were then

centrifuged (14500 rpm, 15 min) and clear supernatant obtained was used for further analysis. 250 µL of 100 times diluted decolourized wine was taken in a test tube and 750 µL of distilled water was added. In this mixture 1 mL of DNS reagent was added and mixture obtained was incubated for 15 min in boiling water bath. After incubation, 300 µL of Rochelle salt solution was added and O.D. was measured at 510 nm. Glucose standard curve was prepared in range of 200 to 1000 µg mL^{-1}. Amount of reducing sugar present in wine was calculated using a linear regression equation obtained from glucose standard curve.

Ethanol estimation in wine was carried out by potassium dichromate spectrophotometric assay with suitable modifications (71). Dried alcohol was prepared by distilling over calcium oxide. 10 g of potassium dichromate was dissolved in 100 mL of distilled water and to this mixture 24 mL of concetrated sulfuric acid was added to prepare potassium dichromic acid. For assay, 50 µL of decolourized wine was taken in a test tube and volume was made to 1 mL using distilled water. To this solution 5 mL of potassium dichromic acid was added and resulting mixture was incubated for 15 min in dark. After incubation absorbance was taken at 620 nm. Ethanol standard curve was prepared in range of 5 to 50 µL. Amount of ethanol present in wine was calculated using a linear regression equation obtained from standard curve.

pH was calculated using a hand held electronic pH meter (Eutech instruments, USA) which was calibrated using standard buffer solutions.

2.2.5.2.2 Colour analysis of wine

Chromatic characteristics (Tint and colour intensity) of the wines were determined as per procedure detailed earlier (72, 73) by a direct measurement of the absorbance of the

wines at 420, 520 and 620 nm using a UV-visible spectrophotometer (Helios-α, Thermo Scientific, USA) with a 10 mm path-length quartz cuvette. The CIELAB parameters (a, b, L) were calculated from wine absorbencies obtained at 450, 520, 570 and 630 nm, according to the methodology reported earlier (74). Tint was determined as the proportion of the absorbance measured at 420 nm and 520 nm. Colour intensity (CI) was measured using following equation:

$$CI = A_{420} + A_{520} + A_{630} \quad (1)$$

For calculation of CIELAB parameters absorbance at 450, 520, 570 and 630 nm were converted to their respective transmittance values by following equation:

$$\tau = 10^{(-A)} \quad (2)$$

From transmittance values tristimulus values were calculated using following equations:

$$X = 19.717\tau_{450} + 1.884\tau_{520} + 42.539\tau_{570} + 32.474\tau_{630} - 1.841$$

$$Y = 7.950\tau_{450} + 34.764\tau_{520} + 42.736\tau_{570} + 15.759\tau_{630} - 1.180 \quad (3)$$

$$Z = 103.518\tau_{450} + 4.190\tau_{520} + 0.251\tau_{570} - 1.831\tau_{630} + 0.818$$

Tristimulus values for the blank, with D65 illuminant and CIE 1964 standard observer are $X_{10} = 94.825$; $Y_{10} = 100$ and $Z_{10} = 107.381$

From these values colour coordinates are calculated using following expressions:

$$L^* = 116\ ((Y/Y_{10})^{1/3} - 0.1379)$$

$$a^* = 500\ ((X/X_{10})^{1/3} - (Y/Y_{10})^{1/3}) \quad (4)$$

$$b^* = 200\ (((Y/Y_{10})^{1/3} - (Z/Z_{10})^{1/3})$$

2.2.5.2.3 Wine analysis for total anthocyanin, phenolic and antioxidant content

Total anthocyanin content (TAC) was calculated using the absorbance at 520 nm of diluted wine 1/100 (v/v) with 1% (v/v) of HCl (30). TAC was calculated as mg malvidin-

3-glucoside L^{-1} equivalents. Standard curve of malvidin-3-glucoside was prepared in concentration range of 0.0375 to 5 µg mL^{-1}. TAC in wines was calculated using linear regression equation obtained from standard curve.

Wines were analyzed for total phenolic content by Folins-ciocalteu method and antioxidant capacity by DPPH and FRAP assay as per method described previously (75). Briefly, 40 times diluted wine samples were used for Folin's, DPPH and FRAP assay. 100 µL of diluted sample was mixed with 250 µL of Folins-ciocalteu reagent and 6% sodium carbonate solution each. After 30 min of incubation O.D. was taken at 725 nm. Gallic acid (GA) standard curve was obtained in concentration range of 5-20 µg mL^{-1} using same procedure as above and total phenolics were represented as µg GA equivalents mL^{-1} of wine. For DPPH assay 100 µL of diluted wine was mixed with 1 mL of 105 µM solution of DPPH. Mixtures were then incubated for 20 min in dark and O.D. was taken at 520 nm. Trolox standard curve was obtained in concentration range of 1-10 µg mL^{-1} and total antioxidant capacity was expressed as µg Trolox equivalents mL^{-1} of wine. For FRAP assay in 200 µL diluted sample, 800 µL of phosphate buffer (0.2 M, 7.2 pH) along with 500 µL of 1% potassium ferricyanide was added. Resulting mixture was incubated in dark for 20 min at 50 °C. After incubation a 500 µL of 10 % solution of Trichloro acetic acid (TCA) was added. In 500 µL of this mixture were added 500 µL of distilled water and 100 µL of 0.1% ferric chloride. O.D. was taken at 700 nm after incubation of 10 min in dark. A trolox standard curve was obtained in range of 15-70 µg per reaction and results are expressed as µg Trolox equivalents mL^{-1} of wine.

2.2.5.2.4 HPLC analysis of wine samples

Analysis of wine samples were carried out on a high performance liquid chromatograph (Jasco Corp, Japan) equipped with a quaternary pump, diode array detector and manual rheodyne injector. C-18 reverse phase column (5μM particle size, 4.6 mm I.D. × 250 mm L, Thermo Scientific, USA) having guard column was used for analysis. Wine samples were filtered through 0.22 μm membrane filters (Millipore) prior to direct injection (20 μL) into chromatograph. The mobile phase consisted of 1.5% o-phosphoric acid in deionized water as solvent A and mixture of glacial acetic acid, acetonitrile, o-phosphoric acid and deionized water in proportion (24:20:1.5:54.5) as solvent B. Flow rate of solvent was kept at 1 mL min^{-1} and following elution gradient was applied: A = 80%, T = 0 min; A = 33%, T = 30 min; A = 10%, T = 33 min; A = 0, T = 40 min. Column was conditioned with acetonitrile for 10 min after every run and re-equilibrated with zero time solvent in 15 min. Spectra were recorded between 250 to 650 nm on diode array detector. Identification of the phenolic compounds present in wine samples was done by comparison of the retention times (Rt) and UV-spectrum with standard compounds when possible (Gallic acid, Caffeic acid, Catechin, Epicatechin, Quercitin and Malvidin-3-glucoside procured from Sigma Aldrich, USA) or by their order of elution and matching of UV-visible spectra with already published literature (76). The quantification was done with the help of standard curve obtained in concentration range of 10 to 100 μg mL^{-1} for all compounds. All anthocyanins identified were quantified as malvidin-3-glucoside equivalents.

2.2.5.2.5 Aroma analysis of wine by GC/MS

Wine head space aroma analysis was performed by SPME extraction and subsequent analysis using GC/MS (QP5050A, Shimadzu, Japan) equipped with special SPME glass

liner (Supelco, USA) for injector and RTX-5 column (5% diphenyl-dimethyl-polysiloxane, 0.25 µm I.D., 30 M length, Restek corporation, USA). 15 mL of wine was taken in 40 mL SPME vial (Supelco, USA) and added with 4.5 g of NaCl. Sample was further added with 10 µL of 10^4 diluted 2-octanol (0.824 µg) as internal standard. Samples were equilibrated for 45 min at 30°C with continuous shaking and headspace was subsequently extracted by preconditioned PDMS/DVB/CAR fibres (Supleco, USA) for 20 min at 30°C. After extraction fibres were desorbed (270°C) in injection port of GC/MS. Helium was used as a carrier gas. GC column temperature program was initial: 60 °C for five min, increased to 200 °C at rate of 4 °C min^{-1} and hold for 5 min and then increased to 280 °C at 10 °C min^{-1} and final hold of 10 min. MS parameters were ionization voltage 70 eV, electron multiplier voltage, 1 kV and scan mode from m/z 40 to 350. Peaks were identified by comparing their mass fragmentation pattern and Kovat's indices with that of standard compounds as well as from the data available in the spectral (Wiley/NIST) libraries of the instrument. Quantification was performed by comparing peak areas of compounds with that of internal standard and results obtained as µg L^{-1} of wine.

2.2.5.3 Sensory analysis of wine

Sensory analysis of wine was carried out using quantitative descriptive analysis as per procedure detailed earlier with slight modifications (77). Twelve panellists aged 25–45 years old with previous experience in descriptive sensory analysis, regularly drinking red wine (drinking at least one glass of red wine per week) and being available at the necessary times were chosen for sensory analysis. Five 120 min training sessions were carried out to practice proper techniques for assessing wine and generate attributes for

descriptive analysis. Standards to be used for colour, aroma, taste and mouthfeel were finalized. The panellists rated 2 appearance attributes, 8 aroma attributes and 6 taste and mouth feel attributes (Table 3). The intensity of each attribute was rated using an unstructured 15 cm line scale with extreme left as lowest value and extreme right representing highest value for attribute assessed. The samples (30 mL) labelled with random three digit code were presented in clear covered tasting glasses in isolated, ventilated tasting booths. All wines were assessed in triplicate by each group. Panellists were provided all aroma reference standards prior to each session with water and unsalted crackers to cleanse their palate.

Table 3. Sensory attributes with their description and corresponding reference standards used for descriptive sensory analysis of wine.

S. No.	Sensory Attributes	Description	Reference standards
	Appearance		
1	Red	Assessed by tilting glass at 45°. Low anchor (Light) High anchor (Dark)	
2	Clarity	Transparent or cloudy. Assessed by tilting glass at 45°. Low anchor (cloudy) High anchor (clear)	
	Aroma		
3	Fruity	Grape like	Grape Juice
4	Berry	Cranberry, blackberry	Cranberry Juice
5	Spice	Clove like	Cloves
6	Floral	Flowery, rose like	Rose petals
7	Honey	Honey, caramel	Honey
8	Woody	Pencil shavings	Pencil shavings
9	Smoky	Cigarette smoke like	Burnt cigarette
10	Vinegar	Acetic, sour	10 times diluted vinegar
	Taste		
11	Astringency	Dry, puckering	800 mg/L aqueous alum solution
12	Bitter		800 mg/L anhydrous caffeine dissolved in water
13	Sourness	Sour, acidic	2 g/L tartaric acid dissolved in water

14	Sweetness		15 g/L (D)-fructose dissolved in water
15	Warmth	Warm to hot	150 mL/L vodka in water
		Viscosity, mouthfeel.	
16	Body	Low anchor (Thin)	7 g/L pectin dissolved in water
		High anchor (Thick)	

2.2.6 Radiation processing for inactivation of wine spoilage organism

Radiation processing of wine for inactivation of microorganisms associated with wine spoilage was attempted.

2.2.6.1 Microorganisms chosen and growth conditions

Three microorganisms were chosen for present study which included two strains of bacteria and one yeast. Bacteria chosen for present study were *Pediococcus acidilactici* (NCIM No. 2292) and *Acetobacter aceti* (NCIM No. 2116) while yeast used was *Dekkera bruxellensis* (NCIM No. 3534). All microorganisms were obtained from National collection of industrial microorganism, National chemical laboratory, Pune. All three microorganisms chosen for present study are commonly associated with wine spoilage (41). Microbial cultures were obtained as agar slants from NCL, Pune and were subcultured on agar slants every month to maintain working culture. Media used for yeast was YPD (10 g/L yeast extract, 20 g/L bacteriological peptone and 20 g/L dextrose) while Lactobacilli MRS medium was used lactobacilli strain used. Mannitol medium containing (25 g/L mannitol, 5 g/L yeast extract and 3 g/L peptone) was used for acetic acid bacteria.

The initial culture of each microorganism was propagated in three steps for evaluation of impact of radiation processing. The stock culture of each species was inoculated in 50 mL of broth. Yeasts cultures were incubated at 25 °C and bacterial cultures were

incubated at 37 °C. When the microbial population reached the stationary phase, 1 mL of this culture is added to 50 mL of fresh broth. After again reaching the stationary phase 50 mL of cultures was taken in sterile centrifuged tubes (100 mL) and centrifugation (12000 rpm, 15 min, 10 °C) was done to pellet out cells. Media was discarded and 50 mL of 0.9 percent saline was added. After vortexing to completely disperse cell pellet, centrifugation was again performed to obtain pellet and saline was discarded. Fresh 50 mL of saline was again added and cells were completely dispersed by vortexing. This cell dispersion was used for evaluation of radiation inactivation of microorganism in saline.

For evaluation of microbial inactivation in wine, microbial cultures grown in broth from stock cultures as described above were added 1 mL in 50 mL of fresh broth containing 6 percent alcohol. Cultures were grown in media containing alcohol to prevent them from alcohol shock after addition in wine. Cultures thus obtained were washed from saline to remove traces of media as described above and final pellet obtained was dispersed in 50 mL of wine. All microbial culture handling was performed under aseptic conditions using a laminar flow.

2.2.6.2 Radiation processing of microbial cultures and inactivation kinetics

Radiation inactivation of microorganisms was evaluated both in saline as well as wine. 10 mL of cell dispersions as obtained above were dispersed in sterile vials and subjected to radiation processing using a cobalt-60 irradiator (GC-5000, BRIT, Vashi, India) having a dose rate of 3.14 kGy/h and dose uniformity ratio of 1.1. Doses given to each microorganism were different and were decided based on preliminary experiments and literature data (78, 79). Yeasts cultures were subjected to various doses (0.5, 1, 1.5 and 2 kGy) up to 2 kGy while cultures of *P. acidilactici* and *A. aceti* were subjected to doses of

250, 500, 750 and 1000 Gy. Post irradiation microbial cultures were serially diluted in saline (0.9 percent) and appropriate dilutions were spread plated (100 µL) on respective media. Log CFU mL^{-1} was plotted against radiation dose and D_{10} values were then calculated from linear regression equation obtained.

2.2.6.3 Effect of radiation processing on wine quality

Effect of radiation processing on wine was also evaluated. Post irradiation wine was evaluated for colour characteristics (as described in section 2.2.5.2.2), total anthocyanin, phenolic and antioxidant content (as described in section 2.2.5.2.3), aroma analysis by GC/MS (as described in section 2.2.5.2.5) and sensory analysis (2.2.5.3)

Chapter 3: *Results and Discussions*

3.1 Effect of radiation processing on free and bound aroma precursors of grapes

Consumer acceptability of food is governed by the nature of flavoring principles that contribute to aroma and taste. Volatile constituents, mainly of terpenoid origin, contribute to the aroma, while nonvolatile compounds account for taste. In addition to these, there exists a class of glycosidically bound tasteless nonvolatile terpene derivatives that are shown to contribute finer notes to food. This class of compounds exists mainly as *O*-β-D-glucoside and *O*-diglycoside derivatives of volatile compounds. Aglycone moiety is often dominated by monoterpenes, benzene derivatives and C-13 norisoprenoids (1). Although, odorless, they are able to release free aroma compounds by enzymatic or chemical hydrolysis during processing and storage (3). In past few years analysis of flavor precursors in fruits, vegetables and spices have received increased interest and attention. These precursors in many cases have been shown to be more abundant than free form to the extent of 70-90%. In view of the importance of aroma to the final product quality and consumer acceptance, characterization of free forms liberated from aroma precursors assumes importance. However, despite their important role, very few studies exist on the stability of these compounds during postharvest processing.

Recently, there are reports demonstrating hydrolysis of aroma glycosides and corresponding increase in free aroma volatiles due to radiation processing. Radiation induced hydrolysis of aroma glycosides was successfully demonstrated previously in products such as saffron, nutmeg, fenugreek, and papaya (45, 47-49). However, no reports exist on the effect of radiation processing on content of aroma glycosides in grapes. Possible hydrolysis of aroma glycosides due to radiation processing and subsequent enhancement in free aroma could be very useful for improving wine quality.

3.1.1 Optimization of extraction and analysis procedures for aroma glycosides and free volatiles

3.1.1.1 Optimization of extraction procedures for aroma glycosides

Optimization of protocols for extraction and analysis of aroma glycosides were carried out using Sonaka variety. This variety was chosen for optimization because of its easy availability in local markets throughout the season. A basic scheme followed for the extraction of aroma glycosides is shown in Figure 4 (Materials and methods). A detail of the extraction scheme is also described in materials and methods section 2.2.2.

3.1.1.1.1 HPLC analysis of the aroma glycoside fraction obtained using various extraction techniques

Aroma glycosides were extracted from the grapes using different methodologies. Both aqueous methanol extract as well as grape juice was used for extraction of aroma glycosides as described in section 2.2.2.1. HPLC chromatograms obtained for various extracts are shown in Figure. 5. A distinct HPLC profile was observed for juice directly extracted either with SPE or XAD column. Further, the chromatographic profile obtained for both these methods were similar. In case of n-butanol fraction, very few compounds were detected in HPLC chromatogram. No separation could be observed in the glycosidic fraction isolated from the aqueous methanol extract using XAD column with only a single peak detected at Rt of 29 min. The observed single peak might be due to enhanced extraction of phenolic compounds which may have resulted in interference with HPLC separation. To further confirm interference due to phenolic compounds this glycosidic

fraction was treated with polyvinylpolypyrrolidone (PVPP) and again analyzed using HPLC. The results obtained are shown in Figure. 6.

Figure 5. HPLC chromatograms obtained for different extraction procedures

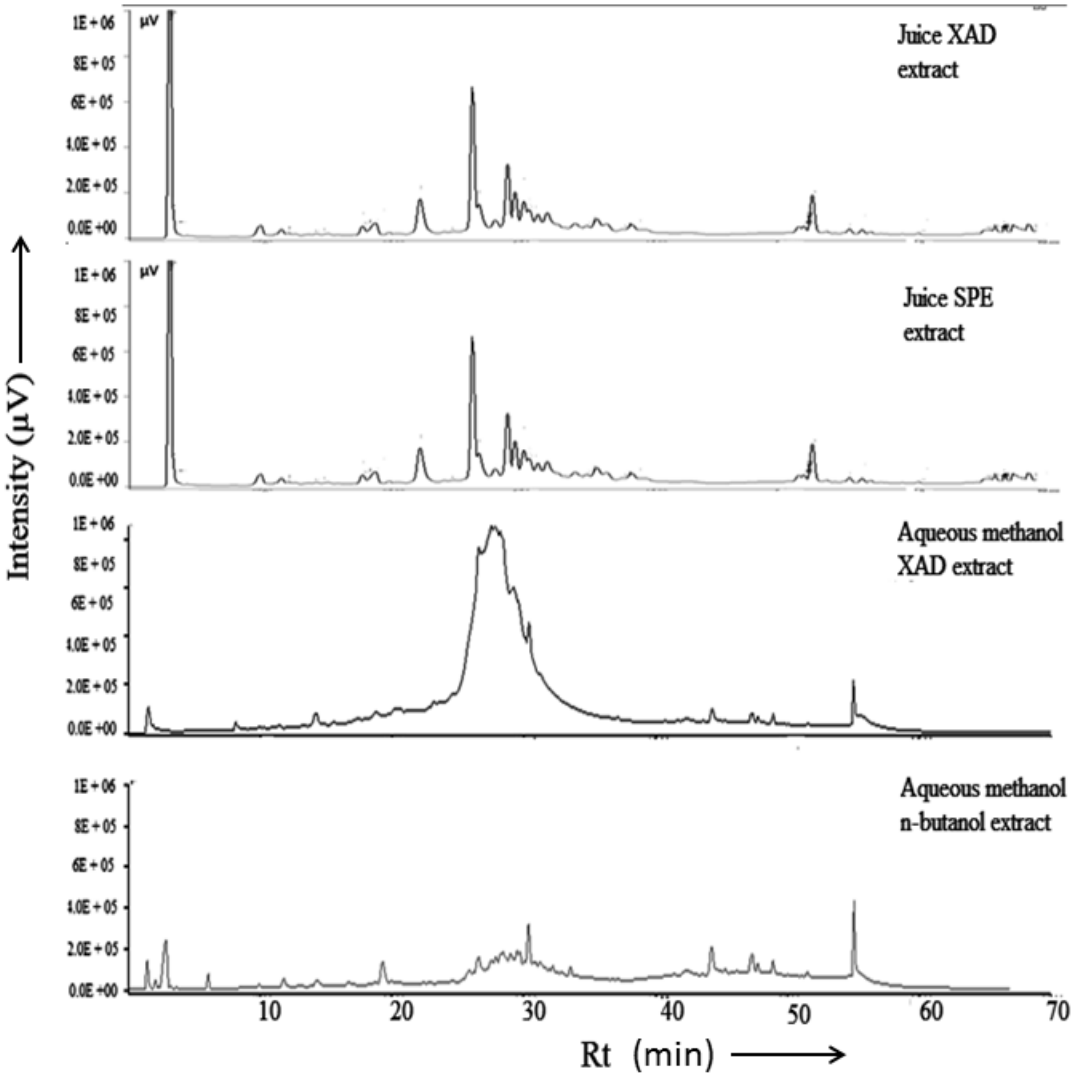
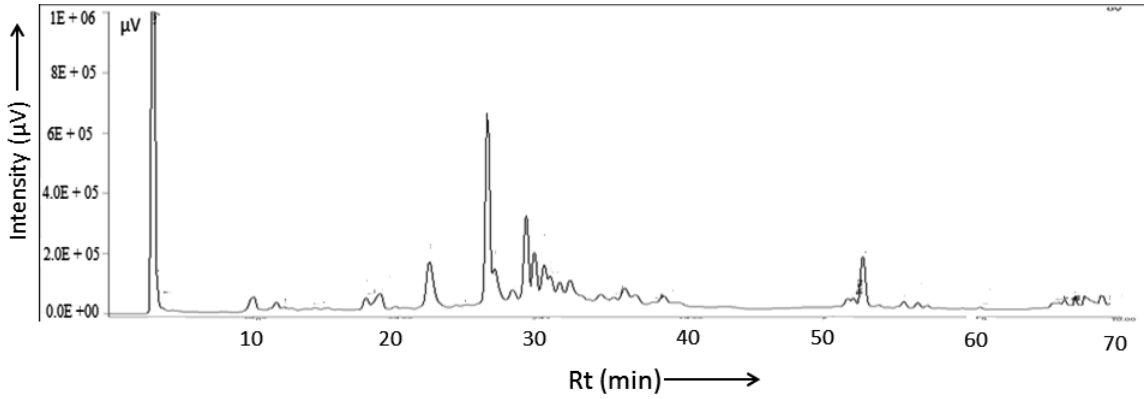


Figure 6. HPLC chromatogram of XAD purified aqueous methanol extract treated with PVPP.



Interestingly, after treatment with the PVPP, the profile became clearer and the chromatogram was similar to that obtained from juice using XAD or SPE extraction. These results confirm that interference in the aqueous methanol extract was due to extraction of large amounts of phenolics components.

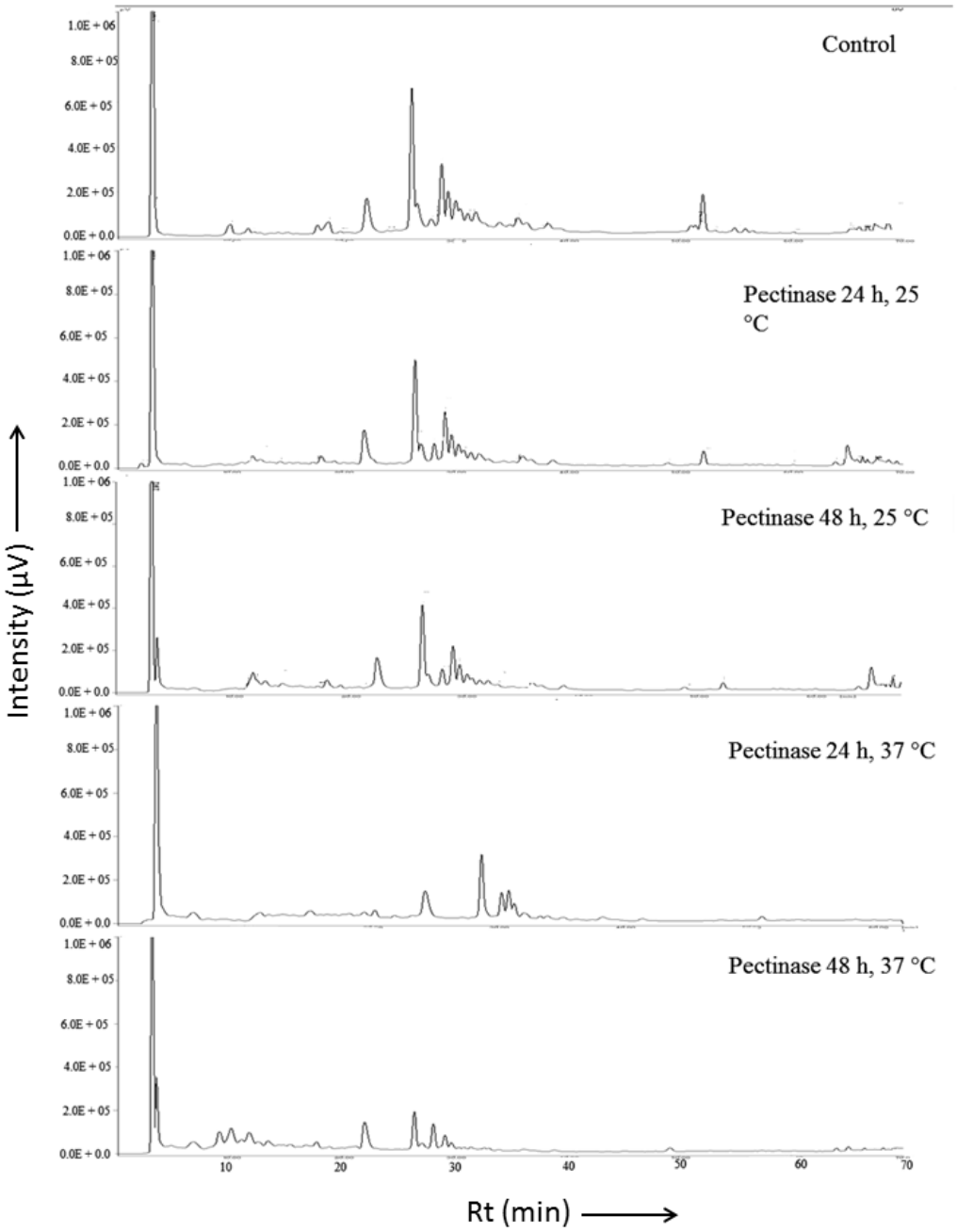
Thus, the above results suggest that best separation of aroma glycosides could be achieved by direct extraction of grape juice using either a XAD column or SPE cartridges. Use of both XAD and SPE cartridges for extraction of aroma glycosides is widely reported in literature (80). Significantly, lesser number of peaks was observed in the n-butanol fractionation indicating poor extraction efficiency of this method for glycosides extraction. It was reported earlier on work on nutmeg that n-butanol fractionation has similar efficiency as that of XAD-16 for extracting aroma glycosides (47). However, in present work significant less number of peaks was observed for n-butanol extract as compared to XAD extract. This might be due to the different composition of glycosidic precursors in fruits as compared to spices such as nutmeg.

With regard to aqueous methanol extract, although, the profile obtained was similar to that of XAD or SPE fractions from juice, it contained high concentrations of phenolics compounds. Further, direct extraction of juice offered a comparatively simpler technique for obtaining a glycosidic fraction as compared to that from aqueous methanol extract. Among the XAD and SPE methods, SPE extraction is a relatively simple technique as compared to the latter since, ready-made pre pack SPE cartridges are available and lesser amount of solvents are required for SPE extraction. Thus, due to these reasons for the identification of glycosides present in grapes and for elucidating the impact of radiation processing, direct extraction of juice using SPE was carried out.

3.1.1.1.2 Optimization of hydrolysis procedures for aroma glycosides

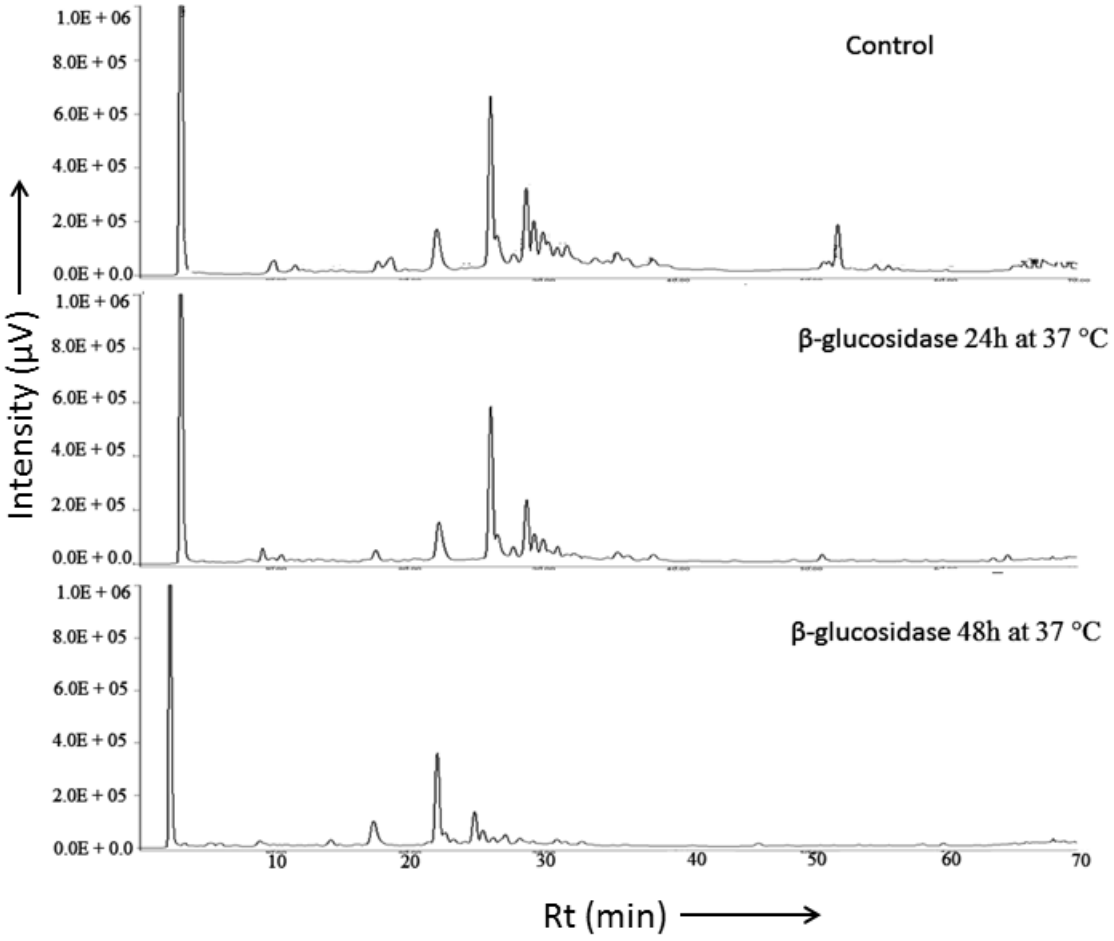
As described in materials and methods section (2.2.2.3), extracted glycosides were subjected to either acidic or enzymatic hydrolysis. In case of enzymatic hydrolysis both β-glucosidase and pectinase were used. In literature, an optimum temperature for β-glucosidase activity was shown to be 37 °C (47). However, for pectinase, the optimum working temperature for hydrolysis of aroma glycosides was has been reported to be between 25 to 50 °C (81). Higher temperatures of beyond 40 °C cause rapid denaturation of pectinase enzymes, therefore, in the present study two temperatures, i.e. 25 and 37 °C were chosen for optimization of method for hydrolysis of aroma glycosides. HPLC chromatograms obtained after hydrolysis of aroma glycosides are shown in Figure. 7 and 8. Enzyme treatment under all conditions resulted in a decrease in the peaks for glycosides. It can be clearly observed from these chromatograms that pectinase treatment at 25 °C even up to 48 h did not result in complete hydrolysis of aroma glycosides.

Figure 7. HPLC chromatograms of aroma glycosides hydrolyzed using pectinase



However, for samples kept at 37 °C a complete hydrolysis of aroma glycosides was observed after 48 h of incubation. For samples treated with β-glycosidase complete hydrolysis was not observed even after 48 h of incubation.

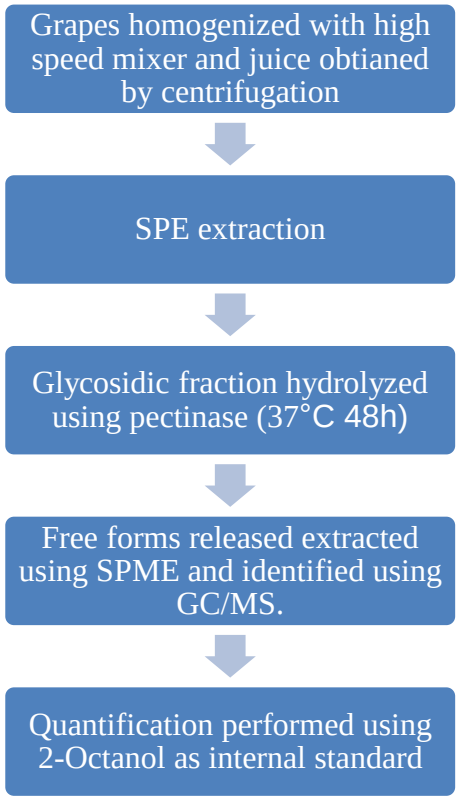
Figure 8. HPLC chromatograms of aroma glycosides hydrolyzed using β-glucosidase



These results suggest that for complete hydrolysis of aroma glycosides incubation with pectinase at 37 °C for 48 h was most suitable. Further, pectinase enzyme preparation, apart from β-glucosidase activity also has α-arabinofuranosidase, α-rhamnosidase and β-apiosidase activity, while, β-glycosidase has only β-glucosidase activity (3). Due to

mixed enzyme activities, use of pectinase enzyme resulted in complete hydrolysis of glycosides. To further confirm the efficacy of different enzyme used free aglycones released by pectinase, β-glycosidase and acid hydrolysis were analyzed using GC/MS. Maximum number of compounds were detected in samples hydrolyzed with pectinase enzymes and least number of compounds was detected in acid hydrolyzed samples. Acid hydrolysis is known to cause significant transformation in released aglycones (47). Thus, the suitability of pectinase hydrolysis for identification of free volatiles released after hydrolysis of bound precursors was further confirmed. The final protocol followed for extraction, identification and quantification of aroma glycosides is shown in Figure. 9.

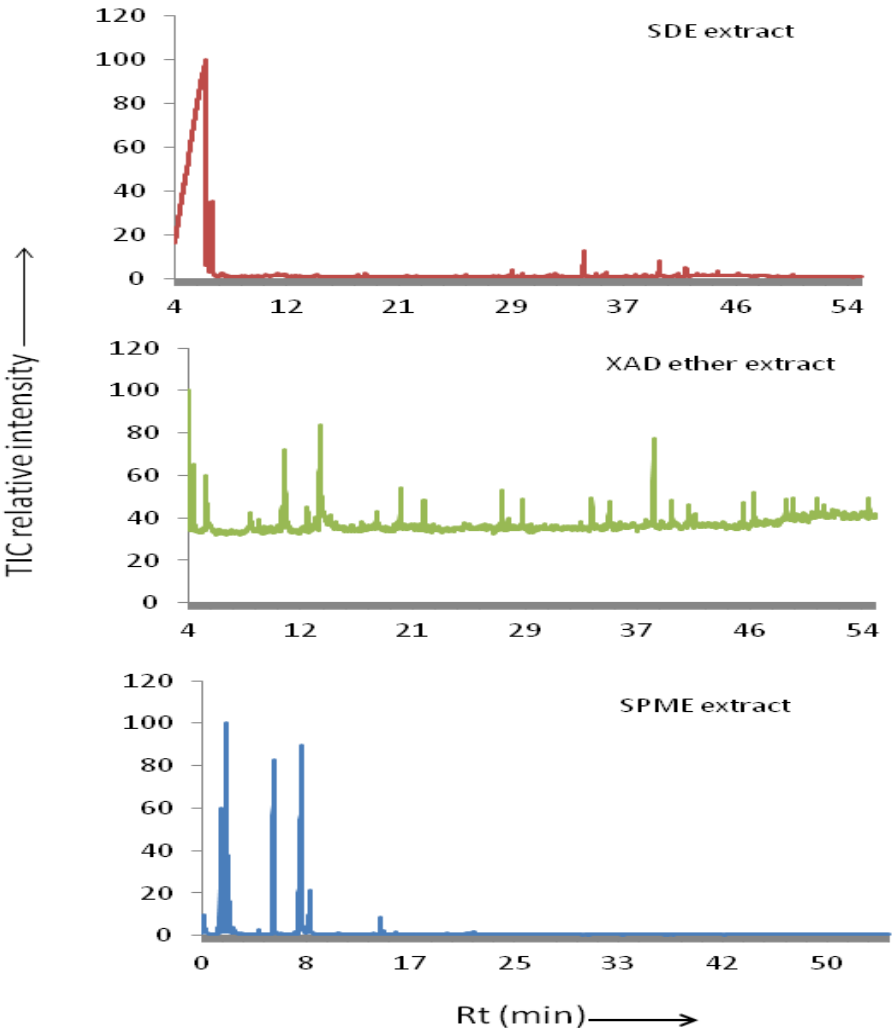
Figure 9. Final optimized scheme for extraction, identification and quantification of aroma glycosides followed in the present study.



3.1.1.2 Optimization of extraction of free volatiles

Free volatiles were extracted using three techniques. A) XAD ether extract B) Steam distillation (SDE) C) SPME. Methodology followed for these techniques is described in section 2.2.3.1. GC/MS profile obtained for these techniques is shown in figure 10.

Figure 10. GC/MS profiles of free volatiles extracted using different techniques



Maximum number of compounds was detected in SPME extraction. Profiles obtained for Steam distillation (SDE) extract and XAD ether extracts were characterized by presence of interfering compounds such as free fatty acids and hydrocarbons. Particularly in XAD ether profile large number of interfering components such as fatty acids and hydrocarbons could be observed. Moreover, both these extracts have traces of solvent (diethyl ether) present even after concentration using gentle stream of nitrogen. Therefore, samples have to be analyzed using appropriate solvent cut in GC. Thus, information regarding lower boiling compounds is thus not available in analysis of SDE and XAD extracts. Moreover, both these methods are tedious, require lot of solvents and are time consuming. On the contrary SPME is solvent less rapid extraction technique. Therefore, for these reasons identification and quantification of free volatiles was performed using SPME.

3.1.2 Effect of radiation processing on bound flavor precursors in grapes

Several reports exists demonstrating radiation induced breakdown of aroma glycosides in various food products. Since, flavor precursors play an important role in final aroma quality of grape products such as wine; it was of interest to elucidate the influence of radiation processing on these precursors. Compounds identified in all three varieties i.e. Chenin Blanc, Cabernet Sauvignon and Shiraz are presented in table 4, 5 and 6, respectively. Profile obtained in the present study is similar to already reported literature data for these varieties (82-84). Maximum numbers of compounds were detected in Cabernet Sauvignon variety while the minimum numbers of bound flavor precursors were observed in Chenin Blanc. Data obtained for bound flavor precursors in control and irradiated samples was analyzed using principal component analysis to better understand their differences among them.

Table 4. Effect of radiation processing on bound flavor precursors of Chenin Blanc variety

Name of compound	Rt	Control µg kg^{-1}	0.5 kGy µg kg^{-1}	1 kGy µg kg^{-1}	1.5 kGy µg kg^{-1}	2 kGy µg kg^{-1}
Aldehydes						
Hexanal	5.766	0.16 ± 0.08	0.19 ± 0.07	0.12 ± 0.05	0.06 ±0.01	0.12 ± 0.07
2-Hexenal	7.871	0.24 ± 0.02	0.18 ± 0.01	0.1 ± 0.06	0.09 ±0.08	0.12 ± 0.02
Heptanal	9.911	0.05 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ±0.01	0.03 ± 0.02
Octanal	14.376	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ±0.01	0.04 ± 0.01
2E-Octenal	16.825	0.02 ± 0.01	0.02 ± 0	0.02 ± 0	0.04 ±0.02	0.02 ± 0.01
Nonanal	18.726	0.09 ± 0.01	0.13 ± 0.04	0.27 ± 0.05	0.12 ±0.02	0.19 ± 0.05
Decanal	22.799	0.04 ± 0.03	0.05 ± 0.01	0.09 ± 0.04	0.21 ±0.15	0.1 ± 0.01
2E,4E-Decadienal	27.042	0.35 ± 0.07	0.39 ± 0.02	0.51 ± 0.06	0.39 ±0.08	0.8 ± 0.25
2-Undecenal	28.684	0.48 ± 0.03	0.43 ± 0.18	0.57 ± 0.03	0.43 ±0.01	0.68 ± 0.07
Trans-2-nonenal	21.059	0.05 ± 0.01	0.04 ± 0.01	0.09 ± 0.02	0.08 ±0.03	0.13 ± 0.01
Alcohols						
1-Hexanol	8.917	0.4 ± 0.03	0.49 ± 0.06	0.89 ± 0.09	0.18 ±0.09	0.56 ± 0.02
1-Heptanol	13.451	0.17 ± 0.03	0.24 ± 0.06	0.3 ± 0.01	0.29 ±0.1	0.34 ± 0.09
1-Octen-3-ol	13.616	0.04 ± 0.01	0.09 ± 0.01	0.08 ± 0.05	0.04 ±0.01	0.09 ± 0.04
2-Ethyl hexanol	16.017	0.39 ± 0.02	0.23 ± 0.05	0.11 ± 0.03	0.35 ±0.2	0.51 ± 0.1
Benzyl alcohol	16.331	0.01 ± 0.01	0.01 ± 0.01	0.04 ± 0	0.08 ±0.08	0.04 ± 0.01
1-Octanol	17.782	0.12 ± 0.02	0.25 ± 0.05	0.23 ± 0.1	0.06 ±0.04	0.23 ± 0.01
3,6-dimethyl-3-Heptanol	20.757	0.01 ± 0	0.08 ± 0.01	0.04 ± 0.01	0.11 ±0.01	0.07 ± 0
2-methyl-3-hexanol	6.727	0.01 ± 0	0.01 ± 0.01	0.01 ± 0.01	0.01 ±0.01	0.01 ± 0.01
Terpenes						
α-Pinene	11.152	0.46 ± 0.05	0.13 ± 0.01	0.01 ± 0.01	0.07 ±0.01	0.13 ± 0.03
Sabinene	12.959	0.47 ± 0.01	0.44 ± 0.01	0.13 ± 0.01	0.03 ±0.01	0.15 ± 0.01
β-Pinene	13.045	0.71 ± 0.44	0.19 ± 0.14	0.13 ± 0.07	0.11 ±0.05	0.13 ± 0.04
Myrcene	13.824	0.09 ± 0.05	0 ± 0	0 ± 0	0 ±0	0 ± 0
Limonene	15.416	0.71 ± 0.25	0.28 ± 0.18	0.13 ± 0.09	0.12 ±0.08	0.23 ± 0.14
Nerol	23.974	0.3 ± 0.02	0.58 ± 0.04	0.72 ± 0.06	0.3 ±0.02	0.08 ± 0.01
Geraniol	24.997	0.84 ± 0.04	0.37 ± 0.03	0.92 ± 0.02	0.42 ±0.01	0.76 ± 0.31
α-Terpineol	22.3	0.12 ± 0.07	0.24 ± 0.03	0.52 ± 0.03	0.19 ±0.1	0.3 ± 0.11
Other compounds						
Hexanoic acid methyl ester	10.981	0.42 ± 0.31	0.07 ± 0.02	0.05 ± 0.01	0.05 ±0.01	0.09 ± 0.03
2-Octanone	13.901	0.67 ± 0.12	0.65 ± 0.17	0.65 ± 0.05	0.49 ±0.04	0.5 ± 0.13

Table 5. Effect of radiation processing on bound flavor precursors in Cabernet Sauvignon variety

	Rt	Control	0.5 kGy	1.0 kGy	1.5 kGy	2.0 kGy
Aldehydes		**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**
2-methyl-butanal	2	26.41 ± 1.97	33.66 ± 12.33	21.15 ± 4.86	24.29 ± 3.94	41.68 ± 5.78
Hexanal	5.73	4.16 ± 0.23	3.9 ± 0.19	2.73 ± 0.38	2.04 ± 0.11	3.84 ± 0.66
2-Hexenal	7.81	3.49 ± 0.37	4.07 ± 0.11	3.26 ± 0.28	0.97 ± 0.08	3.1 ± 0.21
Heptanal	9.86	0.12 ± 0.03	0.18 ± 0.07	0.1 ± 0.01	0.09 ± 0.04	0.1 ± 0.04
Benzaldehyde	12.44	0.91 ± 0.07	0.73 ± 0.03	0.64 ± 0.02	0.68 ± 0.02	1.02 ± 0.13
Octanal	14.35	0.1 ± 0.02	0.1 ± 0	0.12 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Nonanal	18.68	0.9 ± 0.03	0.53 ± 0.12	1.13 ± 0.14	0.36 ± 0.14	1.08 ± 0.28
Decanal	22.74	0.55 ± 0.15	0.14 ± 0.08	0.39 ± 0.07	0.32 ± 0.1	0.56 ± 0.11
Alcohols						
1-Hexanol	8.82	6.24 ± 0.72	3.28 ± 0.85	3.24 ± 0.1	4.12 ± 0.1	4.1 ± 0.52
1-Heptanol	13.33	0.96 ± 0.24	0.46 ± 0.14	0.45 ± 0.04	0.46 ± 0.03	0.55 ± 0.11
1-Octen-3-ol	13.53	0.89 ± 0.14	0.17 ± 0.03	0.26 ± 0.06	0.57 ± 0.09	0.22 ± 0.11
2-Ethyl hexanol	15.87	1.81 ± 0.1	2.05 ± 0.78	0.43 ± 0.06	0.87 ± 0.14	1.25 ± 0.45
Benzyl alcohol	16.08	2.02 ± 0.11	2.02 ± 0.05	0.63 ± 0.68	0.56 ± 0.04	0.11 ± 0.02
2Z-Octen-1-ol	17.45	0.11 ± 0.01	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0.01
1-Octanol	17.61	1.94 ± 0.07	0.98 ± 0.09	1.35 ± 0.1	1.2 ± 0.14	1.12 ± 0.26
1-Decanol	20.25	2.63 ± 0.52	0.1 ± 0	0.42 ± 0.01	0.14 ± 0.02	0.1 ± 0.01
3Z-Nonen-1-ol	20.96	0.2 ± 0.04	0.45 ± 0.18	0.1 ± 0	0.1 ± 0	0 ± 0.06
Terpenes						
α-Pinene	11.13	0.36 ± 0.03	0.54 ± 0.06	0.35 ± 0.01	0.5 ± 0.13	0.68 ± 0.11
β-PINENE	13.02	0.53 ± 0.17	0.4 ± 0.02	0.6 ± 0.14	0.56 ± 0.01	0.56 ± 0.08
l-Limonene	15.37	0.86 ± 0.17	1.15 ± 0.18	1.25 ± 0.08	1.62 ± 0.34	1.52 ± 0.19
Trans-Linalool oxide	18.03	0.11 ± 0.03	0.12 ± 0.05	0.08 ± 0.01	0.1 ± 0	0.17 ± 0.02
Linalool	18.57	0.04 ± 0.01	0.02 ± 0	0.02 ± 0.01	0.02 ± 0.01	0.06 ± 0.01
α-Terpineol	22.27	1.98 ± 0.36	0.41 ± 0.22	0.68 ± 0.16	0.29 ± 0.03	0.21 ± 0.19
Nerol	24.91	0.55 ± 0.09	0.38 ± 0.13	0.7 ± 0.21	0.52 ± 0.22	0.5 ± 0.16
Ketones						

2-Pentanone	2.93	0.23 ± 0.07	0.19 ± 0.1	0.18 ± 0.09	0.09 ± 0.01	0.37 ± 0.07
3-Hexanone	5.26	0.08 ± 0.03	0.15 ± 0.02	0.12 ± 0.09	0.21 ± 0.04	0.08 ± 0.01
2-Hexanone	5.43	0.41 ± 0.06	0.07 ± 0.01	0.17 ± 0.01	0.16 ± 0.08	0.11 ± 0.07
6-Methyl-5-hepten-2-one	13.7	0.25 ± 0.05	0.4 ± 0.02	0.25 ± 0.09	0.33 ± 0.02	0.55 ± 0.16
2-Octanone	13.86	0.82 ± 0.04	1.01 ± 0.28	0.56 ± 0.19	0.5 ± 0.02	0.41 ± 0.02
Geranyl acetone	31.67	0.22 ± 0.01	0.28 ± 0.11	0.38 ± 0.09	0.66 ± 0.03	0.2 ± 0.02
Esters						
Acetic acid ethyl ester	2.14	19.84 ± 3.45	41.78 ± 7.87	36.04 ± 10.46	33.93 ± 4.71	13.95 ± 4.12
Butanoic acid methyl ester	3.55	0.23 ± 0.01	0.39 ± 0.05	0.1 ± 0	0.19 ± 0.02	0.05 ± 0.07
Pentanoic acid methyl ester	6.69	0.35 ± 0.07	0.26 ± 0.1	0.21 ± 0.03	0.31 ± 0.02	0 ± 0.06
4-ethoxy-benzoic acid ethyl ester	34.14	0.4 ± 0.02	0.38 ± 0.11	0.32 ± 0.08	0.47 ± 0.02	0.49 ± 0.16
Hexanoic acid methyl ester	10.94	0.18 ± 0.08	0.19 ± 0.01	0.14 ± 0.02	0.42 ± 0.02	0.49 ± 0.09
Methyl salicylate	22.36	2.83 ± 0.91	5.88 ± 1.44	6.32 ± 0.05	6.18 ± 0.71	6.52 ± 0.78

Table 6. Effect of radiation processing on bound flavor precursors of Shiraz variety

Name of compound	**Rt**	**Control µg kg^{-1}**	**0.5 kGy µg kg^{-1}**	**1 kGy µg kg^{-1}**	**1.5 kGy µg kg^{-1}**	**2.0 kGy µg kg^{-1}**
Aldehydes						
Hexanal	5.758	0.4 ± 0.02	1.05 ± 0.09	0.68 ± 0.02	0.82 ± 0.05	0.9 ± 0.07
2-Hexenal	7.85	1.55 ± 0.05	0.73 ± 0.19	1.49 ± 0.38	1.37 ± 0.19	1.04 ± 0.12
Heptanal	9.883	0.03 ± 0.01	0.12 ± 0.02	0.06 ± 0.01	0.12 ± 0.01	0.02 ± 0.01
Nonanal	18.708	0.63 ± 0.11	0.5 ± 0.06	0.37 ± 0.08	0.56 ± 0.12	0.86 ± 0.01
Decanal	22.775	0.35 ± 0.05	0.11 ± 0.04	0.13 ± 0.04	0.19 ± 0.04	0.19 ± 0.03
Alcohols						
3Z-Hexen-1-ol	8.192	0.28 ± 0.03	0.56 ± 0	0.83 ± 0	1.34 ± 0.1	0.38 ± 0.07
2E-Hexen-1-ol	8.642	1.15 ± 0.06	1.97 ± 0	1.24 ± 0	0.91 ± 0.22	0.6 ± 0.1
1-Hexanol	8.75	5.9 ± 0.28	5.71 ± 0.93	2.68 ± 0.33	2.39 ± 0.95	3.02 ± 0.25

2-Heptanol	10.075	0.14 ± 0.03	0.18 ± 0.13	0.36 ± 0.27	0.58 ± 0.11	0.56 ± 0
1-Heptanol	13.242	0.43 ± 0.03	0.35 ± 0.02	0.54 ± 0.08	0.75 ± 0.03	0.48 ± 0.09
1-octen-3-ol	13.525	0.05 ± 0	0.09 ± 0	0.16 ± 0.01	0.42 ± 0.01	0.14 ± 0
2-ethyl-1-Hexanol,	15.767	1.04 ± 0.06	1.24 ± 1	0.7 ± 0.40	2.7 ± 0.76	1.96 ± 0.15
Benzyl alcohol	16.075	0.37 ± 0	0.28 ± 0	0.32 ± 0	0.08 ± 0	0.08 ± 0
1-Octanol	17.558	0.36 ± 0.15	0.17 ± 0.05	0.55 ± 0.05	0.59 ± 0.1	0.56 ± 0.15
Terpenes						
α-Pinene	11.142	1.46 ± 0.29	2.24 ± 0.48	1.03 ± 0.68	1.57 ± 0.03	1.5 ± 0.84
β-Pinene	13.042	1.01 ± 0.09	1.78 ± 0.63	0.66 ± 0.05	0.17 ± 0.24	0.14 ± 0.01
l-Limonene	15.383	1.13 ± 0.56	1.54 ± 0.37	1.3 ± 0.25	1.39 ± 0.37	2.07 ± 0.29
γ-Terpinene	16.725	0.08 ± 0.04	0.21 ± 0.07	0.09 ± 0.03	0.1 ± 0.04	0.11 ± 0.01
trans-Linalool oxide	17.408	0.07 ± 0.04	0.13 ± 0	0.1 ± 0.04	0.28 ± 0.02	0.05 ± 0
cis-Linalool oxide	18.075	0.03 ± 0.01	0.18 ± 0.14	0.06 ± 0.05	0.4 ± 0.08	0.15 ± 0.1
Linalool	18.608	0.08 ± 0.04	0.05 ± 0.01	0.13 ± 0.1	0.18 ± 0.04	0.2 ± 0
α-Terpineol	22.308	0.42 ± 0.29	0.44 ± 0.26	0.45 ± 0.29	1.49 ± 0.33	0.62 ± 0.48
Nerol	23.842	0.26 ± 0.01	0.5 ± 0.06	0.31 ± 0.02	0.25 ± 0.04	1.1 ± 0.08
Isogeraniol	24.025	0.18 ± 0	0.07 ± 0	0.13 ± 0.03	0.1 ± 0.06	0.07 ± 0
Geraniol	24.833	3.79 ± 0.14	2.38 ± 0	2.76 ± 2.14	2.73 ± 0.05	2.9 ± 0
1,8-Cineole	15.542	0.79 ± 0	0.32 ± 0.03	0.71 ± 0	1.01 ± 0.08	1.03 ± 0
Ketones and esters						
6-methyl-5-hepten-2-one	13.733	0.16 ± 0.04	0.06 ± 0.04	0.16 ± 0.12	0.3 ± 0.01	0.32 ± 0.02
2-Octanone	13.892	0.8 ± 0.07	0.7 ± 0.1	0.45 ± 0.03	0.45 ± 0.07	0.67 ± 0.09
2-Nonanone	18.225	0.29 ± 0.02	0.44 ± 0.02	0.13 ± 0.07	0.2 ± 0.08	0.31 ± 0.05
Geranyl acetone	31.7	0.13 ± 0.06	0.05 ± 0	0.11 ± 0	0.09 ± 0.05	0.17 ± 0.01
2-Pentanone	2.942	0.19 ± 0.03	0.35 ± 0.02	0.14 ± 0.01	0.08 ± 0.02	0.24 ± 0.11
Methyl salicylate	22.383	2.37 ± 0.59	1.57 ± 0.3	3.06 ± 0.34	3.88 ± 0.48	3.39 ± 0.68
Hexanoic acid methyl ester	10.975	0.1 ± 0.09	0.36 ± 0	0.22 ± 0	0 ± 0.08	0.23 ± 0.2

PC score plots obtained for Chenin Blanc, Cabernet Sauvignon and Shiraz are shown in Figure 11, 12, and 13, respectively. In case of Chenin Blanc first two principal components (F1 and F2) cumulatively explained 54.15 % of data variation while in case

of Cabernet Sauvignon data a 48.5% variation was accounted for by F1 and F2. For Shiraz variety, 60.45 % of total variation was explained by first two principal components (PCs). In Chenin Blanc and Cabernet Sauvignon varieties a complete segregation of control sample from irradiated samples was observed. However, no segregation between control and irradiated samples could be observed in Shiraz variety. To know the nature of the constituents responsible for these differences factor loading data was analyzed.

Figure 11. PC score plots for bound aroma volatiles of Chenin Blanc variety between first two PCs. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)

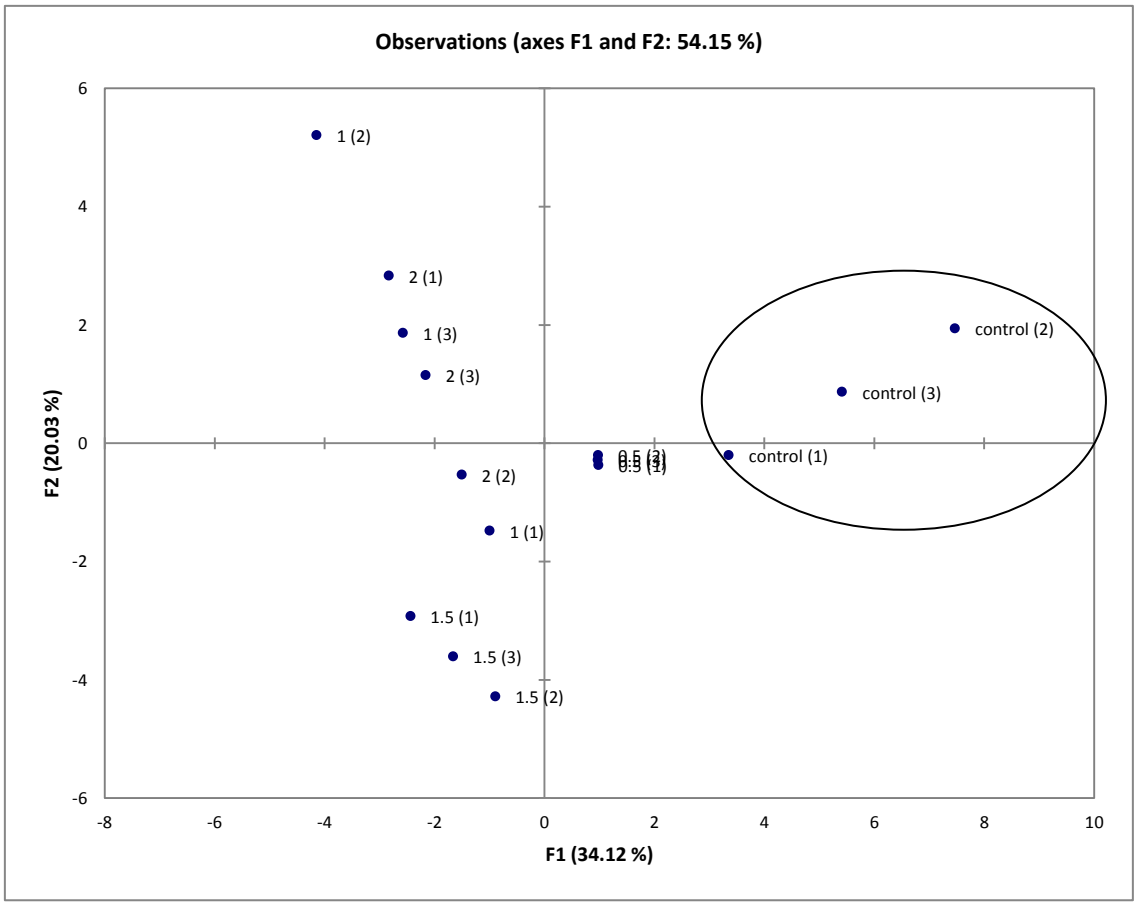


Figure 12. PC score plots of bound aroma volatiles of Cabernet Sauvignon variety. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)

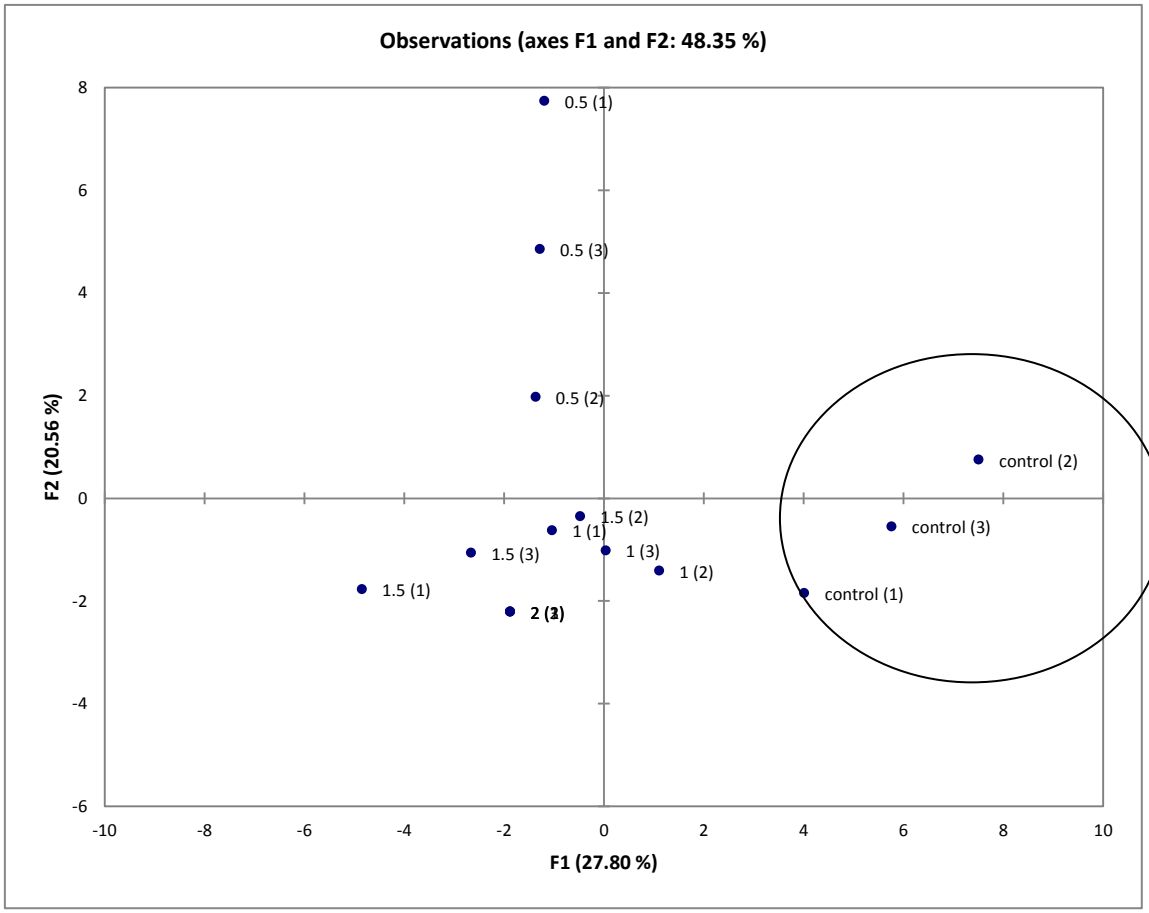
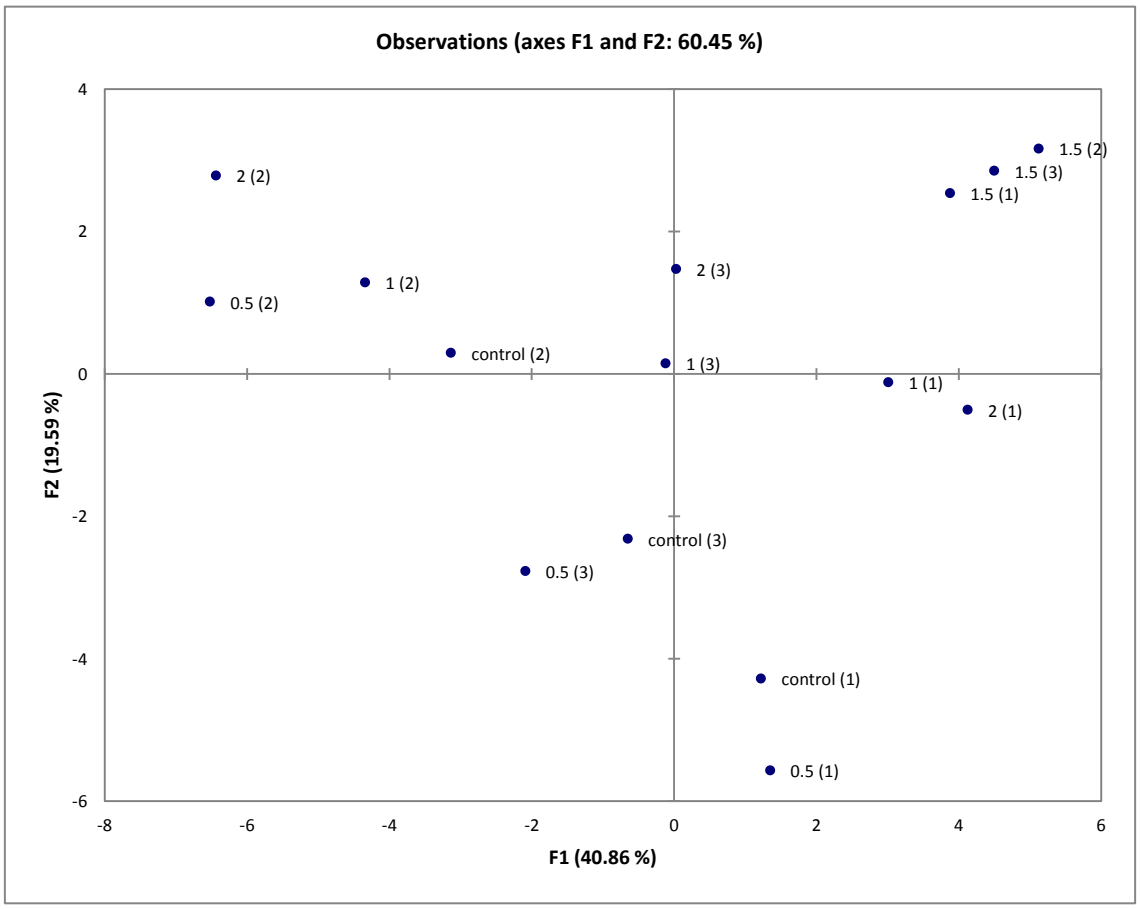


Figure 13. PC score plots of bound aroma volatiles of Shiraz variety. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)



In Chenin Blanc variety, a radiation induced decrease in content of various terpenes such as α-pinene, β-pinene, myrcene, limonene and α-terpineol could be clearly observed. However, no significant changes were observed in other compounds such as alcohols and aldehydes. Maximum decrease of 100 percent was observed for glycosidic precursor of myrcene, followed by limonene, α-pinene and β-pinene with a maximum decrease of 87,

84 and 81 percent, respectively. Generally, maximum decrease in the content of these compounds was observed for radiation doses between 1 – 2 kGy. Interesting results were obtained for monoterpenes alcohols such as nerol and α-terpineol. An increase in content of these two compounds was observed up to a dose of 1 kGy, with a decrease thereafter at higher doses. In control samples, content of nerol and α-terpineol was 0.3 and 0.12 µg kg^{-1}, respectively which increased to 0.72 and 0.52 at dose of 1 kGy. However, content of these compounds decreased to 0.08 and 0.3 µg kg^{-1}, respectively at 2 kGy. Increase in content of these compounds at lower doses up to 1 kGy might be due to their enhanced extractability due to radiation processing. While decrease in content at higher doses of beyond 1 kGy is due to radiation induced hydrolysis of these compounds.

Interestingly, for Cabernet Sauvignon variety in case of terpenes, a decrease in content of bound precursors of only α-terpineol was observed. Its content decreased by 89 percent upon radiation dose of 2 kGy. No changes were, however observed for other terpenes such as α-pinene and β-pinene. A radiation induced decreased content of glycosidic precursors of various alcohols such as hexanol, heptanol, 1-octen-3-ol, 2-ethyl hexanol, benzyl alcohol and 1-decanol was also observed. Glycosidic precursors of various aliphatic alcohols generally demonstrated a decrease in the range of 48 to 75 percent. However, bound precursor of benzyl alcohol, which is an aromatic alcohol decreased up to 94 percent upon exposure to a radiation dose of 2 kGy. Similar results were obtained for Shiraz variety where the decrease in alcohols such as hexanol and benzyl alcohol and terpenes such as geraniol and isogeraniol was observed. Glycosidic precursors for hexanol, benzyl alcohol, geraniol and isogeraniol demonstrated a decrease of 59, 78, 37 and 61 percent, respectively, at radiation doses between 1.5 – 2 kGy. Among other

terpenes content of trans-linalool oxide, cis-linalool oxide, linalool and α-terpineol demonstrated an increase up to dose of 1.5 kGy, with a decrease thereafter. Control sample had 0.07, 0.03, 0.08, 0.42 μg kg^{-1} of trans-linalool oxide, cis-linalool oxide, linalool and α-terpineol which increased to 0.28, 0.4, 0.18 and 1.49 μg kg^{-1} at dose of 1500 Gy. A radiation induced enhanced extraction could be suggested as possible reason for this observation. Apart from terpenes, an a increased content of bound precursor of methyl salicylate (ester) was observed in case of Shiraz as well as Cabernet Sauvignon variety due to radiation processing. Its content increased by 145 and 63 percent, in Cabernet Sauvignon (2 kGy) and Shiraz (1.5 kGy), respectively. Methyl salicylate has flavor note of peppermint. An increase content of its glycosidic precursor might be due to increased extraction of this compound from plant tissues in irradiated samples.

A decrease in content of aroma glycosides due to radiation processing has been reported earlier in several food products such as coffee, nutmeg, fenugreek, and papaya (46-49). In case of nutmeg, four different glycosidic precursors of p-cymene-7-ol, eugenol, methoxyeugenol and α-terpineol were reported (47). A radiation dose dependent decrease in content of all glycosides was reported by these authors. In case of fenugreek, radiation dose dependent decrease in content of phenol glycoside was reported (48). In coffee beans, it was reported that a decreased content of glycosidic precursors of isoeugenol and 4-vinylguaiacol as a result of radiation processing was reported (46). Thus, our results are in accordance with already published literature data. In the present study, a maximum reduction was observed in the content of glycosidic precursor of benzyl alcohol for both Cabernet Sauvignon and Shiraz varieties. A higher resonance stabilization of the phenoxy

(PhO•) radical intermediate formed from this compound compared to the radical from other compounds could explain the low stability of the glucoside of this compound. Furthermore, it was also observed that the glycoside of α-terpineol, a tertiary alcohol, was more sensitive to radiation treatment as compared to primary alcohols. It was previously reported (47), that glycosides of tertiary alcohols are more sensitive to radiation treatment as compared to primary alcohols. A greater stabilization of the radical intermediate of α-terpineol, a tertiary alcohol, formed during the radiolysis of its glucoside as compared to primary alcohols was also suggested (47).

A considerable difference in the effect of radiation processing on the content of glycosidic precursors was observed among the three varieties studied. A radiation dose dependent decrease in content of maximum number of compounds was seen in Cabernet Sauvignon variety, while least number of glycosidic precursors was affected in Shiraz variety. Two types of radiation effects were observed in present study. Both radiation induced increased extractability and degradation was observed. It can be concluded that both these changes can occur simultaneously and independent of each other. Lastly, food products have complex mixture of various compounds. Nature of these compounds and their content could vary significantly among different varieties of same species. Furthermore, different varieties could have different contents of total antioxidants and different pH. The presence of antioxidants and difference in pH could alter the effect of radiation. Thus, variation in composition, total antioxidants and pH could possibly accounts for the different response observed towards radiation processing.

Table 7 summarises the changes in bound flavor precursors as a result of radiation processing in different grape varieties. Thus, it can be concluded from this data that radiation processing results in enhanced content of free aglycones responsible for imparting floral, mint, green, spice, rose and peppermint odors. Free volatiles present in these varieties were therefore analyzed.

Table 7. Summary of changes observed in bound flavor precursors due to radiation processing

Name of compound	Variety	Odor	Effect
α-pinene	Chenin Blanc	Pine, turpentine	Decrease
β-pinene	Chenin Blanc	Pine, resin, turpentine	Decrease
Myrcene	Chenin Blanc	Balsamic, must, spice	Decrease
Limonene	Chenin Blanc	Citrus, mint	Decrease
Nerol and α-terpineol	Chenin Blanc	floral, mint	Increase till 1 kGy, decrease thereafter
α-terpineol	Cabernet Sauvignon	Oil, anise, mint	Decrease
Hexanol	Cabernet Sauvignon, Shiraz	Floral, green	Decrease
Heptanol	Cabernet Sauvignon	herb	Decrease
1-octen-3-ol	Cabernet Sauvignon	Earthy	Decrease
2-Ethyl hexanol	Cabernet Sauvignon	Rose, green	Decrease
Benzyl alcohol	Cabernet Sauvignon, Shiraz	Sweet, floral	Decrease
1-Decanol	Cabernet Sauvignon	Fat	Decrease
1-Octanol	Cabernet Sauvignon	nut	Decrease
Geraniol	Shiraz	Rose, Germanium	Decrease
Isogeraniol	Shiraz	Rose	Decrease
trans and cis-linalool oxide	Shiraz	floral	Increase till 1.5 kGy, decrease thereafter
Linalool, α-terpineol	Shiraz	lavender and mint	Increase till 1.5 kGy, decrease thereafter
Methyl salicylate	Shiraz, Cabernet Sauvignon	Peppermint	Increase

Data on flavour notes taken from flavournet database (85).

3.1.3 Effect of radiation processing on free aroma constituents

Apart from release of aglycones, radiation processing can also result in significant changes in volatile profile of food products (86). To best of our knowledge there are no reports on effect of radiation processing on volatile flavor constituents of grapes. It was therefore, of interest to determine changes in volatile aroma components during radiation processing of grapes. Compounds identified in all three varieties i.e. Chenin Blanc, Cabernet Sauvignon and Shiraz are presented in table 8, 9 and 10, respectively. Profile obtained in present study is similar to already reported literature data for these varieties (83-84, 87). Maximum number of compounds was detected in Cabernet Sauvignon variety while minimum number of aroma compounds was observed in Chenin Blanc. The data obtained for both control and an irradiated sample was analyzed using principal component analysis to better understand differences among them.

Table 8. Effect of radiation processing on free volatiles of Chenin Blanc variety

Aldehydes	**Rt**	**control** **µg kg^{-1}**	**0.5 kGy** **µg kg^{-1}**	**1.0 kGy** **µg kg^{-1}**	**1.5 kGy** **µg kg^{-1}**	**2.0 kGy** **µg kg^{-1}**
3-Methylbutanal	2.465	0.18 ± 0.09	0.2 ± 0.08	0.41 ± 0.03	0.47 ± 0.18	0.88 ± 0.24
Hexanal	5.74	495.91 ± 7.25	335.78 ± 119.45	844.42 ± 135.54	620.45 ± 168.33	714.52 ± 85.52
trans-2-Hexenal	7.486	232.62 ± 16.98	909.56 ± 129.72	904.27 ± 135.07	819.44 ± 174.64	522.98 ± 31.07
Heptanal	9.818	0.13 ± 0.01	0.6 ± 0.03	0.11 ± 0.02	0.24 ± 0.07	0.26 ± 0.01
2E,4E-Hexadienal	10.367	0.08 ± 0.01	0.1 ± 0.02	0.26 ± 0.02	0.12 ± 0.03	0.1 ± 0.02
Benzaldehyde	12.351	0.27 ± 0.02	0.14 ± 0.01	0.1 ± 0	0.1 ± 0	0.1 ± 0.01
Phenylacetaldehyde	16.061	0.14 ± 0.05	0.22 ± 0.03	0.11 ± 0.05	0.43 ± 0.02	0.36 ± 0.1
Nonanal	18.617	0.35 ± 0.01	0.35 ± 0.03	0.3 ± 0.01	0.21 ± 0.02	0.34 ± 0.16
Alcohols						
1-Butanol	2.591	0.74 ± 0.11	1.88 ± 0.21	0.59 ± 0.19	0.19 ± 0.02	1.24 ± 0.16
1-Penten-3-ol	2.819	0.33 ± 0.02	0.12 ± 0.01	0.48 ± 0.03	0.48 ± 0.02	0.36 ± 0.01
3-methyl-1-Butanol	3.812	0.35 ± 0.07	1.03 ± 0.33	1.18 ± 0.08	0.91 ± 0.23	2.5 ± 0.7
cis-2-Pentenol	4.892	0.04 ± 0.02	0.04 ± 0.01	0.37 ± 0.03	0.14 ± 0.03	0.12 ± 0.09
3E-Hexen-1-ol	8.064	6.61 ± 0.15	6.39 ± 2.53	8.39 ± 1.52	7.16 ± 0.22	7.18 ± 0.37
2E-Hexen-1-ol	8.484	28.98 ± 6.66	42.53 ± 11.57	40.21 ± 7.02	43.6 ± 3.66	41.2 ± 6.86
1-Hexanol	8.638	88.23 ± 2.81	84.44 ± 20.55	62.58 ± 9.82	57.91 ± 14.62	51.17 ± 13.86
2-Heptanol	9.894	0.1 ± 0.03	0.13 ± 0.04	0.15 ± 0.1	0.21 ± 0.05	0.2 ± 0.1
1-Heptanol	13.002	0.12 ± 0.02	0.28 ± 0.06	0.35 ± 0.04	0.1 ± 0	0.1 ± 0
1-Octen-3-ol	13.336	0.67 ± 0.21	0.94 ± 0.05	0.53 ± 0.15	1.61 ± 0.19	1.3 ± 0.42
2-Ethyl-1-hexanol	15.521	3.54 ± 0.11	7.55 ± 1.24	1.54 ± 0.17	3.07 ± 0.12	3.82 ± 0.87
1-Octanol	17.357	0.15 ± 0.01	0.13 ± 0.02	0.19 ± 0.08	0.1 ± 0	0.1 ± 0
Terpenes						
Sabinene	12.893	0.04 ± 0.01	0.1 ± 0.02	0.05 ± 0.03	0.05 ± 0.01	0.09 ± 0.03
l-limonene	15.308	0.07 ± 0.02	0.12 ± 0.05	0.13 ± 0.07	0.1 ± 0.02	0.52 ± 0.04
Linalool	18.451	0.03 ± 0.01	0.14 ± 0.01	0.18 ± 0.03	0.05 ± 0.01	0.01 ± 0
α-Terpineol	22.146	0.02 ± 0.01	0.15 ± 0.08	0.12 ± 0.05	0.05 ± 0.01	0.08 ± 0.03
Esters and ketones						

Acetic acid, ethyl ester	2.098	84.68 ± 1.8	108 ± 2.3	149.87 ± 12.99	53.25 ± 14.19	128.52 ± 13.48
Acetic acid, butyl ester	6.302	0.62 ± 0.21	0.81 ± 0.05	0.51 ± 0.1	0.39 ± 0.06	1.05 ± 0.02
Hexanoic acid, methyl ester	10.898	2.69 ± 0.04	4.22 ± 0.75	0.89 ± 0.26	0.13 ± 0.02	5.34 ± 0.89
6-Methyl-5-hepten-2-one	13.681	0.24 ± 0.01	0.16 ± 0.02	0.1 ± 0	0.09 ± 0.05	0.55 ± 0.09

Table 9. Effect of radiation processing on free volatiles in Cabernet Sauvignon variety

Name of compound	**Rt**	**Control µg kg^{-1}**	**500 µg kg^{-1}**	**1000 µg kg^{-1}**	**1500 µg kg^{-1}**	**2000 µg kg^{-1}**
Aldehydes						
3-methyl-butanal	2.48	7.6 ± 1.84	2.2 ± 0.01	4.1 ± 1.5	1.84 ± 0.4	2.1 ± 0.09
Pentanal	3.03	2.51 ± 0.4	3.64 ± 0.97	1.77 ± 0.63	1.66 ± 0.2	2.78 ± 0.67
Hexanal	5.71	253.23 ± 11.4	338.13 ± 59.2	366.23 ± 36.63	361.88 ± 38.88	314.25 ± 38.63
cis-2-Hexenal	7.5	4.63 ± 0.05	5.87 ± 0.52	5.33 ± 0.33	6.39 ± 0.09	5.82 ± 0.27
trans-2-Hexenal	7.83	286.95 ± 51.37	364.48 ± 25.25	430.69 ± 63.72	379.72 ± 89.28	454.07 ± 38.73
Heptanal	9.83	1.85 ± 0.78	1.62 ± 0.58	1.31 ± 0.34	0.72 ± 0.13	1.27 ± 0.52
2E,4E-Hexadienal	10.35	0.03 ± 0.02	0.06 ± 0.04	0.03 ± 0.02	0.09 ± 0.09	0.01 ± 0.01
2E-Heptenal	12.25	2.56 ± 1.27	0.97 ± 0.09	0.22 ± 0.06	0.72 ± 0.08	0.15 ± 0.07
Benzaldehyde	12.37	0.89 ± 0.03	0.25 ± 0.07	0.22 ± 0.01	0.4 ± 0.01	0.47 ± 0.02
Benzeneacetaldehyde	16.04	2.45 ± 0.57	1.08 ± 0.06	1.06 ± 0.15	0.56 ± 0.15	0.53 ± 0.02
Nonanal	18.63	3.74 ± 1.3	2.5 ± 1.38	2.26 ± 0.23	2.28 ± 0.28	2.83 ± 0.2
Decanal	22.69	1.34 ± 0.17	0.45 ± 0.3	0.14 ± 0.04	0.17 ± 0.08	0.73 ± 0.05
Alcohols						
1-Penten-3-ol	2.84	0.53 ± 0.12	0.17 ± 0.02	0.24 ± 0.01	0.22 ± 0.02	0.25 ± 0.01
3-methyl- 1-Butanol	3.8	6.68 ± 2.33	2.67 ± 0.01	5.33 ± 1.87	0.87 ± 0.05	1.23 ± 0.18
2-methyl-1-Butanol	3.89	1.05 ± 0.31	0.35 ± 0.01	1 ± 0.22	0.15 ± 0.04	0.19 ± 0.05
1-Pentanol	4.72	1.89 ± 0.23	0.61 ± 0.14	0.54 ± 0.05	0.7 ± 0.03	1.11 ± 0.04
2Z-Penten-1-ol	4.85	0.18 ± 0.09	0.07 ± 0.02	0.2 ± 0.06	0.18 ± 0.01	0.16 ± 0.1
3Z-Hexen-1-ol	7.97	13.03 ± 2.51	12.88 ± 1.2	13.3 ± 3.58	6.85 ± 0.15	12.62 ± 2.26
2E-Hexen-1-ol	8.52	54.9 ± 17.87	86.98 ± 18.79	52.67 ± 13.85	37.78 ± 1.38	67.81 ± 5.73
1-Hexanol	8.66	240.49 ± 16.6	128.84 ± 24.66	164.47 ± 34.34	82.27 ± 7.1	114.91 ± 13.61

2-Heptanol	9.89	0.46 ± 0.01	0.36 ± 0.05	0.5 ± 0.03	0.21 ± 0.01	0.44 ± 0.01
1-Heptanol	13	0.36 ± 0.04	0.97 ± 0.17	0.52 ± 0.02	0.12 ± 0.02	0.21 ± 0.01
1-Octen-3-ol	13.35	4.75 ± 0.8	3.52 ± 0.39	2.53 ± 0.04	3.03 ± 0.21	2.7 ± 0.46
2-Ethyl- 1-hexanol	15.53	7.23 ± 0.26	6.02 ± 1.63	8.1 ± 1.1	5.92 ± 1.26	7.35 ± 0.37
2Z-Octen-1-ol	17.23	0.26 ± 0.09	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.11 ± 0.01
1-Octanol	17.34	1.46 ± 0.04	0.62 ± 0.06	0.75 ± 0.19	0.3 ± 0.07	0.49 ± 0.02
2-ethyl-2-propyl- 1-hexanol	26.06	3.12 ± 0.68	3.16 ± 0.01	1.17 ± 0.35	1.18 ± 0.5	2.44 ± 0.09
Terpenes						
l-Limonene	15.33	2.44 ± 0.53	1.88 ± 0.08	1.79 ± 0.12	1.69 ± 0.12	1.71 ± 0.67
β-Ocimene	16.66	0.19 ± 0.01	0.16 ± 0.01	0.06 ± 0.01	0.04 ± 0.02	0.12 ± 0.05
Linalool	18.46	0.28 ± 0.05	0.27 ± 0.01	0.21 ± 0.07	0.14 ± 0.01	0.13 ± 0.01
Camphor	20.26	0.11 ± 0.03	0.03 ± 0.02	0 ± 0	0 ± 0	0.04 ± 0.04
α-Citronellol	21.49	0.59 ± 0.46	0.03 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Terpineol-4	21.59	0.09 ± 0.02	0.06 ± 0.04	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
α-Terpineol	22.16	0.29 ± 0.1	0.16 ± 0.01	0.56 ± 0.02	0.06 ± 0.01	0.19 ± 0.07
trans-Geraniol	24.68	0.15 ± 0.02	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
trans-Caryophyllene	30.58	0.06 ± 0.01	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Ketones						
3-methyl-3-Buten-2-one	2.72	0.56 ± 0.16	3.23 ± 0.11	5.83 ± 0.94	2.12 ± 0.01	2.75 ± 0.62
2-Pentanone	2.91	0.68 ± 0.22	0.37 ± 0.09	0.37 ± 0.21	0.18 ± 0.02	0.35 ± 0.01
6-Methyl-5-hepten-2-one	13.67	1.19 ± 0.09	0.65 ± 0.25	0.83 ± 0.09	0.6 ± 0.11	0.67 ± 0.15
2-Octanone	13.84	0.44 ± 0.21	0.25 ± 0.22	0.13 ± 0.1	0.08 ± 0.04	0.2 ± 0.01
Damascenone	29	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Esters						
Acetic acid butyl ester	6.32	0.89 ± 0.03	0.82 ± 0.01	1.01 ± 0.03	0.62 ± 0.01	0.9 ± 0.16
1-Butanol-3-methyl-acetate	8.86	1.46 ± 0.08	5.25 ± 0.85	4.35 ±0.31	1.35 ± 0.29	1.13 ± 0.18
Hexanoic acid, methyl ester	10.92	0.3 ± 0.03	0.41 ± 0.02	0.38 ± 0.09	0.69 ± 0.08	0.59 ± 0.09

Table 10. Effect of radiation processing on free volatiles of Shiraz variety

Name of compound	**Rt**	**control**	**500**	**1000**	**1500**	**2000**
Aldehydes		**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**	**µg kg^{-1}**
3-methyl-butanal	2.48	5.31 ± 1.47	2.06 ± 1.09	2.45 ± 1.5	3.54 ± 0.06	3.29 ± 1.23
Hexanal	5.738	584.78 ± 46.21	579.71 ± 49.69	492.69 ± 37.24	447.18 ± 48.67	553.64 ± 11.32
trans-2-Hexenal	7.915	940.3 ± 26.73	262.43 ± 57.79	890 ± 28.78	746 ± 17.86	824.01 ± 19.75
Heptanal	9.828	0.33 ± 0.03	0.21 ± 0.01	0.17 ± 0.01	0.57 ± 0.03	0.5 ± 0.05
2E,4E-Hexadienal	10.323	1.34 ± 0.13	0.9 ± 0.01	1.1 ± 0.07	0.5 ± 0.08	3.13 ± 0.72
2E-Heptenal	12.265	0.2 ± 0.06	0.24 ± 0.01	0.16 ± 0.09	0.36 ± 0.03	0.5 ± 0.06
Benzaldehyde	12.385	1.35 ± 0.44	1.1 ± 0.07	1.33 ± 0.08	1.27 ± 0.06	1.02 ± 0.2
Benzeneacetaldehyde	16.056	3.22 ± 1.6	2.11 ± 0.05	2.76 ± 0.09	1.38 ± 0.34	2.15 ± 0.44
Nonanal	18.65	1.2 ± 0.01	1.83 ± 0.51	1.24 ± 0.06	0.65 ± 0.18	2.06 ± 0.88
Decanal	22.713	0.47 ± 0.37	0.4 ± 0.34	0.03 ± 0.01	0.16 ± 0.07	0.45 ± 0.01
Benzaldehyde, 4-(1-methylethyl)-	24.039	0.25 ± 0.01	0.12 ± 0.01	0.1 ± 0	0.1 ± 0	0.03 ± 0.01
Alcohols						
1-Butanol	2.603	1.41 ± 0.01	0.6 ± 0.03	1.54 ± 0.06	0.65 ± 0.01	1.4 ± 0.03
1-Penten-3-ol	2.837	0.55 ± 0.02	0.3 ± 0.05	0.59 ± 0.07	0.29 ± 0.02	0.43 ± 0.01
3-Methyl-1-butanol	3.798	15.94 ± 2.33	5.7 ± 0.34	5.25 ± 0.01	3.14 ± 0.22	5.31 ± 0.7
2-Methyl-1-butanol	3.892	2.31 ± 0.01	0.85 ± 0.1	1.5 ± 0.01	1.0 ± 0.01	0.78 ± 0.14
3Z-Hexen-1-ol	8.029	128.91 ± 21.43	58.42 ± 13.35	113.68 ± 29.67	136.29 ± 9.25	115.12 ± 43.16
2Z-Hexen-1-ol	8.481	49.63 ± 5.8	24.09 ± 3.99	22.78 ± 2.34	36.26 ± 0.94	43.25 ± 13.18
1-Hexanol	8.641	349.85 ± 66.22	98.09 ± 0.92	260.17 ± 59.83	94.03 ± 31.38	114.34 ± 18.39
2-Heptanol	9.899	2.73 ± 1.25	2.29 ± 0.61	2.52 ± 0.01	1.24 ± 0.56	2.01 ± 0.89
1-Heptanol	13.024	0.55 ± 0.01	0.55 ± 0.01	0.65 ± 0.01	0.96 ± 0.77	0.37 ± 0.01
1-Octen-3-ol	13.364	0.42 ± 0.13	0.75 ± 0.01	0.75 ± 0.18	1.34 ± 0.39	1.15 ± 0.26
Eucalyptol	15.486	0.65 ± 0.01	0.61 ± 0.01	0.6 ± 0	0.61 ± 0.58	0.6 ± 0
1-Hexanol, 2-ethyl-	15.558	9.6 ± 0.86	9.6 ± 1.45	11.34 ± 0.92	10.17 ± 0.54	12.48 ± 3.64
1-Octanol	17.384	0.13 ± 0.01	0.65 ± 0.01	1.1 ± 0.01	1.1 ± 0.01	0.65 ± 0.01
Terpenes						
Sabinene	12.907	0.78 ± 0.01	0.34 ± 0.01	1.17 ± 0.01	0.44 ± 0.01	0.23 ± 0.17

p-Cymene	15.158	0.47 ± 0.03	0.32 ± 0.04	0.05 ± 0.01	0.33 ± 0.01	0.27 ± 0.04
l-Limonene	15.334	3.21 ± 0.96	2.81 ± 0.01	3.81 ± 0.11	2.43 ± 0.06	3.3 ± 0.74
Linalool oxide	17.342	0.15 ± 0.12	0.33 ± 0.01	0.33 ± 0.01	0.42 ± 0.12	0.34 ± 0.21
Linalool	18.476	0.37 ± 0.02	0.33 ± 0.05	0.51 ± 0.08	0.52 ± 0.03	0.83 ± 0.25
Terpineol-4	21.602	0.11 ± 0.01	0.21 ± 0.01	0.05 ± 0	0.03 ± 0.01	0.06 ± 0
α-Terpineol	22.163	0.66 ± 0.07	0.51 ± 0.06	0.55 ± 0.07	0.81 ± 0.15	0.86 ± 0.09
trans-Geraniol	24.673	0.21 ± 0.01	0.14 ± 0.01	0.05 ± 0.01	0.06 ± 0.05	0.14 ± 0.01
Esters and Ketones						
Acetic acid ethyl ester	2.116	142.42 ± 44.49	34.83 ± 10.15	53.91 ± 9.85	65.85 ± 0.31	66.73 ± 16.19
Acetic acid butyl ester	6.32	2.19 ± 0.38	1.19 ± 0.2	1.69 ± 0.28	1.63 ± 0.03	1.58 ± 0.43
3-methyl-1-Butanol acetate	8.862	3.29 ± 0.02	1.55 ± 0.2	3.29 ± 9.19	2.6 ± 0.27	13.4 ± 0.71
Hexanoic acid methyl ester	10.921	1.3 ± 0.28	0.33 ± 0.05	0.64 ± 0.05	0.62 ± 0.07	0.48 ± 0.04
2-Pentanone	2.914	0.62 ± 0.06	0.24 ± 0.01	0.51 ± 0.01	0.43 ± 0.03	0.53 ± 0.16
Cyclohexanone	9.473	0.64 ± 0.03	0.34 ± 0.02	0.69 ± 0.04	0.6 ± 0.02	0.82 ± 0.05
6-Methyl-5-hepten-2-one	13.68	0.31 ± 0.01	0.46 ± 0.2	0.25 ± 0.05	0.73 ± 0.05	0.58 ± 0.01
Damascenone	29.356	0.06 ± 0.03	0.05 ± 0.01	0.1 ± 0	0.22 ± 0.09	0.82 ± 0.02

PC score plots obtained for Chenin Blanc, Cabernet Sauvignon and Shiraz are shown in Figure 14, 15, and 16, respectively. In case of Chenin Blanc first two principal components (F1 and F2) cumulatively explained 98.17 percent of data variation while in case of Cabernet Sauvignon a data variation of 58.69% was accounted for by F1 and F2. For Shiraz variety, 49.75 % of total variation is explained by the first two PCs. In all the three varieties a complete segregation of control sample with irradiated samples was observed. To know the nature of constituents responsible for these differences factor loading data was analyzed. Most remarkable changes in case of free aroma were observed

in content of aldehydes such as hexanal and trans-2-hexenal. These two compounds are the major compounds (>90%) present in the free volatiles fraction of all the three varieties.

Figure 14. PC score plot for free aroma volatiles of Chenin Blanc variety. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)

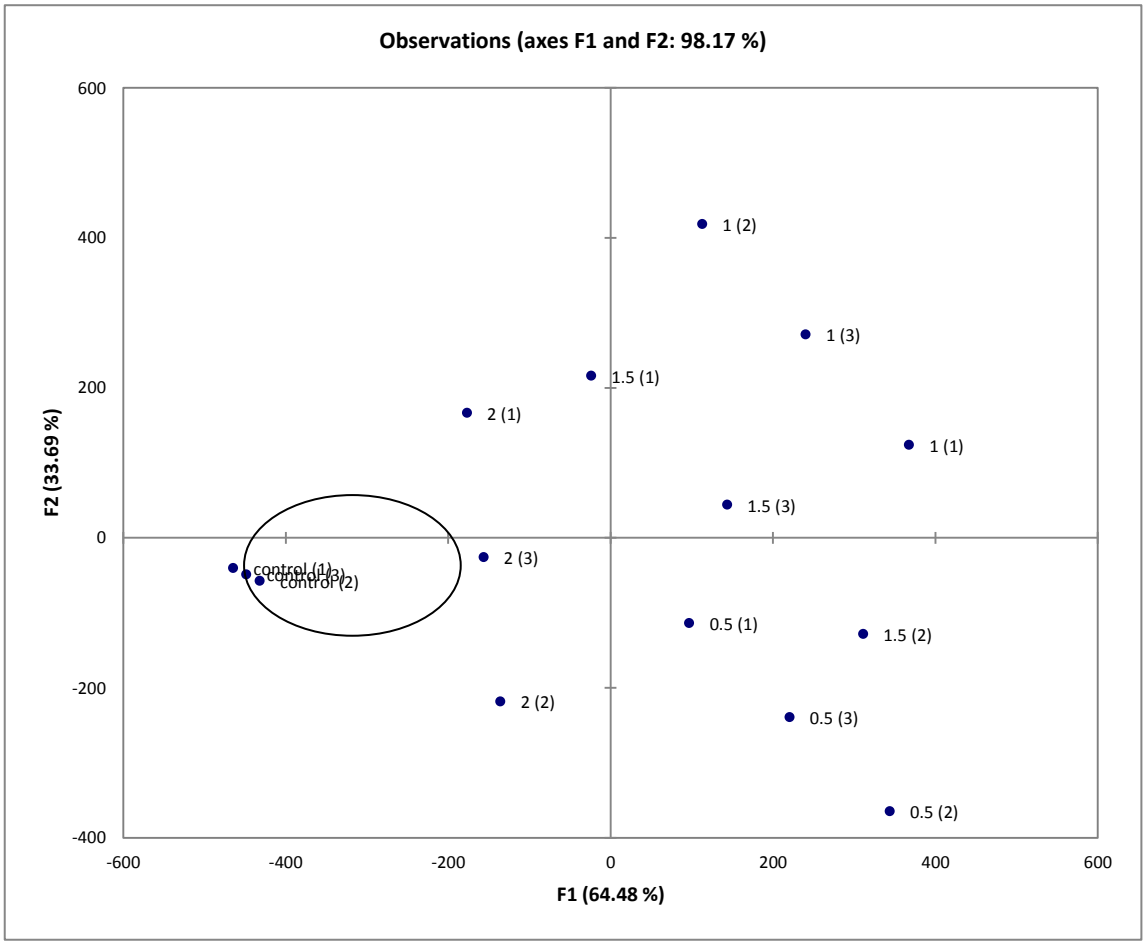


Figure 15. PC score plots for free aroma volatiles of Cabernet Sauvignon variety. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)

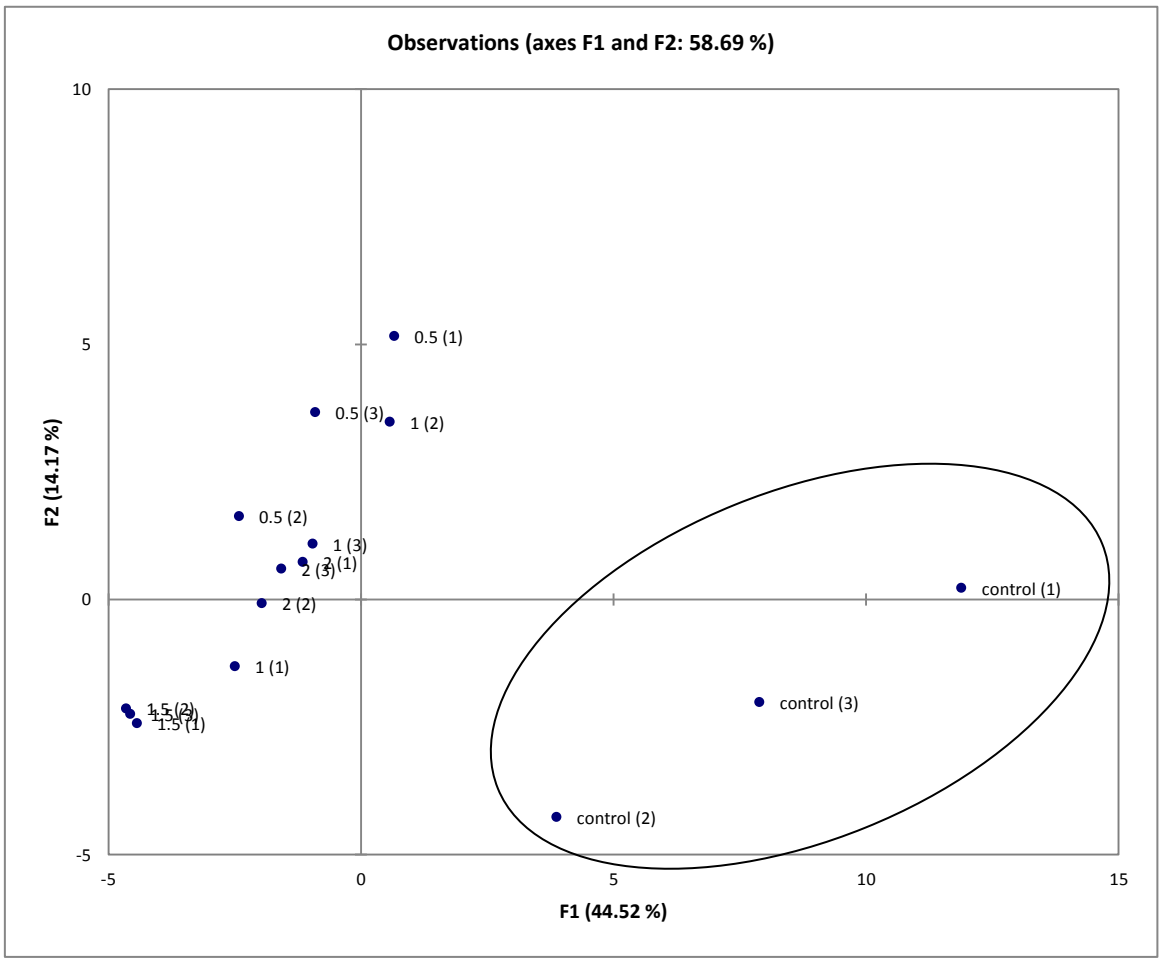
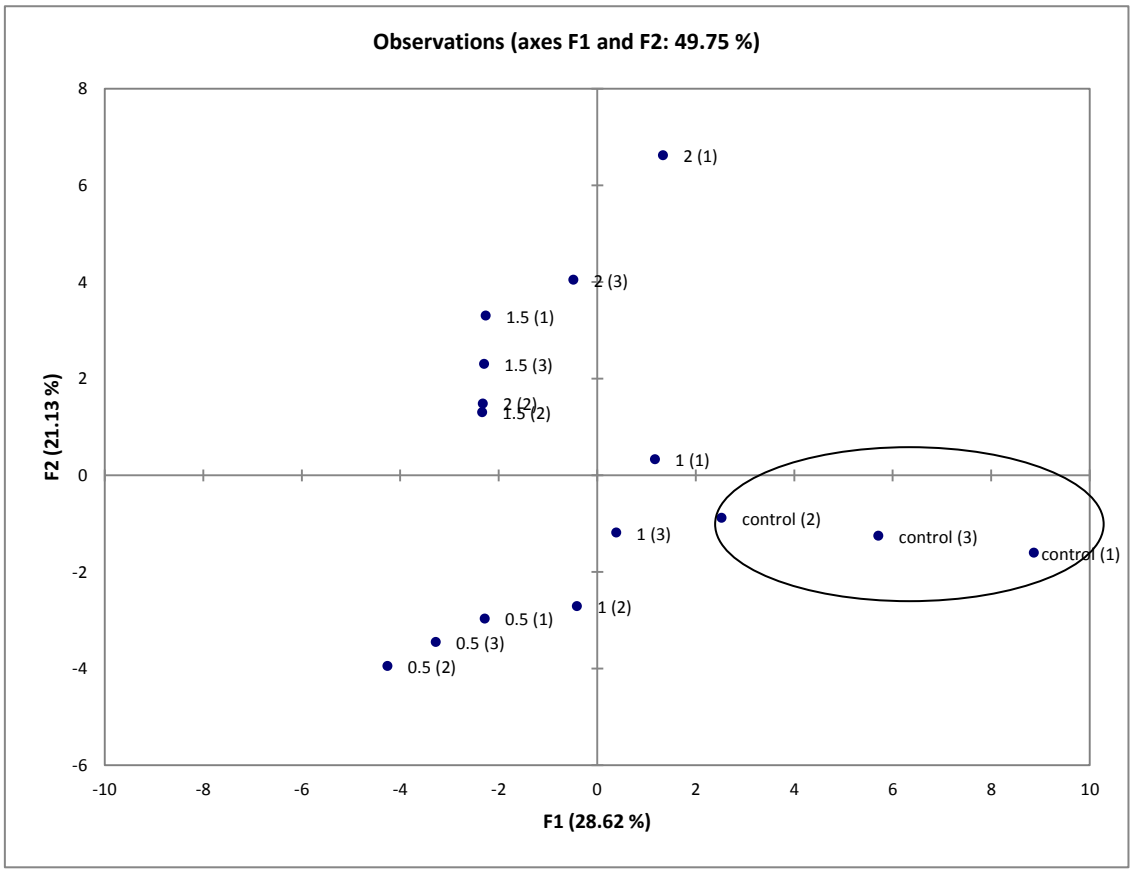


Figure 16. PC score plot for volatile profile data of Shiraz variety. PC score plots for free aroma volatiles of Cabernet Sauvignon variety. Numbers shown in figure represent various doses given to samples in kGy. Numbers in brackets signify different number of independent replicate. (3 statistically independent replicates were analyzed for each sample)



The content of hexanal and trans-2-hexenal increased by 70 and 290 percent, respectively, at a dose of 1 kGy in Chenin Blanc variety. In control Chenin Blanc grapes, the contents of hexanal and trans-2-hexenal was 495.91 ± 7.25 and 232.62 ± 16.98 µg kg $^{-1}$, respectively which increased to 844.42 ± 135.54 and 904.27 ± 135.07 µg kg $^{-1}$, respectively at radiation dose of 1 kGy. Similarly, content of hexanal and trans-2-hexenal

increased by 44 and 58 percent, respectively in Cabernet Sauvignon grapes at a dose of 1 kGy. Thus, extent of increase in the content of these compounds was significantly lower in Cabernet Sauvignon variety as compared to Chenin Blanc. However, surprisingly, no significant change was observed in content of these compounds in Shiraz grapes. Hexanal is characterized by green, grassy odor note, while *trans*-hex-2-enal possess fresh green and leafy aroma. They are reported to play a relatively important role in vegetable flavors (88). The above C6 aldehydes is known to be formed via the lipoxygense pathway from unsaturated fatty acid precursors namely linoleic and linolenic acids liberated mainly from galactolipids. Increased content of these aldehydes due to radiation processing was previously reported. UV irradiation of tomato fruits and leaves was shown to increase the production of n-hexanal as a result of enhanced lipooxygenase and hydroxyperoxidase lyase activity (89). Byun et al, 1995 (90) also reported an increased *trans*-hex-2-enal content in soybeans due to gamma irradiation at a dose above 10 kGy with as high as 5 times increase at 100 kGy. Fan and Sokorai, 2002 (91) on the other hand observed an increase in *trans*-hex-2-enal content of cilantro during post-harvest storage with no significant effect on the content of this compound on irradiation. An increase in content of trans-2-hexenal was also observed in irradiated cabbage (88). Thus, increase in trans-2-hexenal might be attributed to radiation induced lipid radiolysis resulting in release of linolenic acid and its subsequent converts to this compound via lipooxygenase (LOX) pathway. In the present study, it was also observed that content of 1-hexanol, the major alcohol detected decreased in a dose dependent manner in all three varieties. Its content decreases by 34 and 65 percent in Chenin Blanc and Cabernet Sauvignon grapes at dose

of 1 kGy. Radiation induced oxidation of hexanol and its subsequent conversion to hexenal is thus suggested.

Increase in content of hexanal and trans-2-hexenal was highest in Chenin Blanc followed by Cabernet Sauvignon. No increase was observed in Shiraz grapes. Chenin Blanc is a green grape variety and Cabernet Sauvignon and Shiraz are red grape varieties. It is well known that red varieties possess significantly higher antioxidant capacity as compared to white varieties due to the presence of anthocyanins. Presence of higher content of antioxidants could prevent radiation induced lipid peroxidation thereby resulting in less formation of hexanal and trans-2-hexenal.

In case of alcohols, an increased content of 3-methyl-butanol, 2-hexen-1-ol, 1-octen-3-ol and 1-heptanol was observed in Chenin Blanc. Glycosidic precursors of 3-methyl-butanol and 2-hexen-1-ol were not detected while no change was observed in precursors of other two alcohols i.e. 1-octen-3-ol and 1-heptanol. Similarly, the content of 1-heptanol, 1-octen-3-ol and 1-octanol increased in Shiraz variety while no decrease in glycosidic precursors of these compounds was observed. Thus observed increase might be due to increased extractability of these compounds due to radiation processing. However, surprisingly, no increase was observed in content of alcohols in Cabernet Sauvignon variety. Aroma notes of 3-methyl-butanol, 2-hexen-1-ol, 1-octen-3-ol, 1-heptanol and 1-octanol are reported to be whisky, green, lemon, herb and nut like respectively.

Among terpenes, a two fold increase in content of α-terpineol in Cabernet Sauvignon variety was observed up to 1 kGy which however, decreased at higher doses. A radiation induced breakdown of glycosidic precursor of α-terpineol observed in this variety could

explain this observed increase. Decrease at higher dose might be due to radiation induced degradation. Similarly, a 180, 124 and 22 percent higher content of linalool oxide, linalool and α-terpineol was also observed in Shiraz. Terpenes are major contributory compounds towards wine aroma. α-terpineol is known to have a mint odor note while both linalool oxide and linalool impart floral characteristics. No other major radiation induced changes in volatile composition were observed in all three varieties.

Thus, radiation processing resulted in significant changes in aroma profile of all three varieties studied. However, those changes were not similar. Content of hexanal and trans-2-hexenal increased in Chenin Blanc and Cabernet Sauvignon variety which could be attributed to radiation induced lipid peroxidation and oxidation of hexanol. Apart from this increased content of alcohols and terpineols was observed which could significantly influence subsequent wine aroma. Increased content of terpineols might result in enhanced fruity aroma in wines prepared with irradiated grapes.

3.2 Influence of radiation processing on wine quality

Grape wine is the most popular fruit wine consumed around the world with a history of over 5000 years (92). The aroma of a wine is one of the major factors that determine its quality and plays an important role in consumer preference (93). Wine aroma is a result of a combination of chemical compounds that influence its organoleptic characteristics. Over 800 volatile compounds in wines have been identified. Several classes of compounds, mainly alcohols, esters, aldehydes, acids, monoterpenes and other minor components are present in the volatile aroma of wine. The formation of these volatile compounds depends on various factors such as vineyard environmental factors (soil and climate), process of grape and juice production (grape de-stemming, crushing and pressing technology), and the fermentation and ageing procedure (94). Another important group of substances influencing various organoleptic properties such as color, astringency and bitterness of wine are the phenolic compounds (30). Phenolic compounds also have antioxidant properties and are responsible for health promoting properties of wine (31).

As phenolic composition influence the quality of red wine, a great effort has been devoted in recent years to develop different techniques to enhance their extraction during the wine making process. Various techniques proposed to enhance extraction of these compounds are increasing fermentation temperature, extending maceration time, heating grape berries for a short time, freezing grape berries before fermentation, and pulsed electric fields (32, 33). However, using these techniques could lead to wines with poor and unstable color characteristics (95).

Radiation processing is a promising technology for enhancing odor and aroma quality of wines. However, very few studies exists on radiation processing of wine. Gamma

irradiation of Cabernet Sauvignon red wine increased the chemical color age of wine with no perceivable differences in sensory quality up to a dose of 2400 Gy (96). It has also been reported previously that irradiation is a suitable method for improving the taste and quality of the rice and maize wines (66, 67). However, to the best of our knowledge, no report exists on quality evaluation of wine prepared with radiation processed grapes. The aim of the present study was therefore to investigate the effect of radiation processing of grape berries on the antioxidant and aroma quality of Shiraz, Cabernet Sauvignon and Chenin Blanc wines.

3.2.1 Basic chemical parameters of wines

In order to study the effect of radiation processing of grapes on wine, the wines prepared with irradiated grapes were compared to those prepared with non-irradiated grapes. Alcohol and reducing sugar content of control and irradiated wines is shown in Table 11. Wines prepared with control and irradiated grapes had final reducing sugars content of < 1.5 % and alcohol content of more than 10% for all the three varieties. No statistically significant ($p<0.05$) effect of radiation processing was observed on alcohol or reducing sugar content of the wine. pH of the wines was also found to be not significantly ($p<0.05$) affected by radiation processing of grapes (Table 11).

Table 11. Effect of irradiation on ethanol concentration, reducing sugars and pH of wines

	Control	500 Gy	1000 Gy	1500 Gy	2000 Gy
Cabernet Sauvignon wines					
Ethanol (% v/v)	10.55 ± 0.12^a	10.52 ± 0.13^a	10.75 ± 0.5^a	11.0 ± 0.27^a	10.71 ± 0.48^a
Reducing Sugars (g L^{-1})	1.06 ± 0.1^a	1.16 ± 0.05^a	1.16 ± 0.07^a	1.17 ± 0.07^a	1.18 ± 0.06^a
pH	3.3 ± 0.1^a	3.4 ± 0.1^a	3.3 ± 0.1^a	3.4 ± 0.1^a	3.5 ± 0.1^a
Shiraz Wines					
Ethanol (% v/v)	12.21 ± 0.58^a	12.64 ± 0.42^a	12.0 ± 0.2^a	12.48 ± 0.27^a	12.29 ± 0.17^a
Reducing Sugars (g L^{-1})	1.16 ± 0.1^a	1.19 ± 0.08^a	1.1 ± 0.05^a	1.12 ± 0.08^a	1.14 ± 0.05^a
pH	3.2 ± 0.1^a	3.1 ± 0.1^a	3.1 ± 0.1^a	3.0 ± 0.1^a	3.2 ± 0.1^a
Chenin Blanc Wines					
Ethanol (% v/v)	12.1 ± 0.5^a	12.04 ± 0.12^a	12.2 ± 0.26^a	12.35 ± 0.37^a	12.19 ± 0.17^a
Reducing Sugars (g L^{-1})	1.1 ± 0.1^a	1.2 ± 0.2^a	1.1 ± 0.15^a	1.12 ± 0.16^a	1.16 ± 0.15^a
pH	3.5 ± 0.1^a	3.4 ± 0.1^a	3.5 ± 0.1^a	3.5 ± 0.1^a	3.5 ± 0.1^a

Values with same superscript letter in a row are not statistically significantly (0.05) different

3.2.2. Effect of radiation on chromatic characteristics, total phenolics, antioxidant activity, and anthocyanin content of wine.

Data for chromatic characteristics of wine is shown in Table 12. In case of Chenin Blanc wines, which is a white wine a significant ($p<0.05$) radiation dose dependent increase in color intensity up to 1500 Gy with no statistical change thereafter at the higher dose of 2000 Gy was noted. Post maturation, control wine had a color intensity of 0.35 ± 0.04 which increased to 0.54 ± 0.02 at a dose of 1500 Gy. The tint value indicates a relative importance of yellow color on red color (95). Tint value decreased from 5.07 ± 0.04 in control wine to 4.13 ± 0.02 at a dose of 0.5 kGy, however no statistical significant change was observed at higher doses. These results indicate that there is a decrease in yellow color with increase in brown color in white wines due to radiation processing.

Table 12. Effect of radiation processing on chromatic characteristics of wine

	Control	500 Gy	1000 Gy	1500 Gy	2000 Gy
Cabernet Sauvignon wines					
Just after preparation					
Color intensity	10.69 ± 0.15^{a}	14.5 ± 0.15^{b}	15.06 ± 0.15^{b}	20 ± 0.15^{c}	9.18 ± 0.2^{a}
Tint	0.54 ± 0.04^{a}	0.53 ± 0.02^{a}	0.55 ± 0.05^{a}	0.51 ± 0.04^{a}	0.58 ± 0.05^{a}
L	52.88 ± 1.07^{a}	44.06 ± 0.64^{b}	43.44 ± 0.41^{b}	32.13 ± 0.41^{c}	57.19 ± 0.78^{d}
a	53.04 ± 0.95^{a}	59.86 ± 0.67^{b}	58.68 ± 1.12^{b}	57.22 ± 0.44^{b}	46.36 ± 0.68^{c}
b	8.28 ± 2.6^{a}	12.49 ± 0.21^{b}	13 ± 0.42^{b}	13.11 ± 0.44^{b}	7.03 ± 0.06^{a}
Anthocyanin content	61.26 ± 1.61^{a}	82.41 ± 11.32^{b}	91.56 ± 7.28^{b}	120.8 ± 14.21^{c}	58.61 ± 6.02^{a}
After maturation					
Color intensity	11.29 ± 0.17^{a}	13.47 ± 0.2^{b}	13.79 ± 0.17^{b}	16.59 ± 0.17^{c}	10.25 ± 0.24^{a}
Tint	0.62 ± 0.04^{a}	0.62 ± 0.03^{a}	0.59 ± 0.03^{a}	0.61 ± 0.02^{a}	0.61 ± 0.02^{a}
L	50.53 ± 1.07^{a}	46.59 ± 0.64^{b}	45.4 ± 0.41^{b}	40.57 ± 0.41^{c}	56.72 ± 0.78^{d}
a	49.88 ± 0.95^{a}	53.91 ± 0.67^{b}	53.92 ± 1.12^{b}	56.81 ± 0.44^{c}	43.83 ± 0.68^{d}
b	21.26 ± 2.6^{a}	21.83 ± 0.21^{a}	21 ± 0.42^{a}	24.48 ± 0.44^{a}	7.03 ± 0.06^{b}
Anthocyanin content	41.33 ± 3.01^{a}	59.22 ± 3.92^{b}	57.8 ± 0.93^{b}	73.26 ± 0.93^{c}	39.33 ± 3.68^{a}
Shiraz Wines					
Just after preparation					
Color intensity	7.7 ± 0.15^{a}	8.8 ± 0.15^{b}	9.45 ± 0.15^{b}	11.05 ± 0.15^{c}	9.7 ± 0.2^{b}
Tint	0.70 ± 0.05^{a}	0.64 ± 0.03^{a}	0.65 ± 0.04^{a}	0.67 ± 0.03^{a}	0.67 ± 0.04^{a}
L	52.97 ± 0.05^{a}	50.5 ± 0.29^{b}	49.51 ± 1.22^{b}	43.17 ± 1.02^{c}	48.79 ± 0.23^{b}
a	45.84 ± 0.06^{a}	47.28 ± 0.08^{b}	50.26 ± 0.71^{c}	50.28 ± 0.32^{c}	47.66 ± 0.03^{b}
b	14.12 ± 0.06^{a}	16.94 ± 0.03^{a}	9.06 ± 0.06^{b}	20.57 ± 0.1^{c}	16.65 ± 0.02^{a}
Anthocyanin content	98.88 ± 2.89^{a}	111 ± 3.08^{b}	112.3 ± 2.53^{b}	127.1 ± 1.43^{c}	110.27 ± 1.16^{b}
After maturation					
Color intensity	7.73 ± 0.2^{a}	8.68 ± 0.17^{b}	9.47 ± 0.17^{b}	11.06 ± 0.17^{c}	9.34 ± 0.24^{b}
Tint	0.53 ± 0.02^{a}	0.52 ± 0.02^{a}	0.49 ± 0.03^{a}	0.52 ± 0.1^{a}	0.50 ± 0.1^{a}
L	61.48 ± 0.64^{a}	60.32 ± 1.07^{a}	56.47 ± 0.41^{b}	51.91 ± 0.41^{c}	54.8 ± 0.78^{b}
a	47.78 ± 0.67^{a}	48.26 ± 0.95^{a}	52.81 ± 1.12^{b}	55.4 ± 0.44^{c}	54.61 ± 0.68^{c}
b	5.01 ± 2.6^{a}	2.45 ± 0.21^{b}	4.98 ± 0.42^{a}	7.74 ± 0.44^{c}	4.93 ± 0.06^{b}
Anthocyanin content	42.95 ± 1.22^{a}	50.56 ± 1.22^{b}	58.75 ± 0.31^{c}	62.17 ± 0.92^{c}	49.97 ± 0.31^{b}
Chenin Blanc Wines					
Just after preparation					
Color intensity	0.35 ± 0.02^{a}	0.36 ± 0.02^{a}	0.45 ± 0.04^{a}	0.52 ± 0.02^{b}	0.51 ± 0.01^{b}
Tint	5.04 ± 0.02^{a}	4.1 ± 0.02^{b}	4.2 ± 0.04^{b}	4.04 ± 0.04^{b}	4.02 ± 0.04^{b}
L	93.05 ± 0.13^{a}	94.26 ± 0.41^{a}	93.61 ± 0.77^{a}	92.17 ± 0.52^{a}	92.53 ± 0.33^{a}
a	-25.15 ± 0.8^{a}	-4.1 ± 0.1^{b}	4.35 ± 0.13^{b}	-6.1 ± 1.1^{b}	-4.09 ± 0.02^{b}
b	112.5 ± 2.01^{a}	28.2 ± 0.61^{b}	34.3 ± 2.17^{b}	31.44 ± 4.3^{b}	31.04 ± 4.29^{b}
After maturation					
Color intensity	0.35 ± 0.04^{a}	0.4 ± 0.02^{a}	0.42 ± 0.05^{a}	0.54 ± 0.02^{b}	0.51 ± 0.01^{b}
Tint	5.07 ± 0.04^{a}	4.13 ± 0.02^{b}	4.25 ± 0.04^{b}	4.06 ± 0.04^{b}	4.08 ± 0.04^{b}
L	93.05 ± 0.13^{a}	94.26 ± 0.41^{a}	93.61 ± 0.77^{a}	92.17 ± 0.52^{a}	92.53 ± 0.33^{a}
a	-20.15 ± 0.8^{a}	-2.61 ± 0.18^{b}	-2.53 ± 0.11^{b}	-3.11 ± 1.6^{b}	-2.05 ± 1.02^{b}
b	115.5 ± 2.11^{a}	18.51 ± 0.71^{b}	23.4 ± 4.17^{b}	21.22 ± 4.33^{b}	21.94 ± 1.29^{b}

Values with same superscript letter in a row are not statistically significantly ($p<0.05$) different.

This is further confirmed by data obtained for CIELAB parameters for control and irradiated white wines. In CIELAB parameters, L* values indicate lightness or darkness of samples while a* values signifies greenness or redness and b* values depicts yellowness or blueness of samples. Post maturation control white wines had a* value of -20.15 ± 0.8 which increased to -2.61 ± 0.18 on radiation dose of 0.5 kGy. A significant ($p<0.05$) decrease in the b* values from 115.5 ± 2.11 (control) to 18.51 ± 0.71 (0.5 kGy) was also observed. These results further confirm radiation induced browning in white wines. Browning in white wines might be caused due to increased extraction of phenolics due to radiation processing. Browning is not a desirable characteristic of white wines; therefore irradiated white wines were not taken up for further studies.

In case of red wines, a significant ($p<0.05$) radiation dose dependent increase in color intensity was obtained with doses up to 1500 Gy with a reduction thereafter at the higher dose of 2000 Gy for both the varieties. Post maturation, control Cabernet Sauvignon and Shiraz wines had a color intensity of 11.29 ± 0.17 and 7.73 ± 0.2 which increased to 16.59 ± 0.17 and 11.06 ± 0.17 respectively, at a dose of 1500 Gy. The higher color intensity (CI) of wines prepared with irradiated grapes was probably due to increased extraction of oligomeric and polymeric pigments caused by the higher extraction of phenolic compounds. No significant ($p<0.05$) change was observed in the tint values due to radiation processing in both the varieties. Wines prepared with irradiated grapes demonstrated a significant ($p<0.05$) decrease in L* values with increase in a* values in comparison with control up to 1500 Gy (Table 12). However, no trend was observed for b* values. Lower L* and higher a* values obtained for irradiated samples as compared to

control indicate that these wines were darker and have increased redness as compared to control wines.

Increased redness of wines prepared with irradiated grapes might have resulted from increased extraction of anthocyanins during maceration. Therefore, the content of anthocyanins was further investigated. Data for anthocyanin content is shown in Table 10. A significant ($p<0.05$) dose dependent increase in anthocyanin content up to a dose of 1500 Gy in both the varieties was observed. Post maturation 77 and 47 % higher anthocyanin content were observed in Cabernet Sauvignon and Shiraz wines, respectively, at 1500 Gy as compared to their corresponding controls. The increased anthocyanin yield from grape pomace as a result of radiation processing has been reported earlier (56). This increase was attributed to increased membrane and cell wall degradation resulting in higher release of pigments. The increased redness and darkness observed in irradiated wines can be attributed to increased extraction of anthocyanins due to radiation processing.

Results for total phenolic content and antioxidant capacity for Cabernet Sauvignon and Shiraz wines are shown in Figure. 17 and 18, respectively.

Figure 17. Total antioxidant and phenolic content in wine prepared from control and irradiated grapes (Shiraz variety). **(A)** Total antioxidant activity by DPPH assay. **(B)** Total antioxidant activity by FRAP assay. **(C)** Total phenolic content by Folins-ciocalteu method.

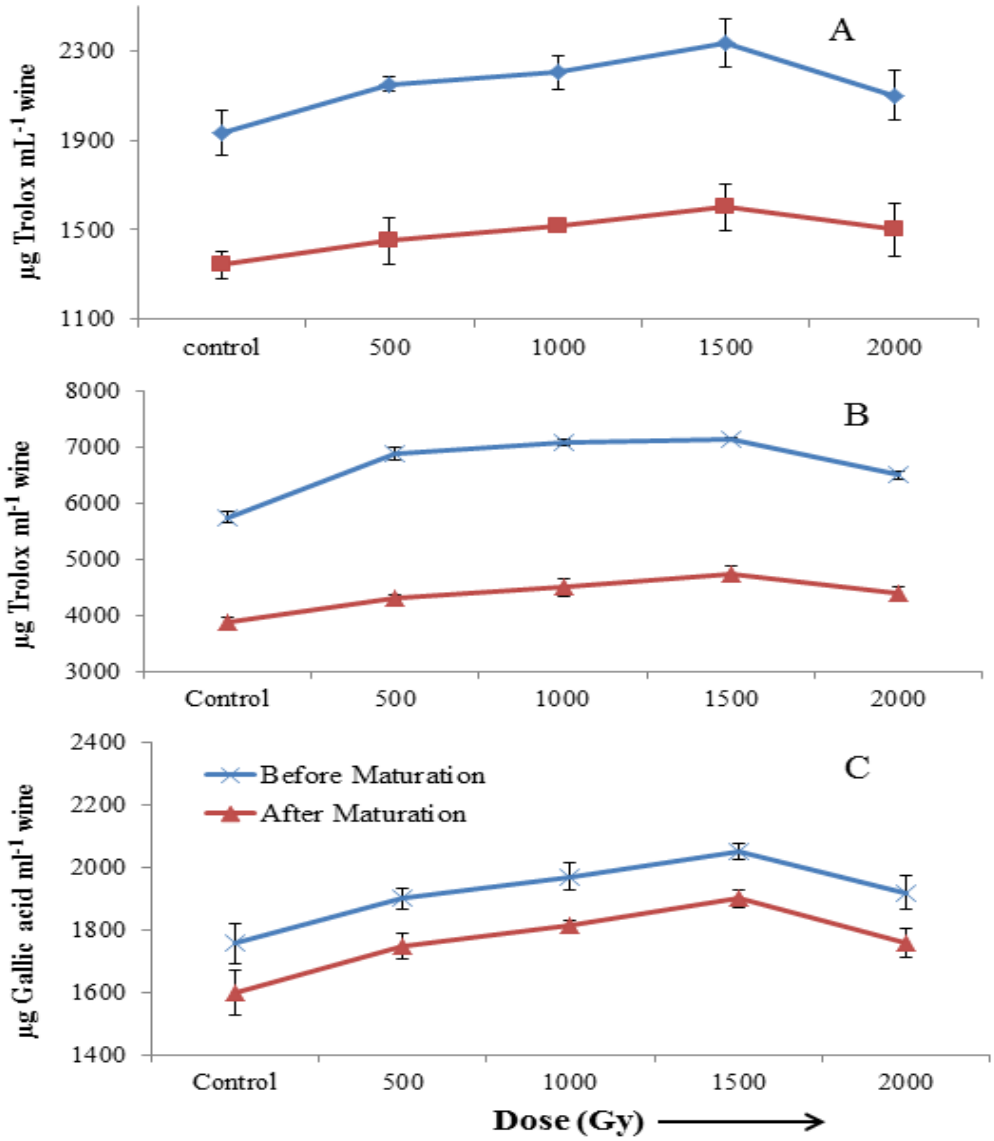
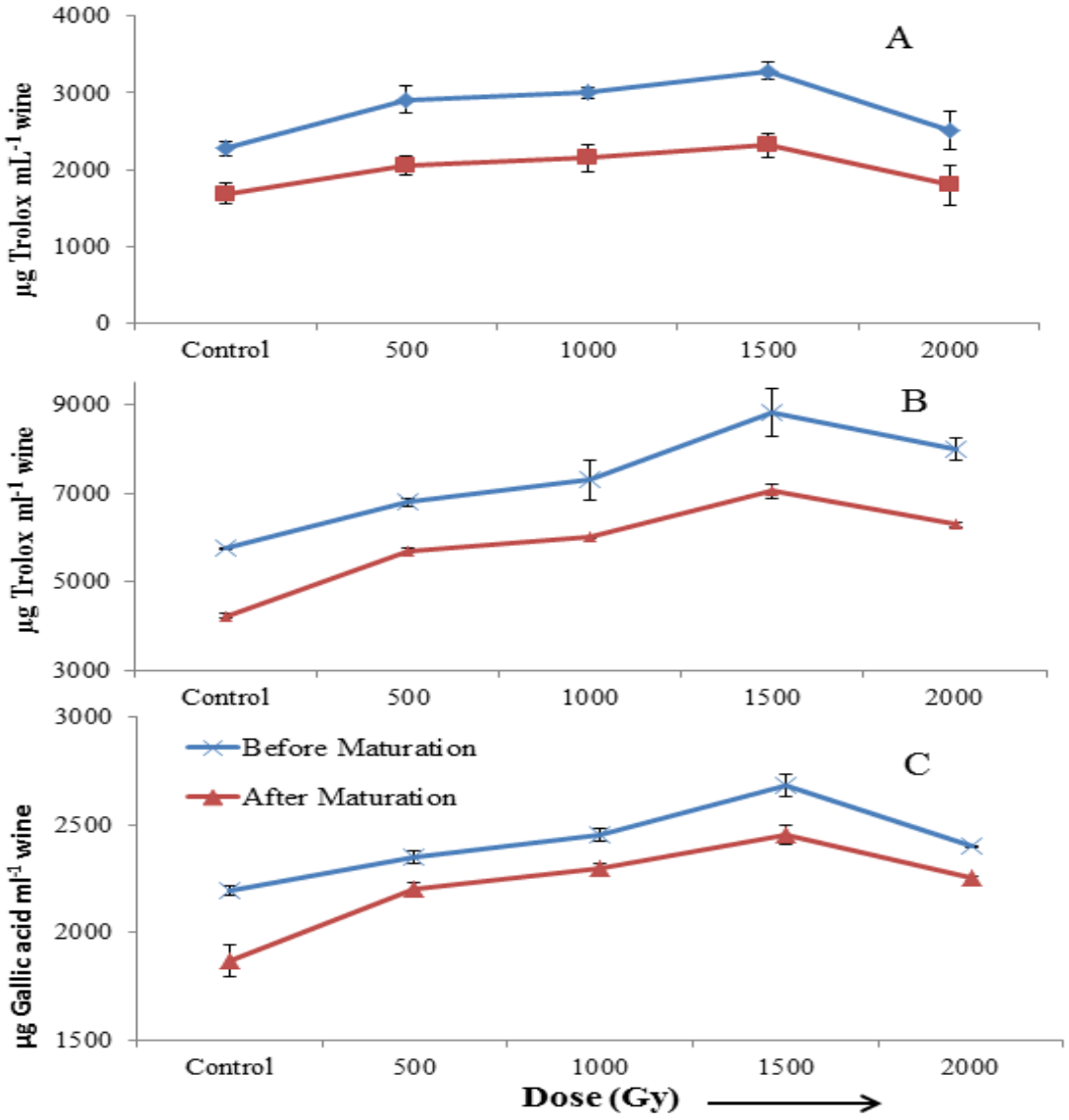


Figure 18. Total antioxidant and phenolic content in wine prepared from control and irradiated grapes (Cabernet Sauvignon variety). (A) Total antioxidant activity by DPPH assay. (B) Total antioxidant activity by FRAP assay. (C) Total phenolic content by Folins-ciocalteu method.



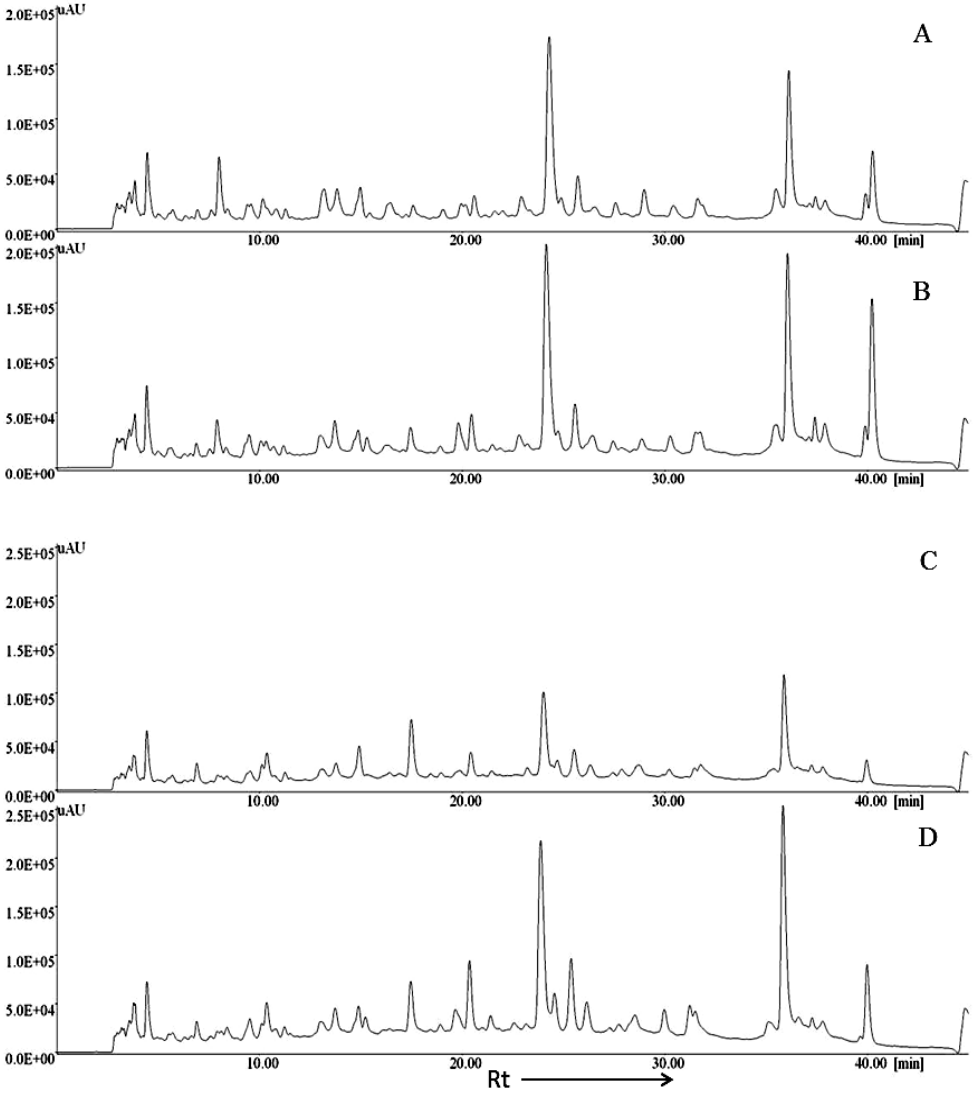
A significant ($p<0.05$) radiation dose dependent increase in phenolic and antioxidant content of both the wines was observed up to a dose of 1500 Gy with a reduction thereafter. Wines prepared with grapes irradiated at a dose of 1500 Gy demonstrated 18

and 31 % higher total phenolic content for Shiraz and Cabernet Sauvignon wines, respectively, as compared to the control wine, post maturation (Figure 17A and 18A). Similar results were also obtained for total antioxidant capacity (Figure 17B and C, 18B and C). A 19 and 22 percent higher antioxidant activity was obtained in Shiraz wines at 1500 Gy as compared to control when analyzed by DPPH and FRAP assays, respectively. Cabernet Sauvignon wines also demonstrated a 37 and 66 percent increase in total antioxidant activity for DPPH and FRAP assays, respectively at a dose of 1500 Gy. Phenolic compounds are mainly responsible for providing antioxidant characteristics to wine and increased phenolic content leads to increased antioxidant properties (31, 97). Therefore, it can be inferred from the above results that increased antioxidant activity was due to the higher proportion of phenolic compounds present in wine samples prepared from irradiated grapes as compared to the control.

It was also noted in the present study that wines just after vinification process had significantly ($p<0.05$) higher total phenolics and antioxidant content as compared to samples after completion of the maturation process (Figure 17 and 18). This reduction in phenolic content during maturation has been attributed to different phenomena that occur simultaneously, such as oxidation and condensation reactions of anthocyanins with other polyphenols and precipitation of anthocyanins and phenolics in the lees (32, 98). Interestingly, wines prepared from irradiated grapes retained higher phenolics and antioxidant properties in comparison to non-irradiated samples even post maturation.

To examine the effects of radiation processing on the qualitative and quantitative composition of individual phenolic compounds, both control and irradiated wine samples were analyzed by HPLC. Figure 19 demonstrates HPLC chromatograms of control and

Figure 19. HPLC chromatograms of wines prepared with control and radiation processed (1500 Gy) grapes. **(A)** Control Shiraz wine. **(B)** Wine prepared from radiation processed (1500 Gy) grapes (Shiraz variety). **(C)** Control Cabernet Sauvignon wine. **(D)** Wine prepared from radiation processed (1500 Gy) grapes (Cabernet Sauvignon variety).



wines prepared with irradiated grapes for both the varieties. No qualitative changes in phenolic composition of wines could be observed as a result of radiation processing.

Amount of various phenolic compounds identified in control and irradiated (1500 Gy) wines is shown in Table 13.

Table 13. Content of phenolic compounds identified in wines using HPLC

	Cabernet Sauvignon		Shiraz	
	Control µg/ml	1500 Gy µg/ml	Control µg/ml	1500 Gy µg/ml
Phenolics				
Gallic acid	10.67 ± 0.35[a]	12.02 ± 0.65[b]	13.06 ± 1.06[a]	13.86 ± 1.36[a]
Catechin	3.09 ± 0.25[a]	5.01 ± 0.25[b]	2.7 ± 0.27[a]	6.19 ± 0.6[b]
Caffeic acid	1.92 ± 0.02[a]	2.68 ± 0.15[b]	3.67 ± 0.5[a]	5.81 ± 0.35[b]
Epicatechin	25.38 ± 1.2[a]	42.9 ± 2.4[b]	49.64 ± 5.1[a]	48.12 ± 6.12[a]
Flavonols				
Quercitin	0.26 ± 0.01[a]	2.74 ± 0.15[b]	0.58 ± 0.12[a]	0.93 ± 0.2[b]
Anthocyanins				
Delphinidin 3-monoglucoside	1.78 ± 0.25[a]	14.36 ± 1.12[b]	4.3 ± 0.25[a]	12.82 ± 1.2[b]
Cyanidin 3-monoglucoside	2.61 ± 0.21[a]	17.53 ± 1.5[b]	11.36 ± 1.36[a]	24.2 ± 2.1[b]
Petunidin 3-monoglucoside	0.42 ± 0.01[a]	0.89 ± 0.1[b]	1.09 ± 0.25[a]	1.18 ± 0.2[a]
Peonidin 3-monoglucoside	1.02 ± 0.05[a]	6.09 ± 0.45[b]	12.12 ± 2.63[a]	9.79 ± 1.2[a]
Malvidin 3-monoglucoside	117.28 ± 11.1[a]	267.32 ± 21.4[b]	222.47 ± 8.5[a]	262.96 ± 11.2[b]
Delphinidin 3-monoglucoside-Ac	5.07 ± 0.5[a]	13.45 ± 0.89[b]	3.4 ± 0.74[a]	9.34 ± 0.85[b]
Cyanidin 3-monoglucoside-Ac	9.95 ± 0.7[a]	15.85 ± 0.54[b]	3.98 ± 0.61[a]	5.6 ± 0.6[b]
Petunidin 3-monoglucoside-Ac	0.8 ± 0.1[a]	7.06 ± 0.62[b]	3.7 ± 0.15[a]	10.4 ± 1.2[b]
Peonidin 3-monoglucoside-Ac	2.55 ± 0.25[a]	2.28 ± 0.03[a]	15.85 ± 1.5[a]	17.01 ± 2.1[a]
Malvidin 3-monoglucoside-Ac	111.61 ± 10.2[a]	272.37 ± 26.5[b]	136.43 ± 10.25[a]	191.82 ± 15.2[b]

Values with same superscript letter in a row are not statistically significantly ($p<0.05$) different

An increased content of phenolic compounds in irradiated (1500 Gy) samples as compared to control is clearly observed for both the varieties. The increase in content of gallic acid, caffeic acid, catechin and epicatechin in irradiated wine (1500 Gy) varied from zero to 70 percent for both varieties. The contents of all four compounds increased in irradiated Cabernet Sauvignon wines, however, gallic acid and epicatechin did not significantly ($p<0.05$) change in Shiraz wines. An increased content of quercitin, a flavonol, was observed in irradiated samples from both varieties. With regard to the major anthocyanins malvidin-3-monoglucoside and malvidin-3-monoglucoside-Ac an increase of 127 and 144, percent, respectively, was observed in irradiated (1500 Gy) wines for Cabernet Sauvignon variety. However, in the irradiated (1500 Gy) Shiraz wines this increase was only 18 and 40 percent, respectively for these compounds (Table 13). Similar observations on increased extraction of various phenolic compounds without qualitative changes have also been reported in wine prepared with musts treated with pulsed electric fields (95).

Phenolic compounds and more specifically anthocyanins are present in vacuoles of hypodermal cells (33). Radiation processing might have resulted in a breakdown of the vacuole membrane, thus increasing the release of phenolic compounds in wine. There is no information available in literature on the effect of radiation processing of grapes on the phenolic content of wine. However, for other plant materials, ability of gamma irradiation to increase phenolic content has been reported previously for several products such as nutmeg, almond seed extract, seeds of Nigella staiva, carrot and kale juice. These increases in phenolic contents were associated with the degradation of tannins and

polyphenols as a result of the radiation treatment, thereby releasing soluble phenolics of low molecular weight resulting in increased extraction yields (54).

Several different techniques are employed for preparation of wines with enhanced phenolic content. In wines prepared after pulsed electric field treatment of musts 23 and 34% higher total phenolic content and antioxidant potential was reported (95). Wines treated with enological condensed tannins were reported to have 16% higher total phenolics and freezing grape berries before winemaking has resulted in wines with 52% higher tannins and 50% more anthocyanins (33). In the present study, gamma radiation (1500 Gy) resulted in increased total phenolic content by 31 and 20% for Cabernet Sauvignon and Shiraz wines when analyzed after maturation. Thus, it can be concluded that the increase in total phenolics and antioxidant activity by gamma radiation is comparable to the increase obtained by other previously used techniques. Moreover, employing gamma radiation in large scale can be much easier than some of the other techniques such as freezing or pulsed electric fields since large scale commercial radiators are available. These results show that the use of gamma irradiation for increasing phenolic and antioxidant content can be a practical proposition.

3.2.3. Effect of radiation on volatile aroma constituents and sensory characteristics of wine

Aroma of wine is a major attribute deciding its consumer preference. Volatile compounds identified from both Shiraz and Cabernet Sauvignon wines are shown in Table 14 and 15, respectively. Sixty two aroma volatiles were identified from Shiraz wines while in Cabernet Sauvignon wines presence of sixty nine compounds was observed. Volatile

profiles of Shiraz (99) and Cabernet Sauvignon (93) wines were similar to the previously reported data.

Table 14. Volatile aroma compounds of control and irradiated Shiraz wine

S.No	Compound name	Rt	Control µg L^{-1}	500 Gy µg L^{-1}	1000 Gy µg L^{-1}	1500 Gy µg L^{-1}	2000 Gy µg L^{-1}
Alcohols							
1	Propanol	1.867	14.98 ± 1.48	15.43 ± 1.6	18.98 ± 1.83	16.94 ± 1.66	23.01 ± 1.83
2	2-Methyl-1-propanol	2.313	134.84 ± 17.39	123.2 ± 12.53	136.6 ± 11.03	167.32 ± 15.68	145.55 ± 12.67
3	1-Butanol	2.711	10.86 ± 0.96	12.17 ± 1.46	10.44 ± 1.44	12.35 ± 1.45	11.77 ± 0.65
4	3-Methyl-1-butanol	4.242	2959.07 ± 300.47	2776.26 ± 219.52	2741.6 ± 260.6	3437.18 ± 288.82	3066.09 ± 174.45
5	2-methyl-1-butanol	4.271	113.85 ± 8.55	118.83 ± 2.79	93.95 ± 24.95	128.62 ± 0.98	105.54 ± 21.36
6	1-Pentanol	4.97	0.96 ± 0.06	0.93 ± 0.04	0.92 ± 0.07	0.85 ± 0.25	1.07 ± 0.01
7	2,3-Butanediol	5.428	13.02 ± 0.87	13.06 ± 0.56	14.85 ± 1.99	10.31 ± 0.49	12.04 ± 1.46
8	2,3-Butanediol	5.765	6.94 ± 0.26	6.53 ± 0.28	7.64 ± 0.78	6.33 ± 0.87	4.29 ± 1.11
9	4-Methyl-1-pentanol	7.428	3.6 ± 0	4.4 ± 0.51	4.59 ± 0.13	4.91 ± 1.09	3.48 ± 0.3
10	3-Methyl-1-pentanol	7.734	20.25 ± 1.35	23.79 ± 1.03	16.38 ± 0.96	24.93 ± 1.47	18.19 ± 1.25
11	Trans-3-Hexene-1-ol	8.035	2.6 ± 0.55	2.13 ± 0.79	3.66 ± 0.19	3.98 ± 0.38	5.62 ± 0.32
12	cis-3-Hexene-1-ol	8.161	13.63 ± 2.83	11.46 ± 1.19	10.16 ± 0.74	5.73 ± 0.27	15.25 ± 0.95
13	1-Hexanol	8.797	131.21 ± 9.71	133.79 ± 9.72	150.74 ± 12.74	143.84 ± 8.56	194.43 ± 8.61
14	2-Heptanol	10.12	1.19 ± 0.16	1.16 ± 0.18	1.39 ± 0.1	1.15 ± 0.05	1.07 ± 0.01
15	1-Heptanol	13.224	67.24 ± 10.16	89.76 ± 19.7	77.64 ± 0.36	82.34 ± 4.94	95.14 ± 3.34
16	1-Octen-3-ol	13.59	1.45 ± 0.1	1.4 ± 0.06	0.92 ± 0.07	1.75 ± 0.65	1.07 ± 0.01
17	2-Octanol	14.54	54 ± 0	54 ± 0	54 ± 0	54 ± 0	54 ± 0
18	2-Ethyl hexanol	15.797	4.05 ± 0.45	6.69 ± 1.34	6.68 ± 0.25	7.99 ± 0.19	8.28 ± 0.72
19	1-Octanol	17.618	8.42 ± 0.32	8.6 ± 0.33	7.64 ± 0.78	11.4 ± 0.6	9.61 ± 0.97
20	2-Nonanol	18.858	0.48 ± 0.03	0.47 ± 0.02	0.46 ± 0.03	0.57 ± 0.03	0.53 ± 0.01
21	Ho-trienol	18.957	1.22 ± 0.32	0.71 ± 0.26	1.39 ± 0.1	1.72 ± 0.08	2.14 ± 0.02
22	1-Nonanol	21.777	10.09 ± 0.19	9.08 ± 0.16	9.68 ± 2.75	8.59 ± 0.41	8.02 ± 0.46
23	1-Decanol	25.682	0.96 ± 0.06	1.18 ± 0.28	0.92 ± 0.07	1.72 ± 0.08	0.53 ± 0.01

	Aromatic alcohols						
24	Benzyl alcohol	16.037	1.19 ± 0.16	0.93 ± 0.04	1.67 ± 0.81	2.02 ± 0.38	1.07 ± 0.01
25	Phenylethyl alcohol	19.466	233.78 ± 18.23	302.29 ± 30.95	187.16 ± 33.3	562.58 ± 36.22	314.81 ± 10.25
	Terpene and related compounds						
26	Linalool	18.763	7.65 ± 0.45	7.18 ± 0.85	7.57 ± 0.14	7.39 ± 0.79	4.82 ± 1.12
27	.beta.-Citronellol	24.037	1.45 ± 0.1	1.87 ± 0.08	1.63 ± 0.35	1.99 ± 0.19	2.41 ± 0.29
28	l-Limonene	15.566	1.45 ± 0.1	4.66 ± 0.2	6.93 ± 0.5	0.87 ± 0.33	9.35 ± 0.17
	Short chain organic acids						
29	Iso-valeric acid	8.536	4.4 ± 1.25	5.13 ± 0.22	5.33 ± 0.62	6 ± 0	8.3 ± 1.42
30	Decanoic acid	29.378	5.08 ± 0.58	7.18 ± 0.85	6.11 ± 1.82	14.29 ± 0.11	14.17 ± 0.13
	Esters						
31	Ethyl Acetate	2.169	221.66 ± 16.46	191.9 ± 13.39	236.97 ± 23.12	185.51 ± 13.69	240.18 ± 12
32	Ethyl Propionate	3.395	2.64 ± 0.06	2.56 ± 0.12	2.99 ± 0.01	2.56 ± 0.16	2.67 ± 0.03
33	2-methyl-propanoic acid ethyl ester	4.575	5.46 ± 2.76	7.24 ± 0.54	4.54 ± 2.4	2.29 ± 0.11	5.64 ± 3
34	Ethyl butyrate	5.928	12.44 ± 0.61	12.82 ± 0.32	12.72 ± 1.15	13.12 ± 0.52	9.66 ± 3.3
35	2-hydroxy-propanoic acid ethyl ester	6.479	5.59 ± 1.09	8.19 ± 1.05	8.6 ± 1.31	6.6 ± 0.6	8.3 ± 0.88
36	Hexanoic acid, methyl ester	11.119	1.64 ± 0.61	1.16 ± 0.18	1.39 ± 0.1	1.12 ± 0.52	1.6 ± 0.02
37	Hexanoic acid, ethyl ester	14.489	150.56 ± 45.64	127.58 ± 26.39	147.04 ± 32.1	113.02 ± 32.62	141.81 ± 41.37
38	Octanoic acid, methyl ester	19.819	5.01 ± 0.39	4.85 ± 0.95	9.37 ± 3.92	6.85 ± 0.25	4.81 ± 0.49
39	Butanedioic acid, diethyl ester	22.188	19.9 ± 3.25	18.58 ± 6.41	18.18 ± 3.61	14.37 ± 1.83	29.96 ± 1.9
40	Ethyl octanoate	22.821	310.08 ± 53.97	305.3 ± 88.32	313.79 ± 20.5	326.26 ± 60.46	412.07 ± 96.17
41	Phenethyl acetate	25.12	1.96 ± 0.61	2.09 ± 0.14	2.99 ± 0.01	6.87 ± 0.33	8.83 ± 0.89
42	Nonanoic acid, ethyl ester	26.555	0.96 ± 0.06	0.93 ± 0.04	0.92 ± 0.07	1.15 ± 0.05	1.07 ± 0.01
43	Decanoic acid, methyl ester	27.583	1.19 ± 0.16	1.18 ± 0.28	0.92 ± 0.07	1.42 ± 0.22	1.6 ± 0.02
44	Ethyl 9-decenoate	29.855	14.88 ± 0.03	15.59 ± 0.03	9 ± 6.86	23.89 ± 2.29	24.28 ± 4.84
45	Decanoic acid, ethyl ester	30.148	66.92 ± 3.02	85.45 ± 0.66	62.45 ± 2.45	92.51 ± 10.69	45.32 ± 41.62

46	Ethyl 3-methylbutyl butanedioate	31.355	0.71 ± 0.19	1.18 ± 0.28	0.92 ± 0.07	1.15 ± 0.05	0.8 ± 0.26
47	Isoamyl Octanoate	31.877	1.7 ± 0.35	1.16 ± 0.18	1.39 ± 0.1	1.17 ± 0.63	1.87 ± 0.25
48	Dodecanoic acid, ethyl ester	36.748	11.8 ± 0.55	13.53 ± 0.58	12.49 ± 2.08	13.45 ± 0.35	18.42 ± 2.22
49	3-Methylbutyl decanoate	38.306	1.48 ± 0.58	0.69 ± 0.2	0.68 ± 0.18	0.57 ± 0.03	1.07 ± 0.01
50	Tetradecanoic acid, ethyl ester	42.724	0.71 ± 0.19	0.69 ± 0.2	0.46 ± 0.03	0.57 ± 0.03	0.53 ± 0.01
51	Hexadecanoic acid, ethyl ester	48.453	1.19 ± 0.16	1.62 ± 0.16	1.39 ± 0.1	1.72 ± 0.08	1.6 ± 0.02
	Aldehydes and ketones						
52	2,3-Butanedione	1.984	11.38 ± 1.48	8.88 ± 0.85	20.64 ± 2.64	14.35 ± 1.25	15.52 ± 1.22
53	3-Methylbutanal	2.55	0.96 ± 0.06	0.93 ± 0.04	0.92 ± 0.07	1.15 ± 0.05	1.07 ± 0.01
54	2,3-Pentanedione	3.07	19.09 ± 1.99	15.39 ± 0.66	24.89 ± 7.32	21.76 ± 1.04	17.92 ± 0.98
55	2-Heptanone	9.639	0.48 ± 0.03	2.66 ± 2.21	0.46 ± 0.03	0.57 ± 0.03	0.53 ± 0.01
56	Benzaldehyde	12.584	3.63 ± 0.48	3.51 ± 0.38	0.92 ± 0.07	1.15 ± 0.05	0.8 ± 0.28
57	2-Methyltetrahydrothiophen-3-one	13.818	1.22 ± 0.32	1.87 ± 0.08	1.63 ± 0.35	1.72 ± 0.08	3.46 ± 1.84
58	6-Methyl-5-hepten-2-one	13.911	0.96 ± 0.06	0.47 ± 0.02	0.46 ± 0.03	0.85 ± 0.25	0.53 ± 0.01
59	2-Octanone	14.101	0.48 ± 0.03	0.69 ± 0.2	0.46 ± 0.03	0.57 ± 0.03	1.07 ± 0.01
60	2-Nonanone	18.447	0.93 ± 0.42	0.69 ± 0.2	0.68 ± 0.18	0.57 ± 0.03	0.8 ± 0.26
	Other compounds						
61	Vitispirane	25.914	0.48 ± 0.03	0.71 ± 0.26	0.46 ± 0.03	0.57 ± 0.03	0.8 ± 0.28
62	.Beta. Damascone	32.67	0.96 ± 0.06	1.4 ± 0.06	1.63 ± 0.35	1.6 ± 0.05	1.6 ± 0.02

Table 15. Volatile compounds identified from control and irradiated Cabernet Sauvignon wines

	Rt	Control µg L^{-1}	500 Gy µg L^{-1}	1000 Gy µg L^{-1}	1500 Gy µg L^{-1}	2000 Gy µg L^{-1}
Alcohols						
1-Propanol	1.839	31.3 ± 2.01	45.6 ± 6.43	35.61 ± 4.04	34.25 ± 6.65	37.98 ± 12.99
Isobutyl alcohol	2.296	269.77 ± 0.14	281.01 ± 55.29	279.03 ± 13.22	274.54 ± 50.23	447.68 ± 157.57
1-Butanol	2.697	18.59 ± 0.18	16.55 ± 2.46	15.24 ± 1.99	16.46 ± 0.95	11.39 ± 6.74
3-Methyl-1-butanol	4.304	8651.43 ± 116.41	9244.88 ± 1291.05	8450.87 ± 638.73	8058.92 ± 1308.79	10109.21 ± 3100.27
2-Methyl-1-Butanol	4.33	240.9 ± 59.67	187.55 ± 65.02	270.57 ± 4.27	222.48 ± 75.56	354.96 ± 120.58
1-Pentanol	4.956	2.87 ± 0.53	2.94 ± 1.07	1.87 ± 0.65	1.98 ± 1.15	6.95 ± 1.94
2,3-Butanediol	5.387	24.79 ± 0.56	31.48 ± 3.01	15.8 ± 1.88	20.73 ± 3.02	16.47 ± 6.57
1,3-Butanediol	6.663	3.99 ± 0.21	3.25 ± 0.86	2.93 ± 0	2.92 ± 0.47	18.83 ± 2.75
4-Methyl-1-pentanol	7.378	10.06 ± 0.33	12.47 ± 0.93	10.09 ± 0.12	11.19 ± 2.03	5.16 ± 1.22
3-Methyl-1-pentanol	7.693	68.34 ± 0.27	76.22 ± 5.53	57.34 ± 2.22	70.3 ± 8.01	39.95 ± 11.63
Trans-3-hexen-1-ol	8.001	1.47 ± 0.24	1.31 ± 0.34	0.82 ± 0.01	0.7 ± 0.1	2.67 ± 0.99
Cis-3-Hexene-1-ol	8.176	2.12 ± 0.01	1.77 ± 0.46	0.39 ± 0.39	1.34 ± 0.26	1.17 ± 1.17
1-Hexanol	8.752	225.87 ± 8.81	320.55 ± 30.68	255.02 ± 13.28	232.27 ± 28.68	309.85 ± 71.96
2-Heptanol	10.079	1.03 ± 0.19	1.16 ± 0.45	0.92 ± 0.01	0.72 ± 0	5.41 ± 1.59
Cis-hept-4-enol	12.968	1.62 ± 0.11	3.25 ± 0.26	1.96 ± 0.21	2.06 ± 0.3	3.13 ± 0.42
1-Heptanol	13.162	45.92 ± 2.14	80.72 ± 4.49	51.44 ± 2.15	55.97 ± 4.64	61.7 ± 9.03
Methionol	13.541	6.14 ± 0.03	6.96 ± 1.38	6.21 ± 1.03	4.51 ± 0.61	10.09 ± 3.87
2-Ethyl-1-hexanol,	15.738	8.68 ± 0.41	9.69 ± 1.42	8.69 ± 0.53	10.8 ± 3.4	9.06 ± 1.36
Benzyl alcohol	15.972	5.32 ± 0.7	4.06 ± 0.72	1.94 ± 0.75	2.56 ± 0.74	4.63 ± 0.68
1-Octanol	17.562	3.86 ± 0.14	9.11 ± 0.68	8.28 ± 1.08	7.96 ± 0.66	4.93 ± 0.85
Phenylethyl alcohol	19.716	2994.82 ± 62.14	3735.1 ± 439.59	2587.39 ± 542.28	3090.44 ± 717.91	3076.89 ± 436.04
Nonanol	21.762	8.32 ± 1.1	9.42 ± 1.49	7.88 ± 0.22	10.32 ± 1.14	3.48 ± 3.48
Aldehydes and ketones						
2-Methylbutanal	2.002	44.87 ± 0.5	44.7 ± 6.57	33.54 ±	34.2 ± 7.71	41.89 ± 14.27

				3.81		
3-Hydroxy-2-butanone	3.47	2.02 ± 0.05	1 ± 0.43	0.92 ± 0.09	1.34 ± 0.62	4.46 ± 0.96
2(3H)-dihydro-Furanone	10.579	3.38 ± 1.09	2.28 ± 0.88	0.68 ± 0.04	3.26 ± 0.22	3.47 ± 2.15
2-Methyl-tetrahydrothiophen-3-one	13.77	4.99 ± 0.02	4.67 ± 0.76	4.73 ± 0.37	5.23 ± 0.05	9.47 ± 2.59
Benzeneacetaldehyde	16.278	1.78 ± 0.48	1.75 ± 0.39	0.34 ± 0.34	2.06 ± 0.27	0.45 ± 0.45
Nonanal	18.889	3.06 ± 0.18	3.19 ± 0.56	2.14 ± 0.78	3.64 ± 1.12	3.55 ± 0.16
Esters						
Ethyl propionate	3.361	2.12 ± 0.09	2.41 ± 0.19	3.41 ± 0.05	3.63 ± 0.24	4.06 ± 0.39
Butyric acid ethyl ester	5.889	3.21 ± 0.07	4.5 ± 1.34	6.25 ± 0.23	6.67 ± 0.13	11.3 ± 4.93
Propanoic acid, 2-hydroxy-, ethyl ester	6.452	7.93 ± 0.26	7.39 ± 1.13	5.18 ± 0.41	5.69 ± 1.17	6.52 ± 2.67
Butanoic acid, 3-methyl-, ethyl ester	7.944	0.93 ± 0.03	1.36 ± 0.26	1.87 ± 0.23	1.81 ± 0	0.77 ± 0.77
1-Butanol, 3-methyl-, acetate	8.95	22.22 ± 0.09	23.89 ± 5.98	65.48 ± 11.4	48.61 ± 5.84	48.63 ± 6.58
1-Butanol, 2-methyl-, acetate	9.07	1.7 ± 0.12	2.02 ± 0.87	4.83 ± 1.55	2.87 ± 0.61	5.97 ± 1.2
Hexanoic acid, ethyl ester	14.411	48.35 ± 0.61	102.08 ± 30.53	136.02 ± 44.36	154.55 ± 44.25	114.1 ± 32.91
Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	16.957	4 ± 0.55	6.01 ± 0.81	5.61 ± 0.84	4.34 ± 1.2	3.59 ± 0.65
Butanedioic acid, diethyl ester	22.145	15.63 ± 0.61	17.41 ± 2.56	15.68 ± 4.72	17.98 ± 3.82	16.49 ± 1.07
Octanoic acid, ethyl ester	22.718	56.07 ± 0.3	306.55 ± 50.47	327.46 ± 59.41	457.86 ± 95.69	195.42 ± 46.48
Benzeneacetic acid, ethyl ester	24.603	2.22 ± 0.11	5.73 ± 0.47	1.92 ± 0.46	2.51 ± 0.99	1.4 ± 0.31
Acetic acid, 2-phenylethyl ester	25.067	16.35 ± 1.34	45.57 ± 5.67	40.77 ± 12.89	51.43 ± 6.55	25.53 ± 1.88
Hexanoic acid, 3-hydroxy-, ethyl ester	27.795	3.72 ± 1.11	3.18 ± 0.66	1.93 ± 0.52	1.92 ± 0.28	0.73 ± 0.73
Ethyl 9-decenoate	29.804	8.6 ± 0.92	57.57 ± 2.77	37.06 ± 9.57	50.89 ± 0.59	0 ± 0
Decanoic acid, ethyl ester	30.086	24.78 ± 1	70.52 ± 8.47	64.01 ± 21.16	93.54 ± 5.12	34.08 ± 6.6
ethyl 3-methylbutyl butanedioate	31.296	2.95 ± 0.04	3.17 ± 0.49	3.19 ± 0.79	3.06 ± 0.61	3.05 ± 0.53
Isoamyl octanoate	31.825	1.56 ± 0.05	1.96 ± 0.58	2.04 ± 0.7	3.14 ± 0.58	2.65 ± 1.01
Dodecanoic acid, ethyl	36.695	10.58 ± 1.18	10.63 ±	8.38 ± 4.56	17.24 ± 2.27	10.26 ± 2.7

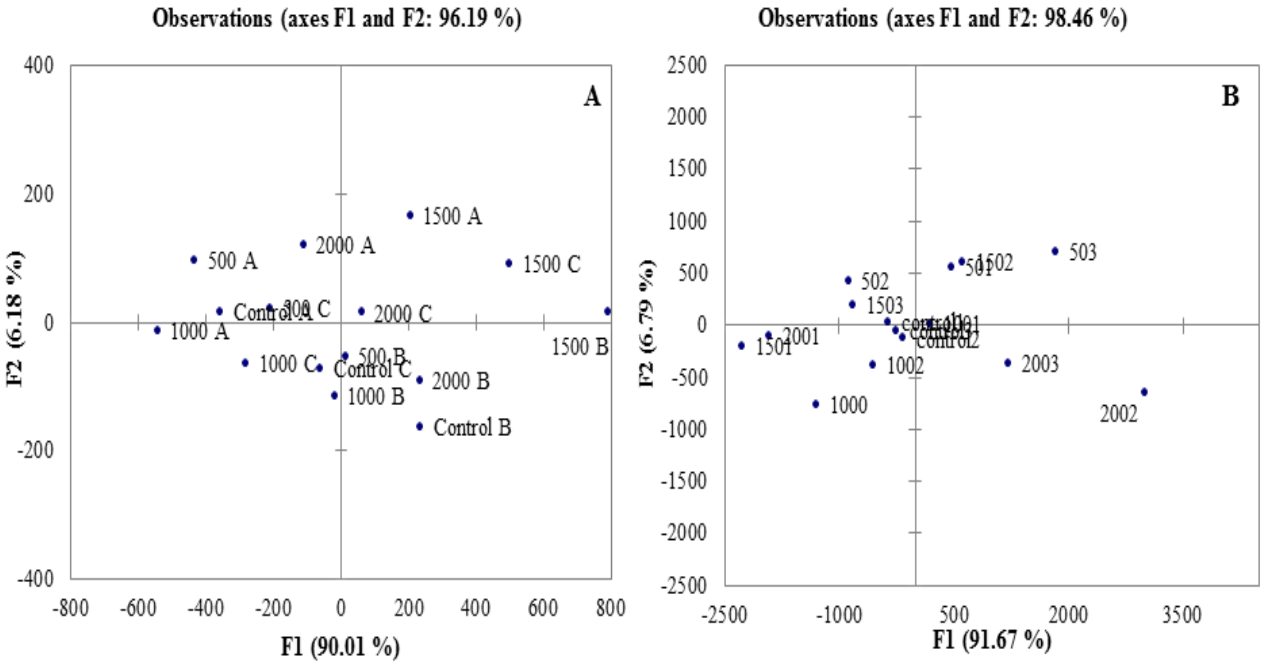
ester			2.68			
3-Methylbutyl decanoate	38.251	1.49 ± 0.06	1.46 ± 0.69	1.78 ± 1.78	2.53 ± 0.19	0.74 ± 0.74
Hexadecanoic acid, ethyl ester	42.681	1.85 ± 0.2	1.68 ± 0.28	0.73 ± 0.73	1.48 ± 0.08	1.26 ± 0.11
Hexadecanoic acid, ethyl ester	48.411	1.96 ± 0.14	2.25 ± 0.48	1.14 ± 1.14	1.76 ± 0.27	0.39 ± 0.39
Short chain organic acids						
Acetic acid	2.39	71.65 ± 9.58	81.2 ± 15.34	53.83 ± 25.21	47.25 ± 10.53	102.92 ± 16.02
Isobutyric acid	5.221	14.48 ± 1.69	16.3 ± 2.85	14.71 ± 0.44	11.69 ± 0.83	24.63 ± 8.43
2-Methylbutanoic acid	9.17	16.27 ± 0.33	19.29 ± 4.9	10.87 ± 3.33	12.72 ± 3.01	50.31 ± 12.92
Hexanoic acid	15.225	95.1 ± 4.41	131.85 ± 21.28	51.57 ± 45.98	123.58 ± 20.22	90.31 ± 14.5
Octanoic acid	23.038	178.07 ± 24.15	118.52 ± 42.63	77.08 ± 77.08	203.19 ± 56.64	47.24 ± 12.64
Decanoic acid	29.495	69.79 ± 7.9	65.75 ± 16.8	56.06 ± 34.62	67.06 ± 19.74	25.92 ± 4.99
Hexadecanoic acid	35.744	2.71 ± 0.31	3.15 ± 0.98	0.3 ± 0.3	13.05 ± 3.04	3.22 ± 0.28
Terpene and related compounds						
l-Limonene	15.503	2.86 ± 0.29	3.73 ± 0.32	2.34 ± 0.45	2.5 ± 0.2	3.54 ± 0.54
Para cymene	17.977	1.51 ± 0.14	1.28 ± 0.36	0.53 ± 0.53	1.09 ± 0.27	0.51 ± 0.51
l-.alpha.-Terpineol	22.502	10.02 ± 0.92	5.57 ± 1.22	7.77 ± 0.89	6.12 ± 2.07	7.56 ± 1.01
Citronellol	23.991	2.61 ± 0.36	5.53 ± 0.89	4.32 ± 1.1	1.73 ± 1.73	5.72 ± 0.37
Other compounds						
Damascone	np	np	1.98 ± 0.33	1.97 ± 0.29	2.12 ± 0.1	0.91 ± 0.91

np = Not present

Aroma profile data for both the wines was subjected to principal component analysis for analyzing changes due to radiation processing. First two principal components (F1 and F2) explained 90 and 6% of data variation for Shiraz wines and 91.6 and 6.8% for

Cabernet Sauvignon wines. Score plot for both the wines is depicted in Figure. 20. A score plot from the first two principal components clearly demonstrated that there was no segregation of control and irradiated wine samples. These results clearly indicated that there were no major differences in aroma constituents of Shiraz and Cabernet Sauvignon wines.

Figure 20. Principal component analysis (PCA) score plots for volatile compounds data. **(A)** Score plot for Shiraz variety. **(B)** Score plot for Cabernet Sauvignon variety.



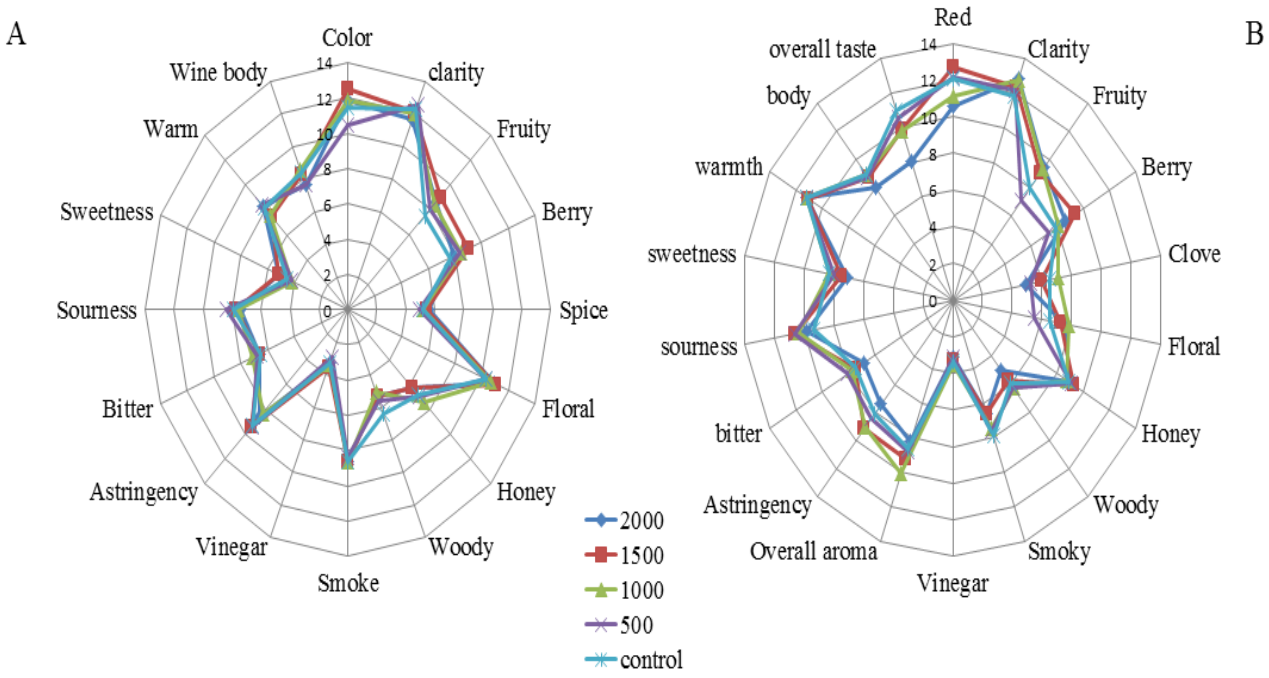
Although, no major radiation induced changes were observed in the aroma of wine obtained from both the varieties, a careful examination of volatile data revealed some changes due to radiation processing. An increased content of 1-heptanol, 2-ethyl hexanol, 1-octanol by 14, 75 and 32 percent, respectively in Shiraz and by 20, 22 and 200 percent, respectively in Cabernet Sauvignon was observed. A decrease in glycosidic precursors of

these compounds is demonstrated in present study which might have resulted in increased content of these volatiles in wines. Aroma notes of 1-heptanol, 2-ethyl hexanol, 1-octanol and benzyl alcohol is described as herb, rose, nut and sweet of floral, respectively.

Interestingly, in Cabernet Sauvignon wines the presence of β-damascone was observed only in irradiated samples beyond a dose of 500 Gy with its content reaching 2.12 ± 0.1 μg L^{-1} at a dose of 1500 Gy. No new radiation induced compounds were observed in Shiraz wines. However, content of β-damascone also increased in a dose dependent manner in Shiraz wines (Table 14). Control Shiraz wines had β-damascone content of 0.96 ± 0.06 μg L-1 that increased to 1.6 ± 0.05 μg L-1 at a dose of 1500 Gy. β-damascone is known to be a degradation product of carotene (100). Thus, radiation induced degradation of carotenes may possibly be the reason for observed presence or increase of this compound in irradiated samples. Odor of β-damascone is reported to be as fruity, honey like, floral and sweet (101, 102). Thus, increased amount of β-damascone observed in irradiated samples in the present study may contribute to an enhanced fruity or floral note.

Increased concentrations of above said volatiles in radiation processed wines cold possibly lead to higher fruity, floral and rose notes in wine. Therefore, to judge the effect of radiation processing, control and irradiated wine samples were subjected to descriptive sensory analysis after four months of maturation. Results are depicted in the form of a web diagram in Figure 21A and 21B for Shiraz and Cabernet Sauvignon wines, respectively.

Figure 21. Cobweb diagram of sensory scores of wines prepared from control and irradiated grapes post four months of maturation. **(A)** Sensory scores of wine prepared from Shiraz variety. **(B)** Sensory scores of wine prepared from Cabernet Sauvignon variety.



Irradiated wines demonstrated higher fruity and berry aroma characteristics as compared to control wines which could be explained by the presence or enhancement in β-damascone, 1-heptanol, 2-ethyl hexanol or 1-octanol in the irradiated samples. Higher red color was also observed in irradiated wines (1500 Gy) as compared to the control. This could be correlated to the higher anthocyanin content observed in irradiated samples. No significant differences were observed in the mean values of all other sensory attributes between control and irradiated samples for both Shiraz and Cabernet Sauvignon wines. Further, sensory panel did not observe any radiation induced off flavors or taste in wines

and the samples were possessing similar sensory quality as that of the non-irradiated samples.

3.2.4. Conclusions

In conclusion, radiation processing of grapes resulted in wines with higher total antioxidants and phenolics in all the three varieties. White wines samples prepared using irradiated grapes were significantly brown in color. However, for red wine making varieties, wines prepared from irradiated grapes demonstrated better red color, higher color intensity and anthocyanin content as compared to the controls. Highest color intensity, anthocyanin content, phenolic and antioxidant content were observed in wines prepared with grapes subjected to a dose of 1500 Gy. There were no major changes in aroma profile of wine samples prepared with irradiated grapes. It was observed in descriptive sensory analysis of wines that irradiated samples (1500 Gy) had more red color and higher fruity and berry odor as compared to control due to the presence of β-damascone. However, no radiation induced off flavor or taste was observed by sensory panel. Thus, application of radiation processing on a large industrial scale is feasible and could be a practical approach to obtain wines with better antioxidant and organoleptic characteristics.

3.3 Radiation processing of wine for inactivation of spoilage microorganisms

Although microorganisms play a major role in wine production, certain species of yeasts and bacteria can also cause spoilage which diminishes the quality and acceptability of the final product. These spoilage are usually recognized by haze formation, increase in acetic acid or volatile acidity, volatile phenols, volatile sulphur and viscosity of wine.

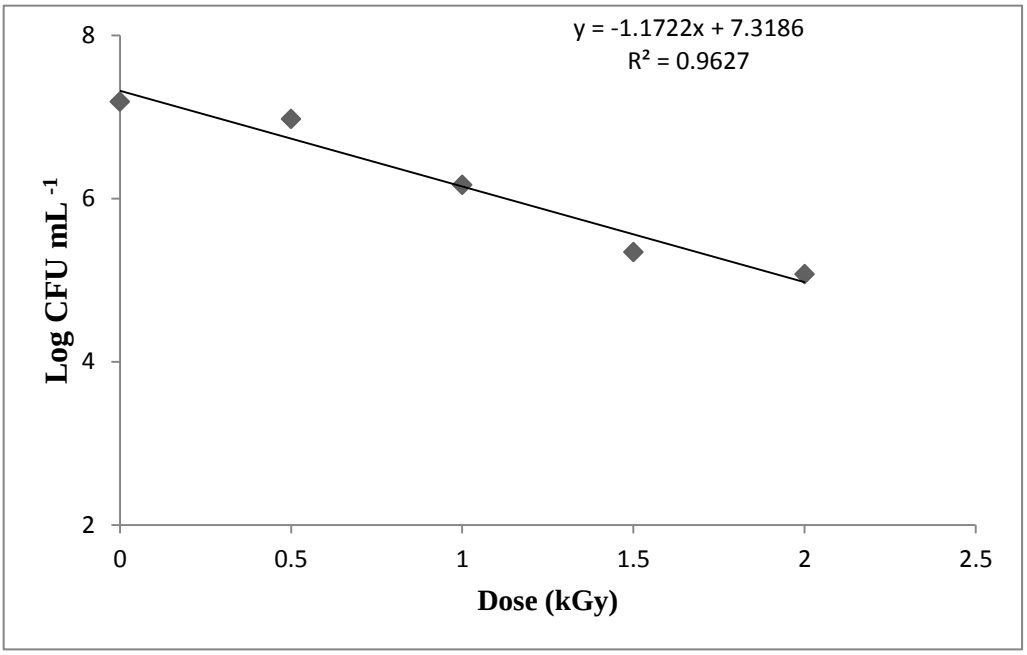
The addition of SO_2 is an effective means of stabilizing grape juice and wine microbiologically and is presently widely used in industry. However, use of SO_2 is associated with possible health risks such as intolerance or allergic reactions. Further, due to its possible health risks International Organization of Vine and Wine (OIV) has progressively reduced the maximum concentration authorized in wines which is now 200 mg/L (39). Apart from SO_2 other techniques such as filtration and fining are also efficient in controlling microbial growth, but unfortunately these techniques have detrimental effects on the sensory properties of the wine (42). Therefore, there is a need to develop alternative strategies to either substitute or enhance the effect of reduced SO_2 levels without modifying the chemical and sensory properties of the wine. Use of UV-C, pulsed electric fields, high hydrostatic pressure, and high power ultrasounds for inactivation of wine and grape juice spoilage microorganisms has been previously reported (42, 41). However, to best of our knowledge there are no reports on use of gamma radiation for inactivation of spoilage microorganisms in wine. Three different microorganisms i.e. yeast (*Dekkerra bruxellensis*), lactobacillus bacteria (*Pediococcus acidilactici*) and acetic acid bacteria (*Acetobacter aceti*) were chosen to demonstrate the efficacy of gamma radiation for inactivation of spoilage organisms in wine. *Dekkerra bruxellensis* is most widely distributed spoilage yeast in wine industry. It transforms hydroxycinnamic acids

such as *p*-coumaric acid and ferulic acid to undesirable volatile phenols namely 4-ethyl-phenol and 4-ethyl-guaiacol. These volatile phenols are associated with 'animal', 'leather' and 'horse sweat' aroma notes in wine (102). *Acetobacter aceti* is an acetic acid bacterium which can oxidize ethanol to acetic acid. Apart from producing acetic acid these microorganisms can also produce other compounds which can adversely affect wine quality (104). Lactic acid bacteria can produce diacetyl which can impart 'buttery' or 'butterscotch' aroma to wine. Radiation processing to inactivate these microorganisms in red wine prepared from Shiraz variety was attempted. Impact of radiation processing on wine quality was also assessed.

3.3.1. Radiation inactivation of various microorganisms

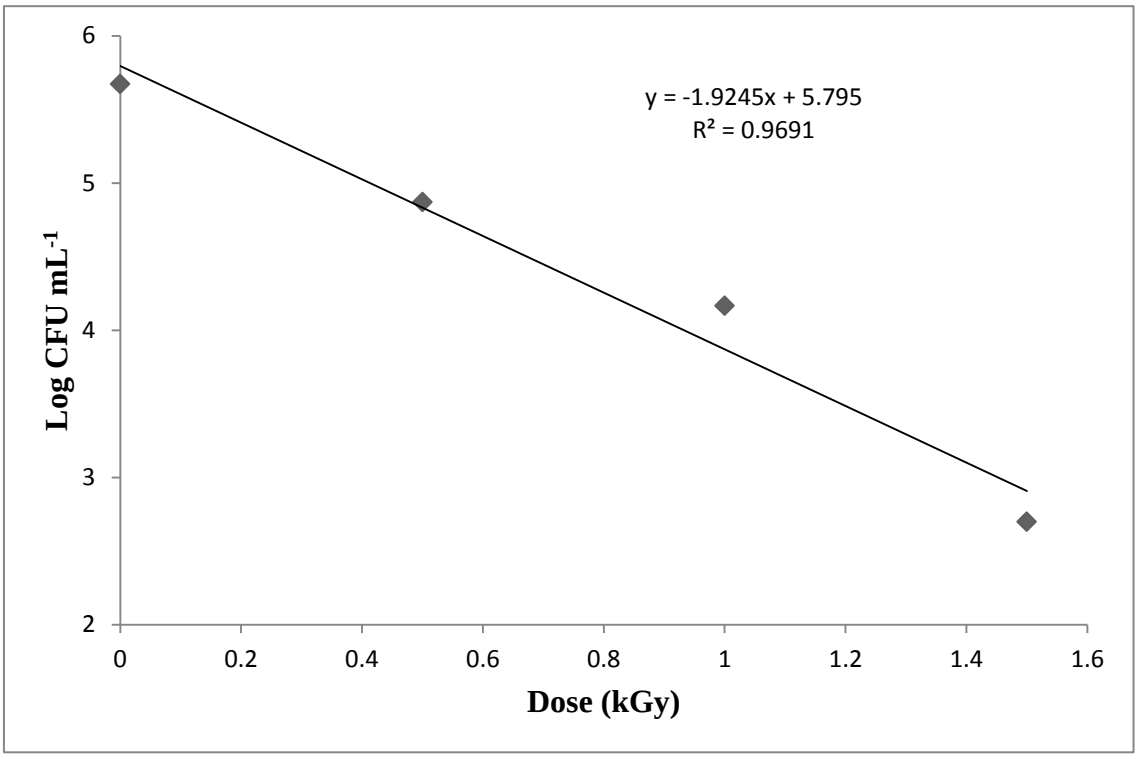
Radiation inactivation of all the three microorganisms was studied in both saline medium and after inoculation in wine. Data obtained for inactivation of *D. bruxellensis* in saline and wine medium is shown in figure 22 and 23, respectively.

Figure 22. Radiation inactivation curve for *D. bruxellensis* in saline



A radiation dose dependent decrease in viable organisms is clearly observed. Data obtained was fitted into a linear curve and equations obtained were solved to calculate D10 values in saline as well in wine. *D. bruxellensis* had D10 value of 0.9 kGy when irradiated in saline, however, surprisingly D10 value reduced to 0.5 kGy when radiation was carried out after inoculation in wine.

Figure 23. Radiation inactivation curve for *D. bruxellensis* in wine



D10 values for various species of yeasts were reported in between 0.76 to 1.76 kGy in saline (79). No reports however, exist for radiation inactivation of *D. bruxellensis*. Nevertheless, D10 values obtained in present study are in close agreement with D10 values of other yeasts reported (79).

Data obtained for inactivation of *P. acidilaciti* in saline and wine medium is shown in figure 24 and 25, respectively. A radiation dose dependent decrease in viable organisms is clearly observed. Data obtained was fitted into a linear curve and equations obtained were solved to calculate D10 values in saline as well in wine. *P. acidilaciti* had D10 value of 0.83 kGy when irradiated in saline, however, D10 value reduced to 0.49 kGy when radiation was carried out after inoculation in wine.

Figure 24. Radation induced inactivation of *P. acidilactici* for irradiation carried out in saline.

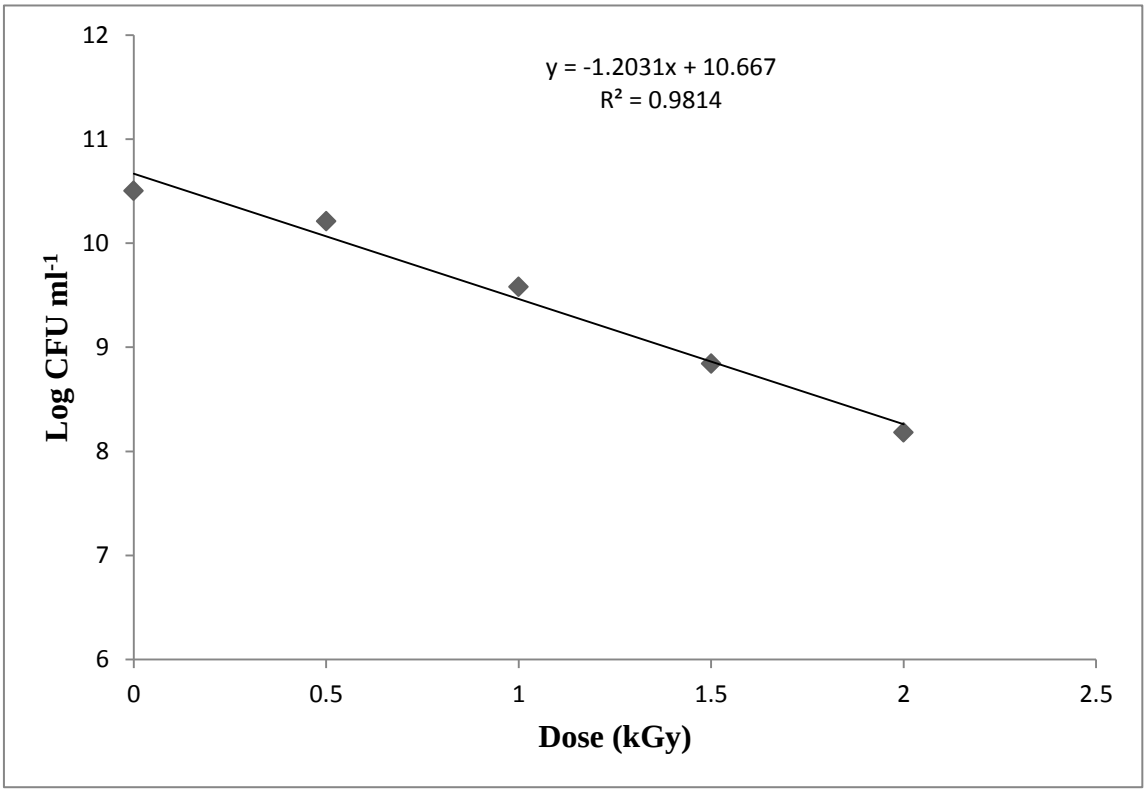
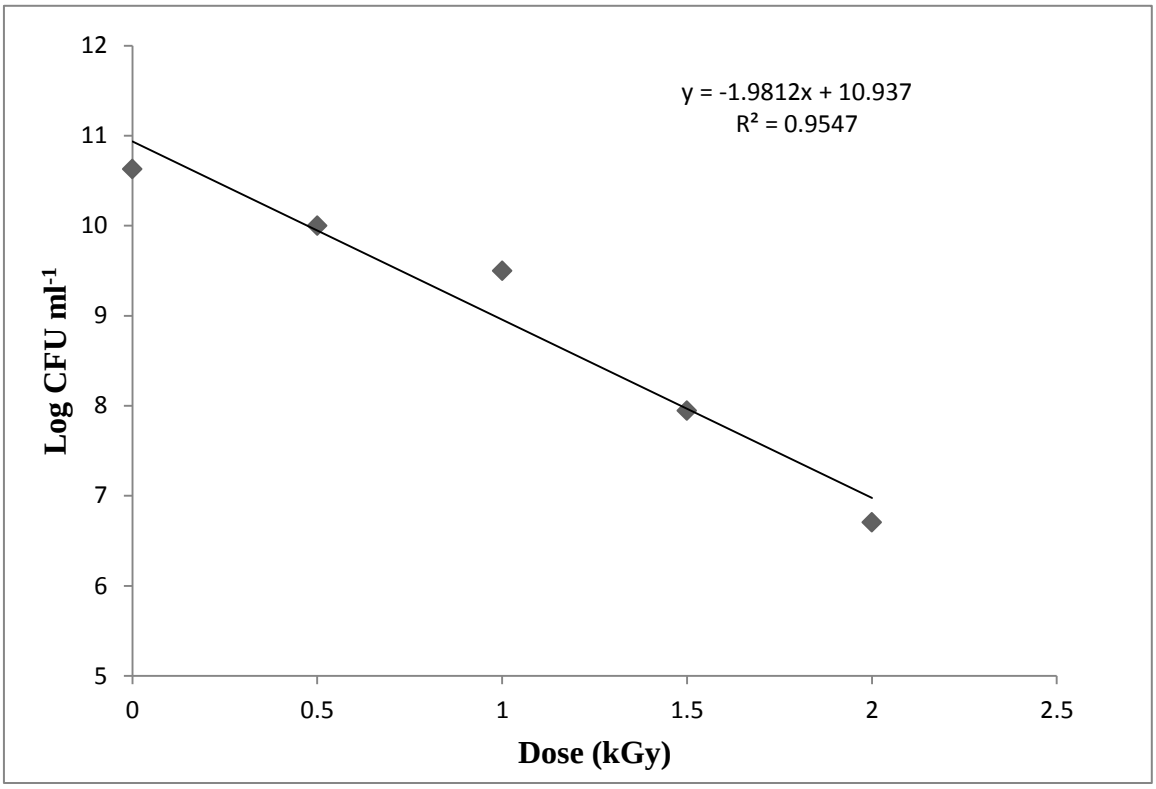


Figure 25. Radiation induced inactivation of *P. acidilactici* for irradiation carried out in wine.



D10 values for various species of lactobacillus were reported in between 0.25 to 0.75 kGy in saline (105). No reports were however, present for radiation inactivation of *P. acidilaciti*. Nevertheless, D10 values obtained in present study are in close agreement with D10 values of other lactobacillus reported (105). Data obtained for inactivation of *A. aceti* in saline and wine medium is shown in figure 26 and 27, respectively.

Figure 26. Radiation induced inactivation of *A. aceti* for irradiation carried out in saline

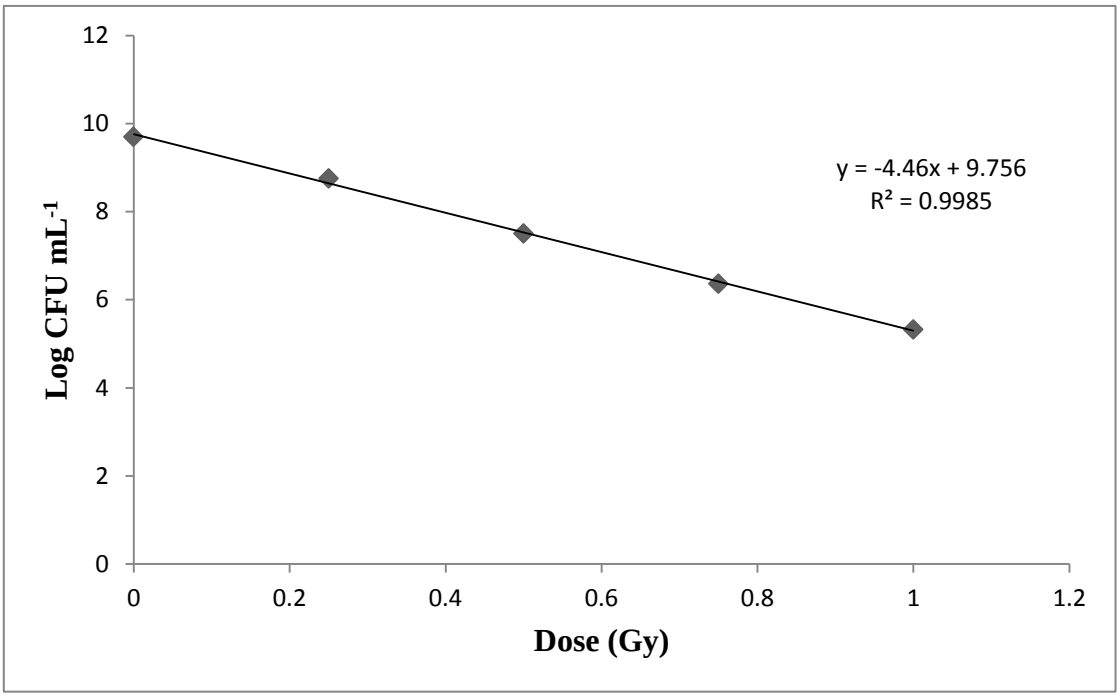
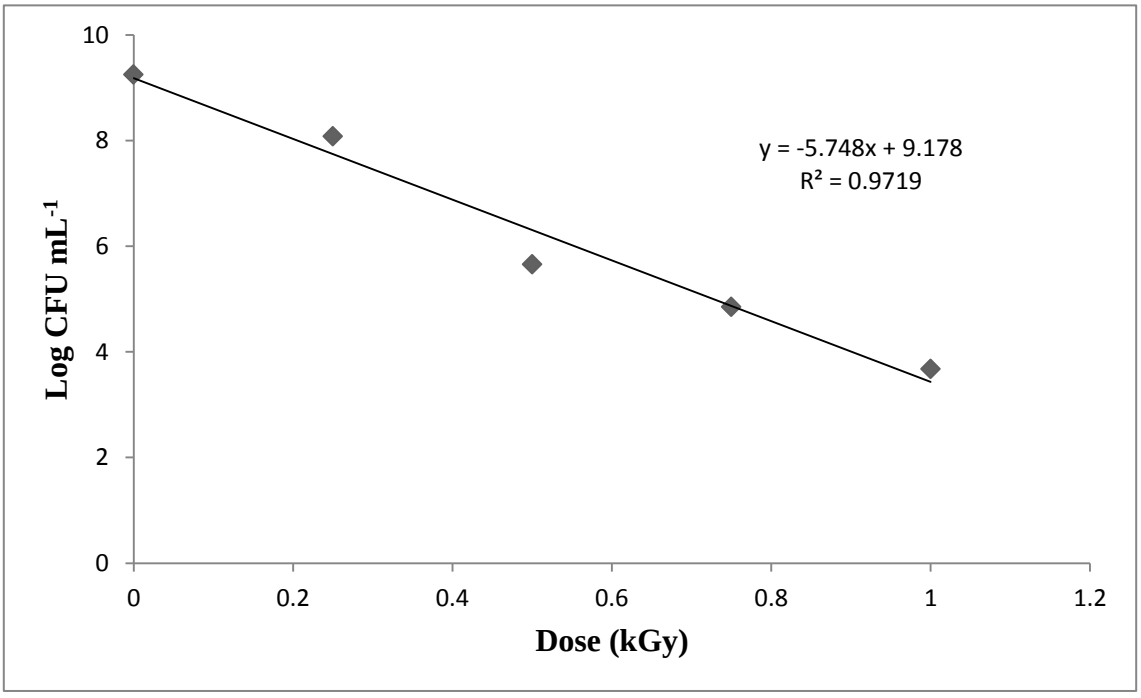


Figure 27. Radiation induced inactivation of *A. aceti* for irradiation carried out in wine.



A radiation dose dependent decrease in viable organisms was clearly observed. Data obtained was fitted into a linear curve and equations obtained were solved to calculate D10 values in saline as well in wine. *A. aceti* had D10 value of 0.22 kGy when irradiated in saline, however, D10 value reduced to 0.17 kGy when radiation was carried out after inoculation in wine. No reports on radiation inactivation of acetic acid bacteria are available in literature.

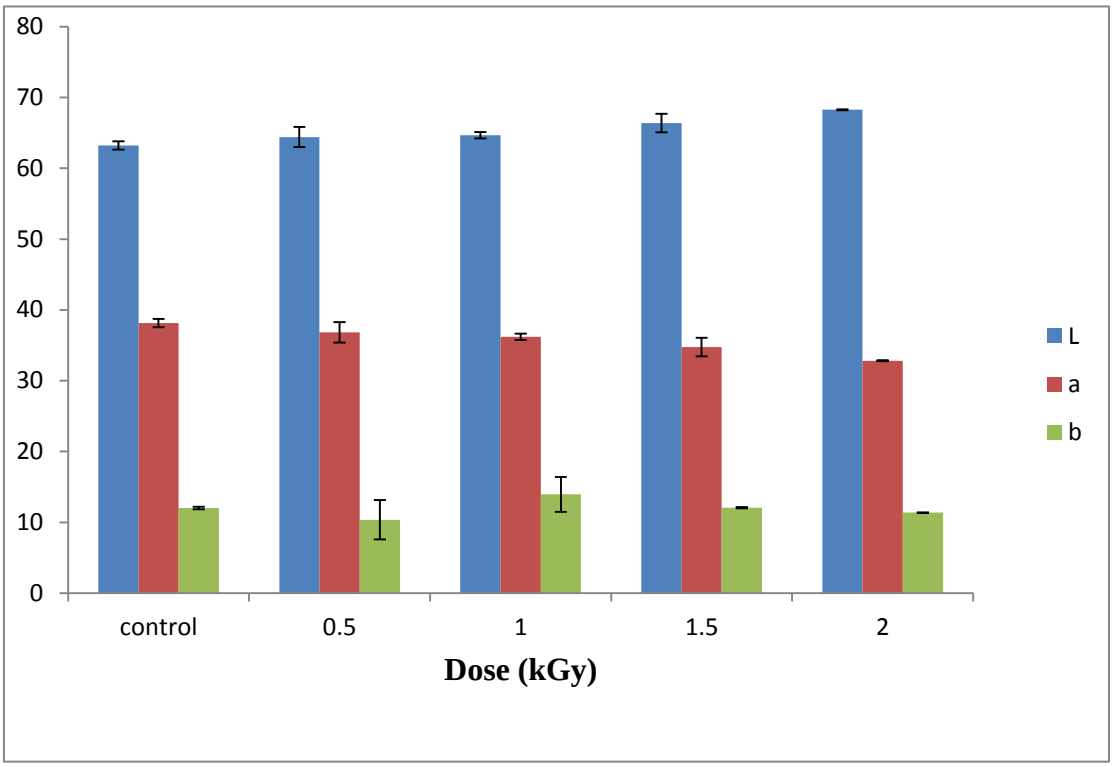
In all three microorganisms studied, a significantly ($p<0.05$) lower D10 values were observed when wine was subjected to radiation processing as compared to saline. Many studies have reported that food matrix offers protection from radiation inactivation to microorganisms and D10 values are higher for microbial inactivation in food matrix as compared to saline (59). However, in contrast to these reports, in the present study a lower D10 values was observed for all the three microorganisms when wine was exposed to radiation as compared to saline. This might be due to the presence of high alcohol content in wine, which may have acted synergistically with radiation processing. Although, no previous reports are available on gamma radiation inactivation of microorganism in alcoholic beverages, synergism between alcohol and UV radiation for microbial deactivation is reported (106).

Thus, results presented here, demonstrate that radiation processing decreases in viability of wine spoilage microorganisms. To further evaluate suitability of radiation treatment for inactivation of spoilage microorganisms, wines subjected to radiation processing was evaluated for total antioxidant, total phenolics, color and sensory characteristics.

3.3.2. Effect of radiation processing on wine quality.

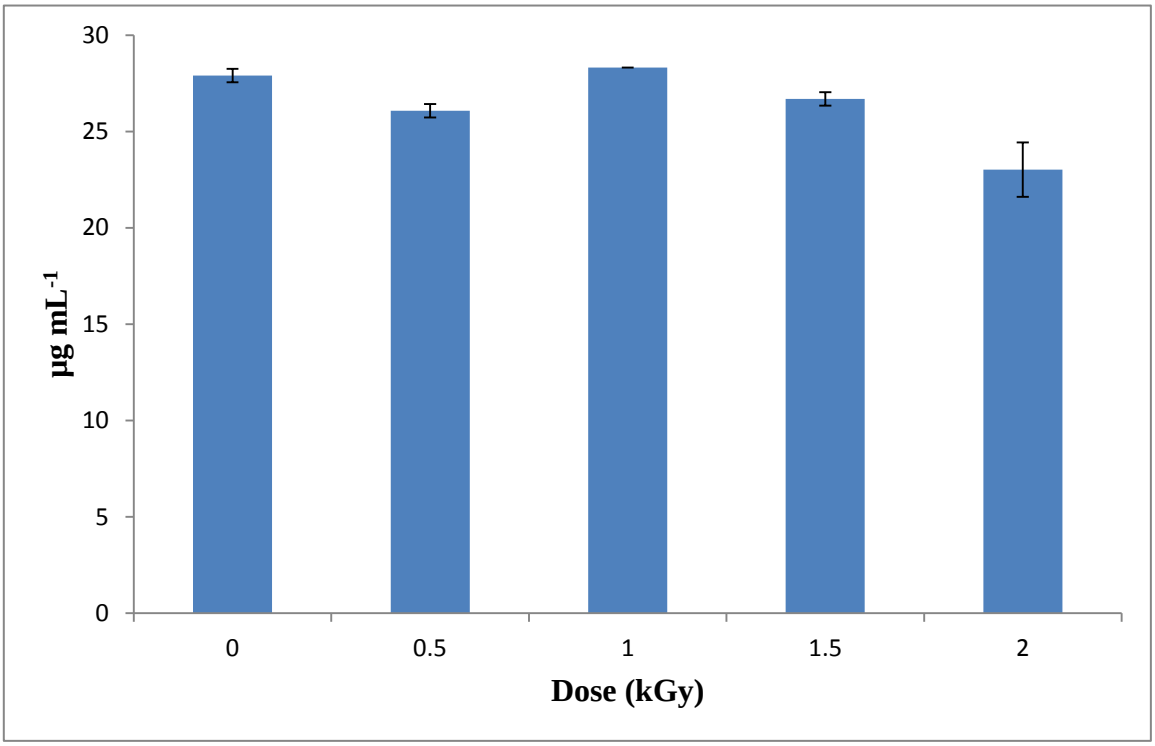
Wine prepared with Shiraz grapes was subjected to radiation processing and subsequently analyzed for various quality parameters. Effect of radiation processing on color characteristics of wine is demonstrated in figure 28.

Figure 28. Effect of radiation processing on color characteristics of Shiraz wine



No significant ($p<0.05$) differences were observed in L* and b* values of control and radiation processed wines. In case of a* values, no significant change ($p<0.05$) was observed up to a dose of 1.5 kGy, however, at higher dose of 2 kGy a small but significant ($p<0.05$) reduction in a* value was observed. Decrease in a* value signifies a reduction in red color intensity of wine. This might be due to oxidation of anthocyanins at higher dose of 2 kGy. Data obtained for total anthocyanins content in control and radiation processed wine is demonstrated in Figure 29.

Figure 29. Effect of radiation processing on total anthocyanin content of Shiraz wine



No statistical significant ($p<0.05$) change up to 1.5 kGy, with significant reduction thereafter was clearly observed (Figure 29). Thus, reduction in anthocyanin content at dose of 2 kGy resulted in decreased red color intensity of wine. Wine after radiation processing was also analyzed for total antioxidant and total phenolics content and results obtained are shown in figure 30 and 31, respectively. Interestingly, no significant ($p<0.05$) effect of radiation processing was observed on either of these quality parameters.

Figure 30. Effect of radiation processing on total antioxidant content in Shiraz wine

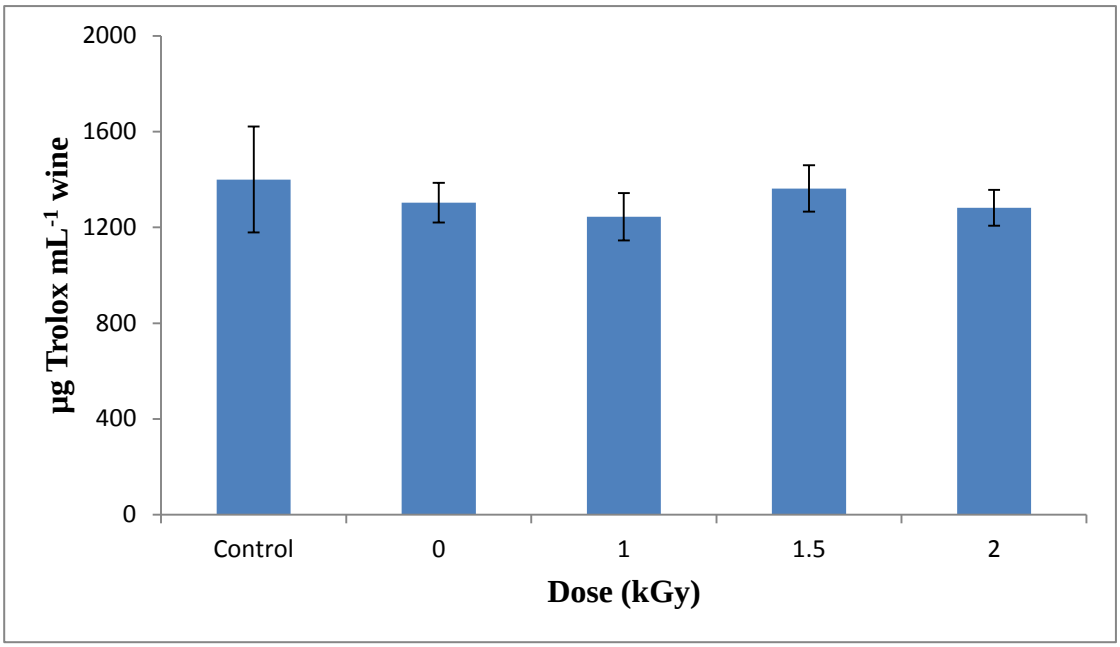
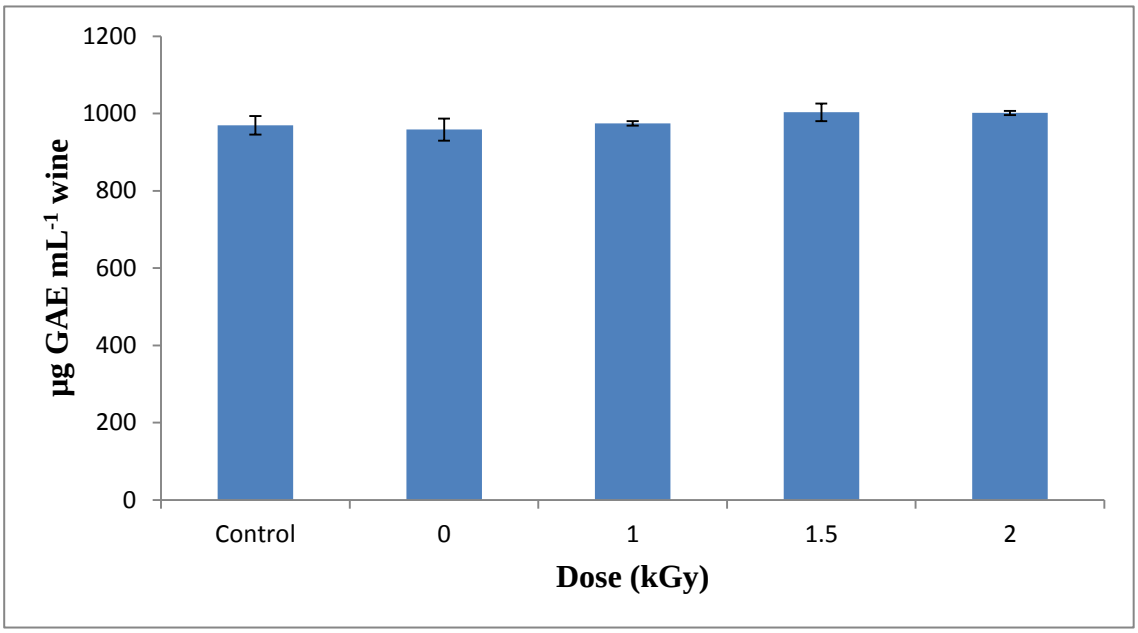
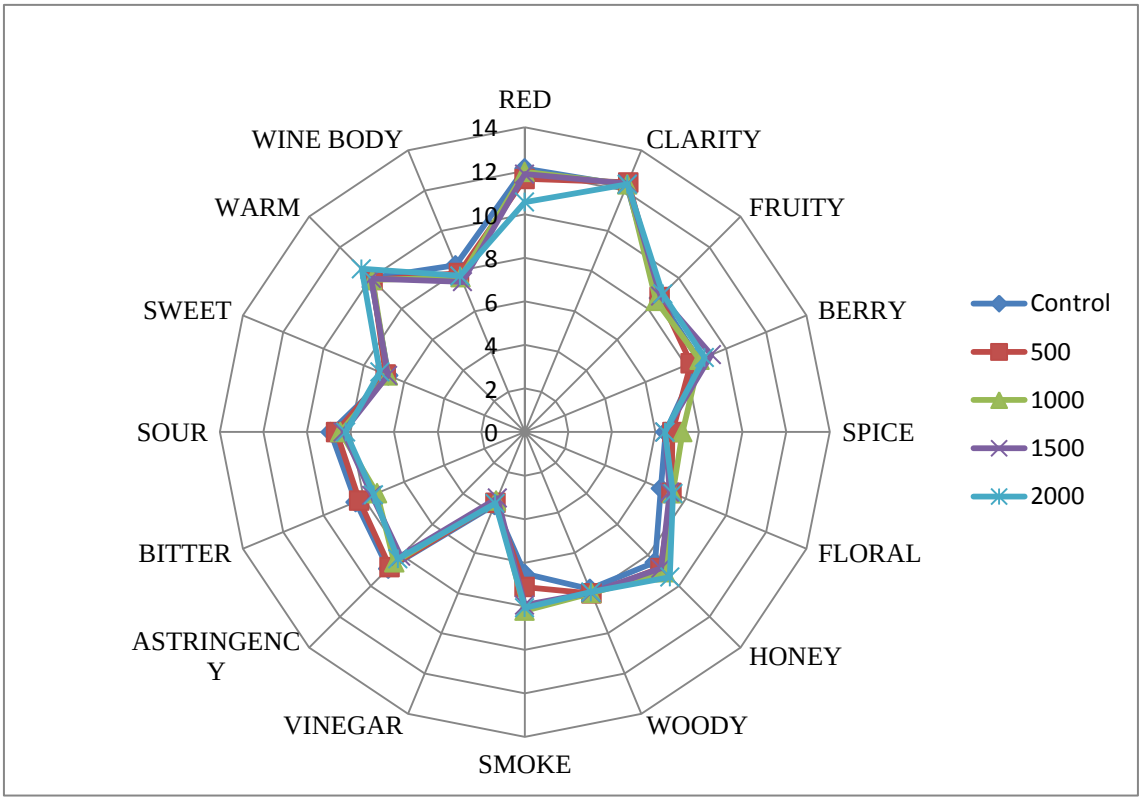


Figure 31. Effect of radiation processing on total phenolics content in Shiraz wine



Finally, sensory analysis of control and radiation processed wines was carried out using quantitative descriptive analysis and results obtained are shown in figure 32.

Figure 32. Effect of radiation processing on sensory quality of Shiraz wine



As can be clearly observed (Figure 32), there were no major radiation induced changes in sensory quality of wine. However, a slight reduction in redness at dose of 2 kGy was noted. There are very few reports on radiation processing on wine. To best of our knowledge there is only one report analyzing effect of radiation processing on quality of red wine (96). These authors have observed no effect of radiation processing on antioxidant and sensory characteristics of wine up to a dose of 2.4 kGy. Moreover, an increase in chemical color age of wine due to radiation processing was also suggested.

However, in present study it was noted that wine was amenable to radiation processing only up to a dose of 1.5 kGy only. Observed differences might be due to different variety of wine chosen for study. Cabernet Sauvignon wine was chosen for earlier study while work was carried out on Shiraz variety in the present study. With regards to other physical treatments for microbial deactivation, effect of high pressure processing of wine on its various quality characteristics has been reported (39). A slight decrease in monomeric anthocyanins along with decrease in antioxidant properties of wine were reported by these authors. High pressure processed wines had slightly more cooked fruit and spicy aroma as compared to untreated wines.

In present study, however, radiation processing caused significant changes in wine quality beyond a dose of 1.5 kGy. It could also be seen from microbial inactivation data that at a dose of 1.5 kGy, 99.9 percent of spoilage yeasts (*D. bruxellensis*) and lactic acid bacteria (*P. acidilactici*) could be inactivated. A much greater reduction (9 log cycles) could be obtained for acetic acid bacteria (*A. aceti*) at 1.5 kGy. Thus, use of radiation processing for inhibition of wine spoilage microorganisms could be a practical proposition.

3.4 GC/MS integrated with chemometrics for rapid identification of radiation treatment

As shown in section 3.1 radiation processing resulted in significant changes in free volatile profile of grapes. Volatile profile data when analyzed by PCA showed a segregation of control samples from irradiated grapes in all three varieties. This offers possibility of identifying radiation treatment in fruits. However, present methodology of identifying and quantifying individual compounds and then subjecting them to chemometrics is tedious and time consuming. Therefore, to make methodology for identification of radiation treatment simpler a chemometric analysis of total mass spectrum of entire volatile fraction was attempted.

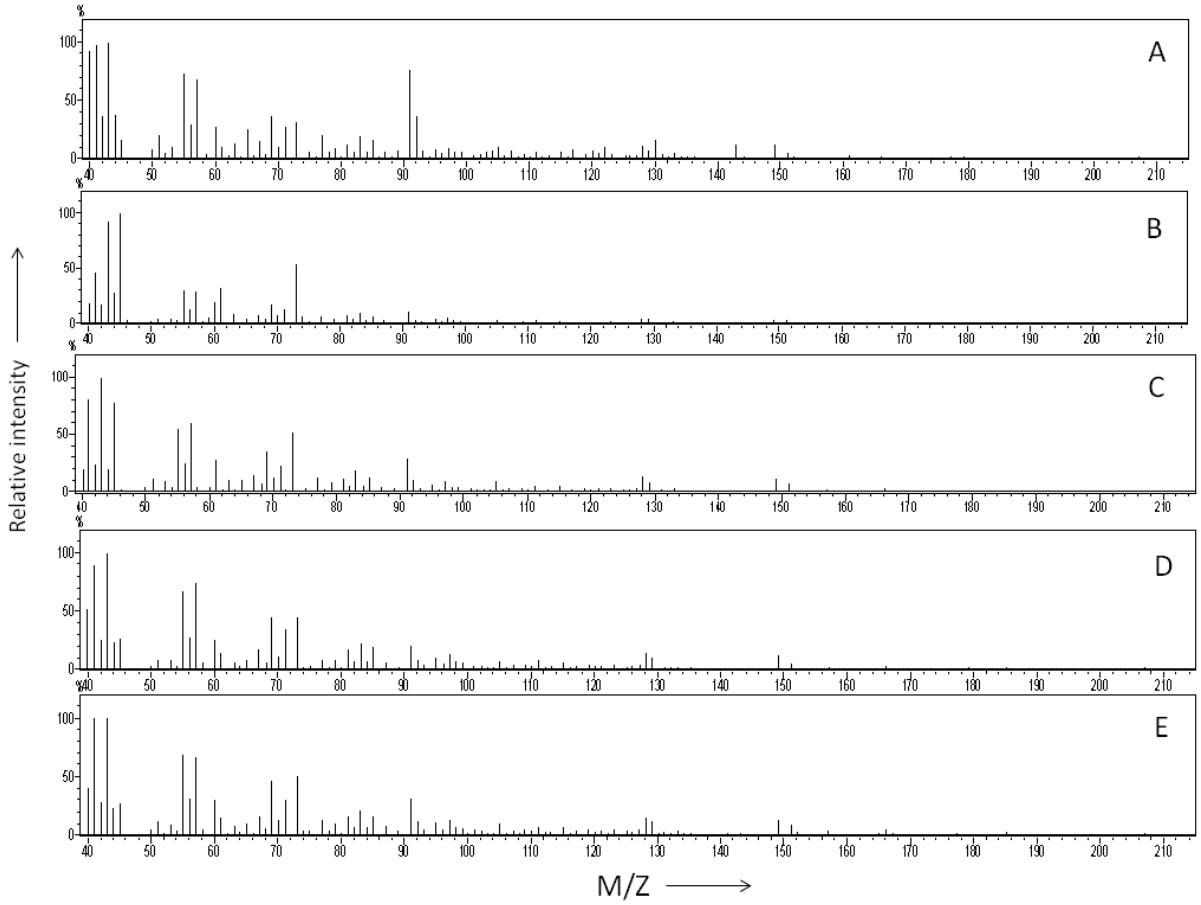
Interest in detection methods for irradiated foods has increased enormously in recent years. Reliable analytical methods help regulatory authorities to check compliance with the labeling requirements. Thus, identifying whether or not a food has been subjected to irradiation is essential for control of international trade in irradiated food and building consumer confidence (107). The ability to analytically identify irradiated food makes it possible to check compliance with existing regulations such as enforcement of labeling. Present methods to detect irradiated foods include GC/MS analysis of hydrocarbons and 2-alkylcyclobutanones, electron paramagnetic resonance (EPR spectroscopy), thermoluminescence, photostimulated luminescence and DNA comet assay screening (108). Above listed methods for detection of irradiated foods are either time consuming sample purification or require sophisticated instruments such as EPR. Thus, there is a need to develop a rapid and simple method for identification of irradiated foods.

To the best of our knowledge, no reports are available demonstrating the use of total mass chromatogram of sample for detection of radiation treatment. In this study, the development of a simple and rapid method for classification of non-irradiated and irradiated grape samples using total mass chromatogram and multivariate techniques is therefore attempted. Grapes were chosen as a model for demonstration of the developed method. Suitability of developed methodology was further demonstrated on apples. To obtain total mass spectrum three different grape extracts, i.e. XAD ether extract, SDE extract and headspace volatiles obtained from SPME were analyzed using GC/MS. Total mass chromatogram obtained was further analyzed using chemometrics to identify radiation treatment.

3.4.1. Analysis of XAD ether extract using GC/MS and chemometrics.

XAD ether extract obtained from the extraction of grapes of Chenin Blanc variety were injected in GC/MS and total mass spectra was obtained as described in materials and methods section 2.2.3 and 2.2.4, respectively. Total mass spectra obtained for control and irradiated samples are shown in Figure. 33.

Figure 33. Total mass spectra of control and irradiated samples obtained from analysis of XAD ether extract. A total mass spectrum was obtained by integrating GC/MS chromatograms from 7 to 55 min. A) Control B) 0.5 kGy C) 1.0 kGy D) 1.5 kGy E) 2.0 kGy.

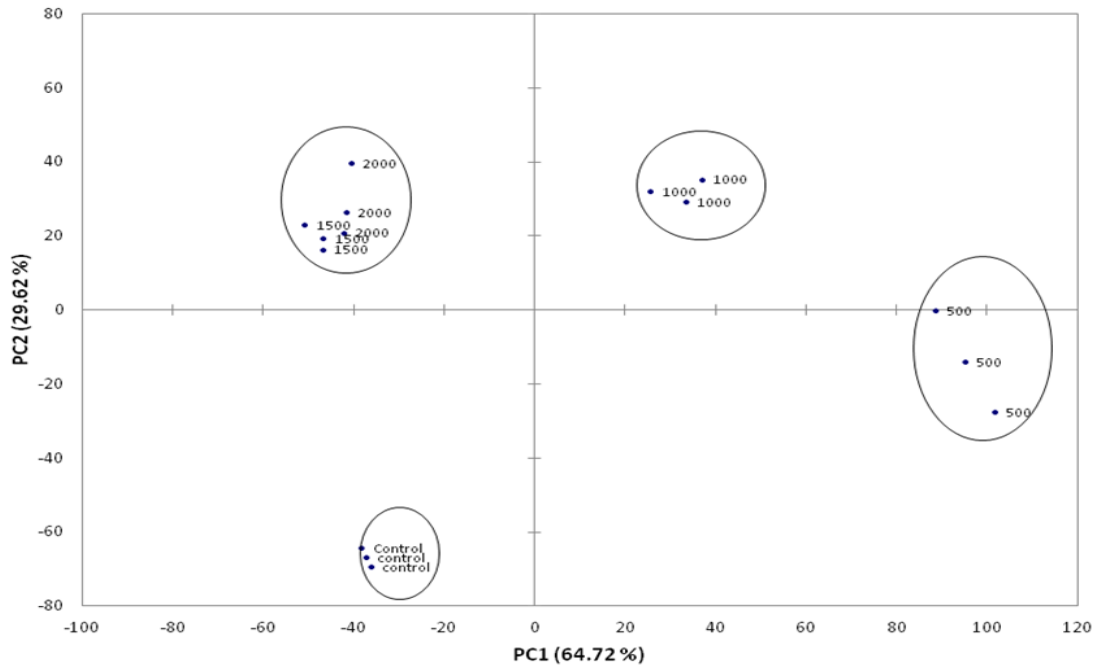


A significant decrease in the relative intensity of m/z 55, 57 and 91 was observed in irradiated samples as compared to the control that could possibly contribute towards differences between the control and irradiated samples. Very few other notable differences were observed visually in control and irradiated samples. Data was therefore,

processed by multivariate statistical analysis in order to reveal further differences among samples.

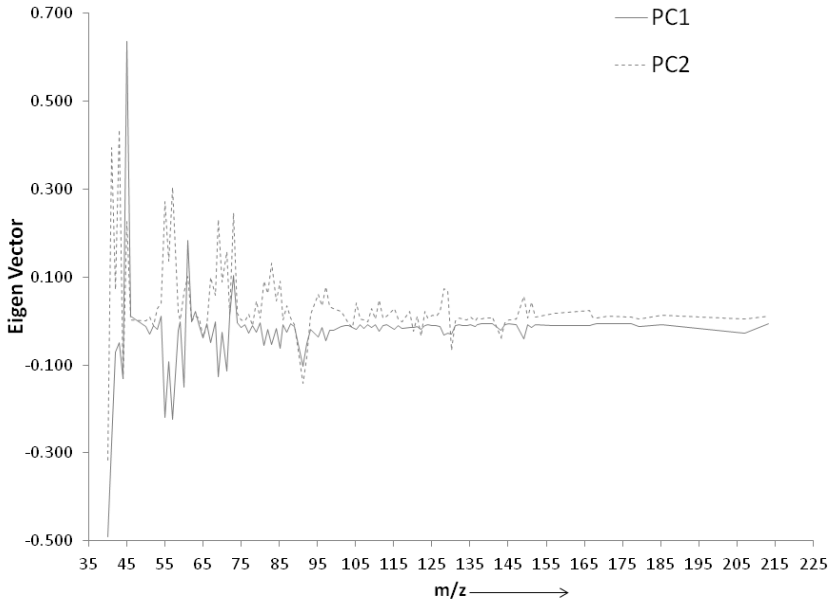
The PCA was applied to total mass spectral data of various samples. Cumulatively first two principal components explained 94.34 % (64.7% by PC1 and 29.6% by PC2) of data variance in the set of spectra of control and irradiated samples. PC score plot (Figure. 34) demonstrates a clear separation of samples according to radiation treatment.

Figure 34. Principal component analysis of total mass spectrum of control and irradiated grape samples (Plots of first two principal components); Score plot depicting distribution of various samples with PC1 and PC2. Three samples for each dose indicate three independent replications



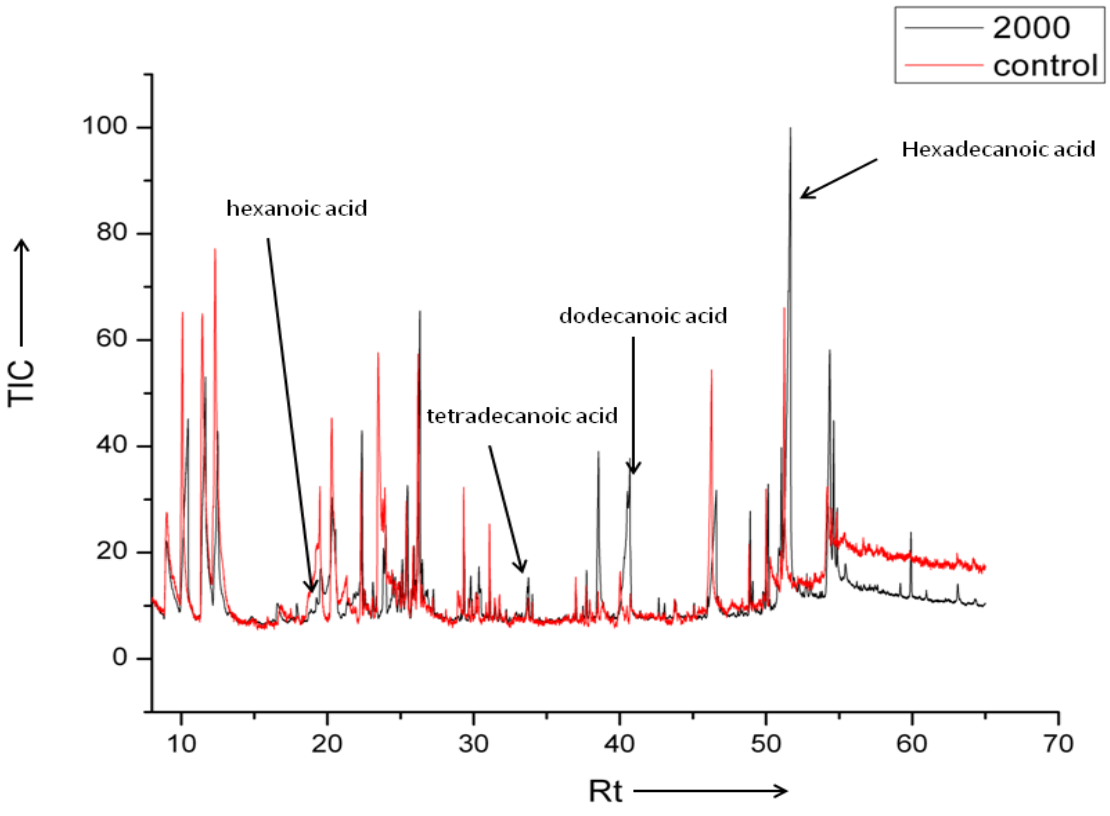
Score distribution from first two PCs demonstrated four separate groups in the samples analyzed. The first group had control samples and was located on negative side of both PC1 and PC2. Samples treated with radiation dose of 0.5 kGy constituted the second group located on positive side of PC1 but negative side of PC2. 1.0 kGy irradiated samples were located on positive side of both PC1 and PC2. However, samples treated with 1.5 kGy and 2.0 kGy, although separated from rest of treatments overlapped with each other and were located on negative side of PC1 but positive side of PC2. Thus, a complete segregation of irradiated samples was obtained from control samples. Further, to identify major ions responsible for observed segregation eigenvectors corresponding to PC1 and PC2 were plotted. m/z fractions having highest eigenvectors are more responsible for observed variations among samples. Figure 35 shows the eigenvectors corresponding to PC1 (64.7%) and PC2 (29.6%).

Figure 35. Eigenvectors of various m/z fragments for first two principal components.



The highest eigenvectors explaining the observed differences are m/z 45, 61 and 73 for PC1 and 41, 43, 55, 57, 69, 73, 81 and 83 for PC2. Majority of work related to the use of HS-MS as e-nose is reported on classification of wines and m/z fragments responsible for the reported variations are 115, 127, 129, 143 and 145 (109). Since, no reports are available for discrimination of irradiation treatments using ms-enose, direct correlation with existing reports was not possible. To identify possible group of compounds responsible for the observed changes in total mass spectra of samples, GC chromatograms of control and irradiated samples were compared. Figure 36 provides the overlaid GC chromatograms of control and irradiated (2 kGy) samples.

Figure 36. GC/MS chromatograms for control and irradiated (2 kGy) samples.



Although changes induced by radiation processing were very negligible as evident from the Figure 36, some differences in the chromatograms could be observed. It was noted that the irradiated samples had several extra peaks at Rt 20.7, 33.7, 40.6 and 51.6 min. All these compounds were free fatty acids like hexanoic acid, tetradecanoic acid, dodecanoic acid and hexadecanoic acid respectively (Figure 36). Moreover, in the present study it was observed that content of trans-2-hexenal increase with radiation dose. The observed differences in mass spectra of samples could possibly be due to the presence of these compounds. Major m/z fragments of these fatty acids are provided in Table 16.

Table 16. Major m/z fractions of various fatty acids and trans-2-hexenal

Compound	Major m/z
trans-2-hexenal	27, 29, 39, 41, 55, 57, 69, 83, 98
Hexanoic acid	41,42,43, 45,55,56,57,60, 61, 73, 74, 87
Dodecanoic acid	41,42,43, 45,53,55,56,57,60, 61,69,70,71, 73, 74,83,84,85, 87,97,101,115,129,157,200
Tetradecanoic acid	41,42,43, 45,55,56,57,60, 61,69,70,71, 73, 74,83,84,85, 87,97, 98,115,129,185,228
Hexadecanoic acid	41,42,43,44,45,55,56,57,60,61,69,70,71,73,83,84,85,87,97,98,129

The m/z responsible for observed variations between control and irradiated samples i.e. 41, 43, 45, 55, 57, 61, 69, 73, 81 and 83 were major fragments of all fatty acids, and trans-2-hexenal (Table 14). These results further suggests role of these compounds in observed variation between control and irradiated samples. However, complete identification of chemical compounds responsible for observed variations was beyond the scope of this study.

MS data was also analyzed using LDA which is a supervised classification technique where the number of categories and samples belonging to each category was previously defined. The method supplies a number of orthogonal linear discriminant functions, equal

to the number of categories minus one, that allow the samples to be classified in one or another category (110). Table 17 demonstrates classification results obtained using LDA classification methods. A very high and accurate classification rate for both control (100 %) and irradiated (90%) samples was obtained using LDA classification models.

Table 17. Classification statistics for linear discriminant analysis (LDA) for irradiation treatment

	Control	Irradiated	Total	% correct
Control	3	0	3	100.00%
Irradiated	1	9	10	90.00%
Total	4	9	13	92.31%

These results demonstrate that differences in control and irradiated samples could be successfully obtained in total mass spectrum and revealed by chemometrics. The total mass spectral fingerprints of samples contained information enabling discrimination of samples based on radiation treatment which was verified by mathematical models of PCA and LDA.

Most of the reports regarding use of mass spectra and chemometrics as an electronic nose employ head space sampler coupled with mass spectrometer as a tool for generating mass spectral data. However, in present study GC/MS with chromatographic separation was used as a tool for obtaining total mass fragmentation pattern for samples. Present approach of using GC/MS can offer three significant advantages over HS-MS based systems. Firstly, use of GC/MS with chromatographic separation could be used to separate solvents from samples; like in present study diethyl ether was separated from sample components. This could be useful for samples having substantial amount of solvents such as ethanol. Secondly, use of GC/MS could enable identification of

compounds responsible for differences among samples. Further, GC/MS is standard equipment present in majority of analytical laboratories and could thus be a cheaper alternative to the costly HS-MS based e-nose. Although chemometric analysis of XAD ether extract was successful for discrimination of radiation treatment of grapes this approach had some drawbacks. Firstly, this methodology employs column purification using solvents and thus making it time consuming. Secondly, GC/MS separation for sample extract has to be performed to separate solvent i.e. ether from rest of the sample as mass fragments (m/z) from solvent could interfere in final chemometric analysis. To overcome these limitations direct probe analysis of extract could offer suitable alternative. Direct probe could offer faster alternative to GC/MS analysis as sample is directly injected in mass spectrometer rather than eluting through the column. Thus, run times can be shortened from 60 min in standard GC/MS analysis to 11 min. However, direct probe analysis of XAD ether extract is not suitable because of possible presence of high boiling point compounds which could result in damage to MS equipment. To, overcome this problem direct probe analysis of steam distillation extract was attempted. In steam distillation only volatiles are extracted thus making it more amenable for analysis using direct probe of mass spectrometer.

3.4.2 Direct probe of steam distilled oil

Steam distilled oil was directly injected into mass spectrometer using a direct probe. During the direct probe analysis whole sample was infused into the mass spectrometer at once; therefore a single peak was obtained in TIC chromatogram. Figure 37 shows a representative TIC chromatogram obtained from direct probe analysis. This single peak

obtained was integrated to obtain a total mass spectrum of the sample. Figure 38 demonstrates total mass spectra obtained for control and irradiated samples.

Figure 37. A representative TIC obtained from direct probe analysis of steam distilled oil.

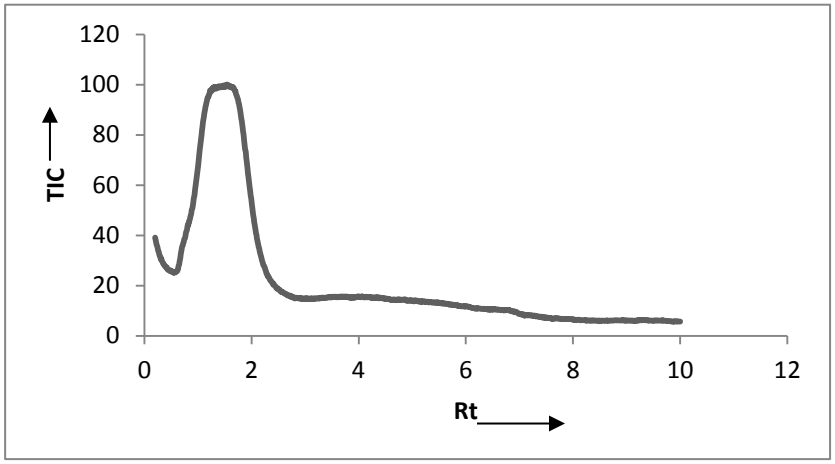
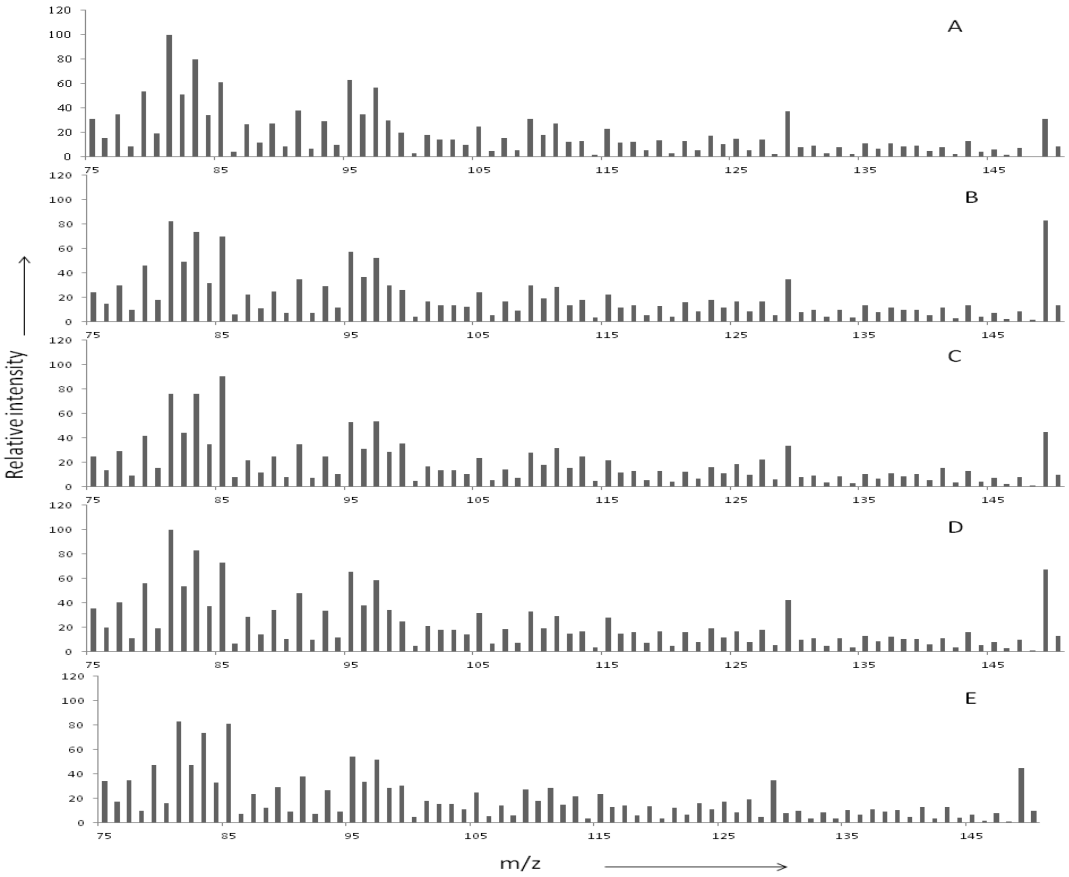
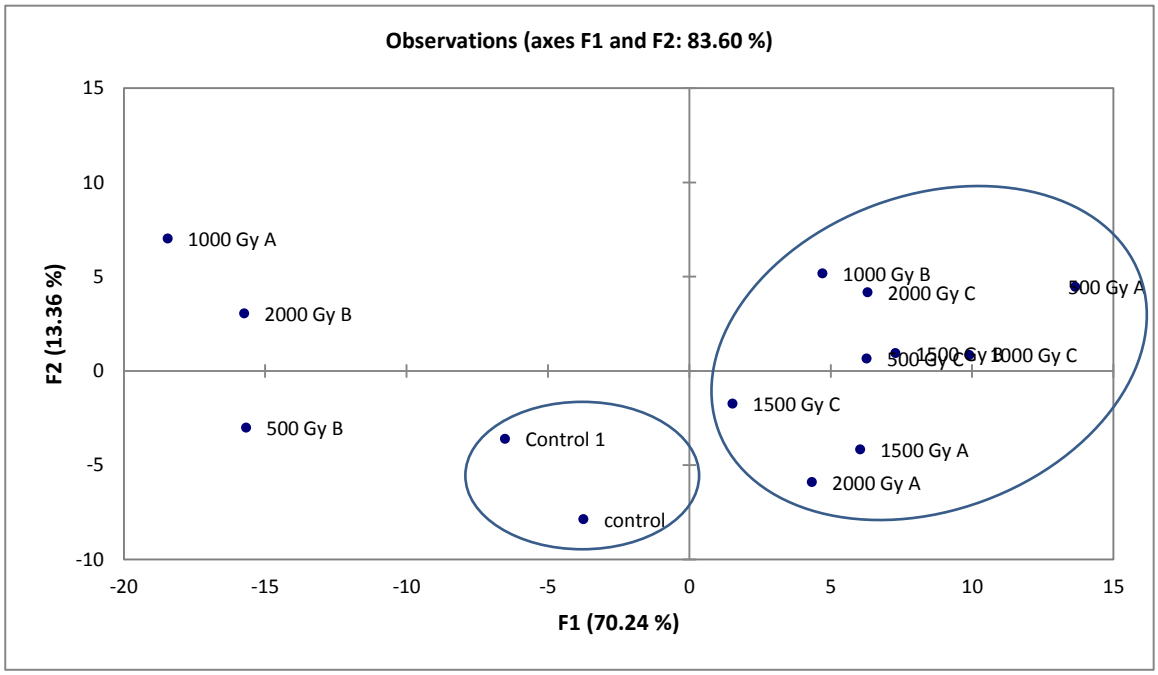


Figure 38. Total mass spectrum of control and irradiated samples. Steam distilled oil analyzed using direct probe mass spectrometer. A) Control B) 500 Gy C) 1000 Gy D) 1500 Gy E) 2000 Gy.



Very few differences were observed in mass spectra of control and irradiated samples when analyzed using direct probe. Nevertheless, data was further analyzed using PCA and score plot obtained is shown in Figure 39.

Figure 39. PCA score plot for direct probe data



PCA score plot (Figure 39) revealed that while a gross separation could be achieved between control and irradiated samples, no segregation could be obtained between various irradiation doses studied. Moreover, there were few outliers in irradiated samples. This might be due to the fact that in direct probe analysis m/z fractions below 75 were not considered. Since diethyl ether contributes to m/z up to 74, fractions below 75 were not considered for analysis to avoid interference due to solvent. However, it was observed during XAD analysis that major m/z fractions responsible for observed variations between control and irradiated samples are 41, 43, 45, 55, 57, 61, 69, 73, 81 and 83 (section 3.4.1). Since majority of fractions responsible for observed variations were below 75 ignoring them for analysis might have led to significant loss of variability in data. Furthermore, in direct probe analysis separation of small amounts of diethyl ether present from rest of the sample was not possible because whole sample is directly infused

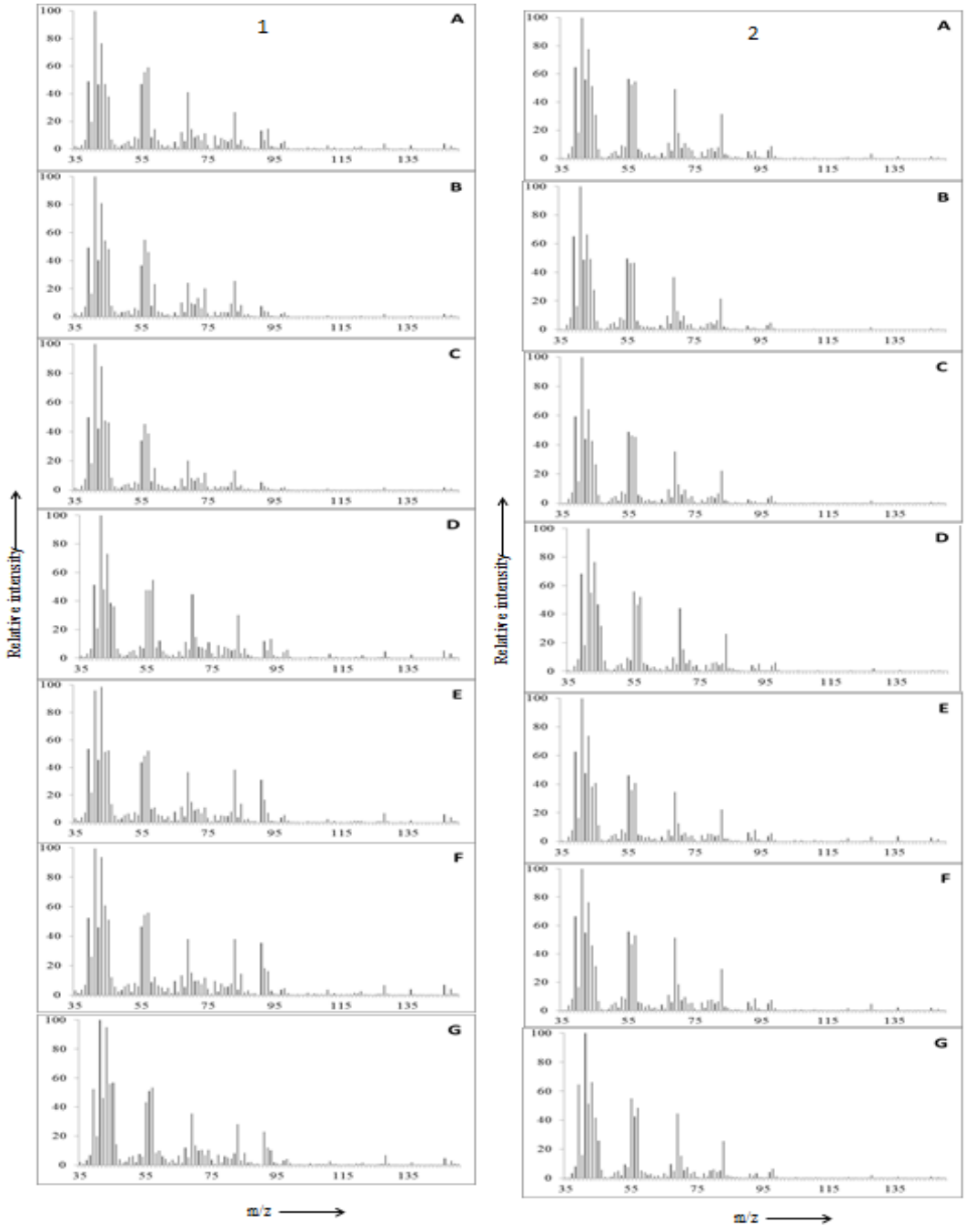
in mass spectrometer rather than separating through the column. Thus, the analysis performed using GC column rather than direct probe analysis was found to be suitable as the solvent ether was initially separated from the column avoiding interference in the final chemometric analysis.

Thus, it can be concluded from MS analysis of XAD ether extract and SDE extract that total mass spectrum in combination with chemometrics could successfully be used to screen radiation treatment. Although, XAD ether extract could be used for screening of radiation treatment, however the method was not rapid. It involved column purification requiring extensive use of solvents, concentration of ether extract and subsequent analysis by GC/MS to obtain total mass spectrum. To overcome these limitations headspace extraction with SPME and subsequent analysis with a GC/MS equipped with a transfer line rather than a column was attempted.

3.4.3 SPME in combination with chemometrics for identification of radiation treatment

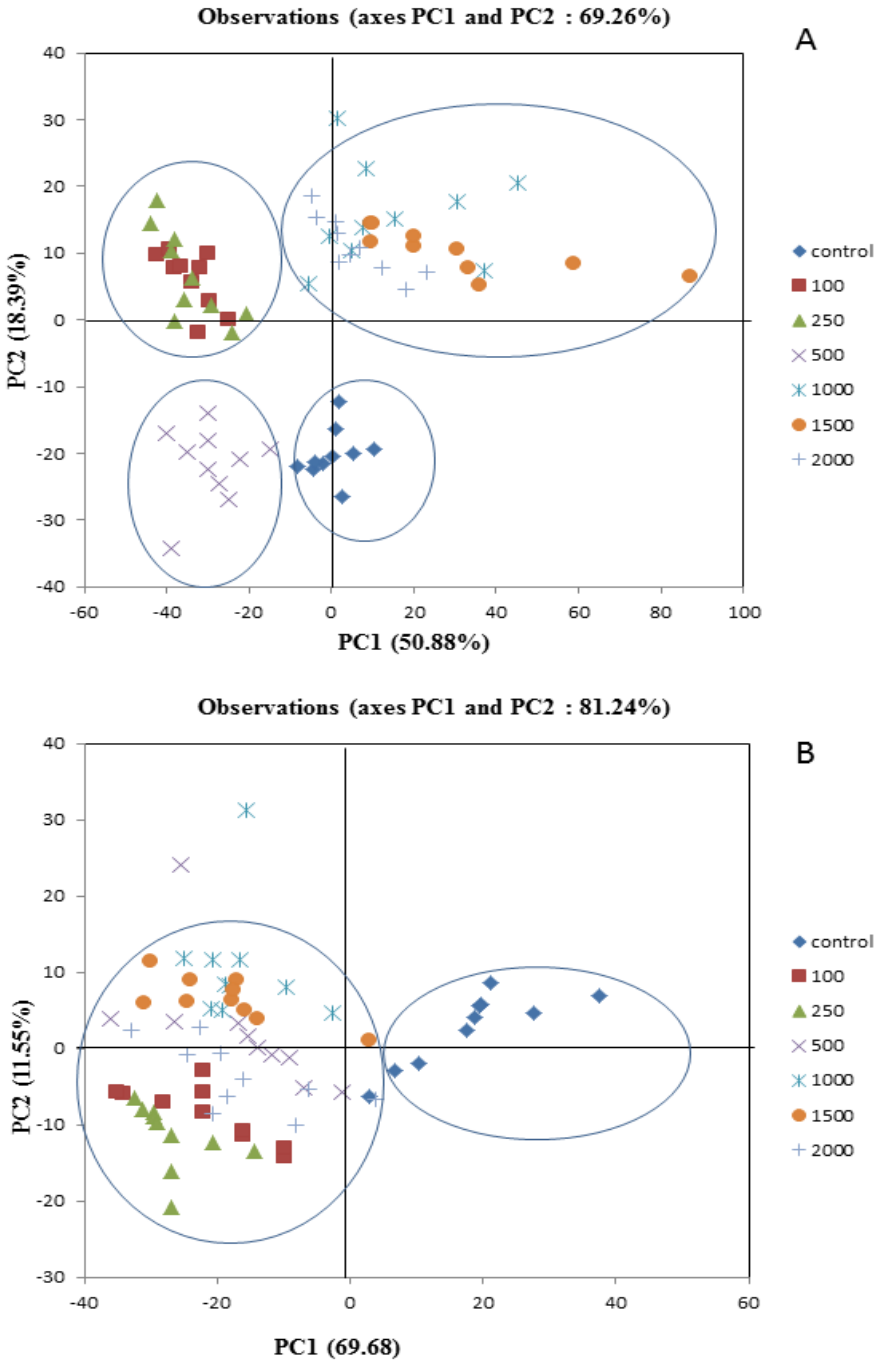
Volatile sample head space was extracted using SPME and fiber was desorbed in split port of GC/MS equipped with a transfer line. A single peak was obtained which was integrated to obtain total mass spectrum. Two varieties of grapes 'Cabernet Sauvignon' and 'Chenin Blanc' were chosen for present work. Total mass spectra obtained for control and irradiated grape samples are shown in Figure 40.

Figure 40. Total mass spectra of grape samples 1) Total mass spectrum of Cabernet Sauvignon samples. 2) Total mass spectrum of Chenin Blanc samples. A) Control B) 0.1 kGy C) 0.25 kGy D) 0.5 kGy E) 1 kGy F) 1.5 kGy G) 2 kGy.



Very few notable differences were observed visually in mass spectrum of control and irradiated samples for both varieties. Data was therefore, processed by multivariate statistical analysis in order to reveal more differences among samples. The PCA was applied to total mass spectral data of various samples. PC score plots for Cabernet Sauvignon and Chenin Blanc are demonstrated in Figure 41A and 41B, respectively.

Figure 41. Principal component analysis of total mass spectrum of control and irradiated grapes A) Score plot depicting distribution of various Cabernet Sauvignon samples with PC1 and PC2 B) Score plot depicting distribution of various Chenin Blanc samples with PC1 and PC2. Each point is average of two replications for a given sample.



Interestingly, Cabernet Sauvignon samples segregated into four groups. First group had control samples and was located on positive side of PC1 and negative side of PC2. Second group constituted 0.5 kGy irradiated samples and was located on negative side of both PC1 and

PC2. Samples subjected to radiation dose of 100 and 250 Gy constituted third group and was located on negative side of PC1 and positive side of PC2, while, fourth group located on positive side of both PC1 and PC2 had samples irradiated to a dose of 1, 1.5 and 2 kGy (Figure 41A). However, samples of second grape variety i.e. Chenin Blanc segregated only into two groups (control and irradiated) based on score distributions of first two PCs (Figure 41B). Among both the varieties studied, segregation between different doses was observed in Cabernet Sauvignon while in Chenin Blanc differences were observed only between control and irradiated samples. This might be due to different nature of volatiles present in Cabernet Sauvignon variety as compared to Chenin Blanc. Since a transfer line is used in present study a detailed volatile profile was not obtained and hence identifying individual volatiles responsible for observed segregation in Cabernet Sauvignon was not possible.

PCA is basically a data reduction technique and is useful for observing differences and similarities among various samples. Results obtained by PCA analysis revealed that there are differences between control and irradiated samples and thus complex mass spectral data could possibly be used for building a supervised classification model.

MS data was also analyzed using LDA which is a supervised classification technique where the number of categories and samples belonging to each category was previously defined. The method supplies a number of orthogonal linear discriminant functions, equal

to the number of categories minus one, that allow the samples to be classified in one or another category (110). LDA models were prepared using total 70 numbers of samples consisting of all doses and verified against test data consisting of six samples which were not used for model building. Test samples were chosen randomly out of total sample set. Discrimination among samples for various doses was achieved by use of first linear discriminant function for both varieties. Table 18 demonstrates classification results obtained using LDA classification methods.

Table 18. Classification results for linear discriminant analysis (LDA) for irradiation treatment for grape samples

	Control	0.1 kGy	0.25 kGy	0.5 kGy	1 kGy	1.5 kGy	2.0 kGy	Total	% correct
Grapes									
Cabernet Sauvignon									
Control	1							1	100
100		1						1	100
250			1					1	100
500				1				1	100
1000					1			1	100
1500						1		1	100
2000									100
Chenin Blanc									
Control	1							1	100
100		1						1	100
250									
500				1				1	100
1000					1			1	100
1500						1		1	100
2000							1	1	100

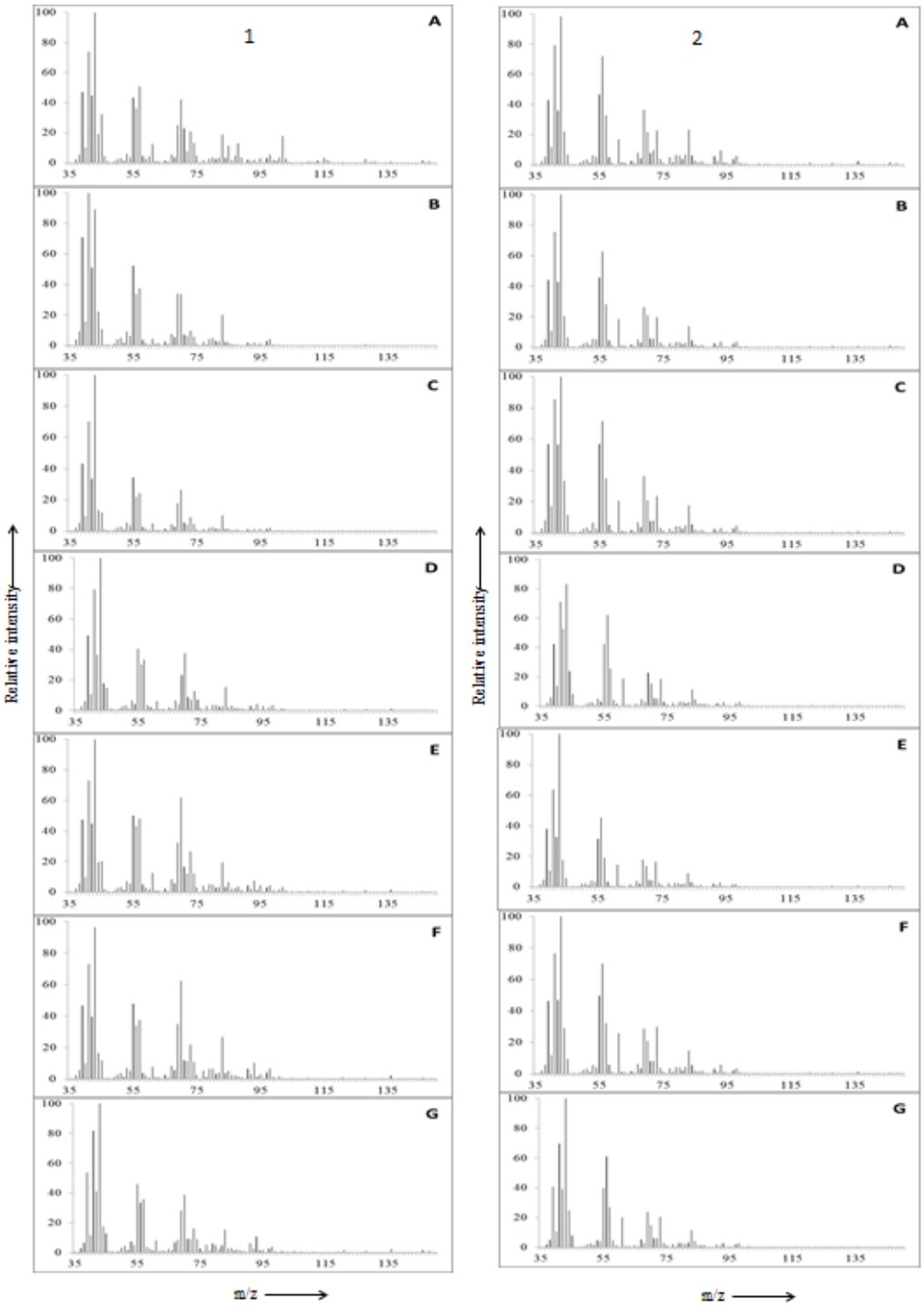
A very high and accurate classification rate for both control (100 %) and irradiated (100%) samples at every dose was obtained using LDA classification models.

Furthermore, major m/z responsible for obtained discrimination in LDA models were identified as 56, 67, 79, 117 and 128. Majority of work related to the use of HS-MS as e-nose is reported on classification of wines and m/z fragments responsible for the reported variations are 115, 127, 129, 143 and 145 (109). Since, no reports are available for discrimination of irradiation treatments using ms-e_nose, direct correlation with existing reports was not possible.

These results demonstrate that differences in control and irradiated samples could be successfully obtained in complex mass spectrum and revealed by chemometrics. The complex mass spectral fingerprints of samples contained information enabling discrimination of samples based on radiation treatment which was verified by mathematical models of PCA and LDA. Unsupervised technique (PCA) segregated control and irradiated samples but no dose wise segregation was achieved. However, use of supervised technique (LDA) aided in separation of samples based on different doses.

Thus, use of GC/Ms as e-nose for rapid identification of radiation treatment was successfully demonstrated in grapes. However, to demonstrate wide applicability of develop methodology studies were also carried out on two varieties of apples (Red Delicious and Royal Gala). Total mass spectra obtained for control and irradiated apple samples are shown in Figure 42.

Figure 42. Total mass spectrum of control and irradiated apple samples. 1) Red Delicious 2) Royal Gala. A) Control B) 0.1 kGy C) 0.25 kGy D) 0.5 kGy E) 1 kGy F) 1.5 kGy G) 2 kGy



Very few notable differences were observed visually in mass spectrum of control and irradiated samples for both fruit samples. Data was therefore, processed by multivariate statistical analysis in order to reveal more differences among samples. The PCA was applied to total mass spectral data of various samples. PC score plots for apple varieties Red Delicious and Royal Gala are shown in Figure 43A and 43B, respectively. In case of Red Delicious segregation between control and irradiated samples was observed in plot between PC2 and PC3 while in case of Royal Gala two separate groups (control and irradiated) were observed in score distribution from first two PCs (Figure 43).

A complete segregation of control and irradiated samples was observed in both the varieties analyzed (Figure 43). PCA analysis discriminated control and irradiated samples in all varieties studied and thus complex mass spectral data could possibly be used for building a supervised classification model. MS data was also analyzed using LDA which is a supervised classification technique. LDA models were prepared using total 70 numbers of samples consisting of all doses and verified against test data consisting of six samples which were not used for model building. Test samples were chosen randomly out of total sample set. Discrimination among samples for various doses was achieved by use of first linear discriminant function. Table 19 demonstrates classification results obtained using LDA classification methods. A very high and accurate classification rate for both control (100 %) and irradiated (100%) samples at every dose was obtained using LDA classification models.

Figure 43. PCA score plots for apples. A) PC score plots for Red Delicious B) PC score plots for Royal Gala

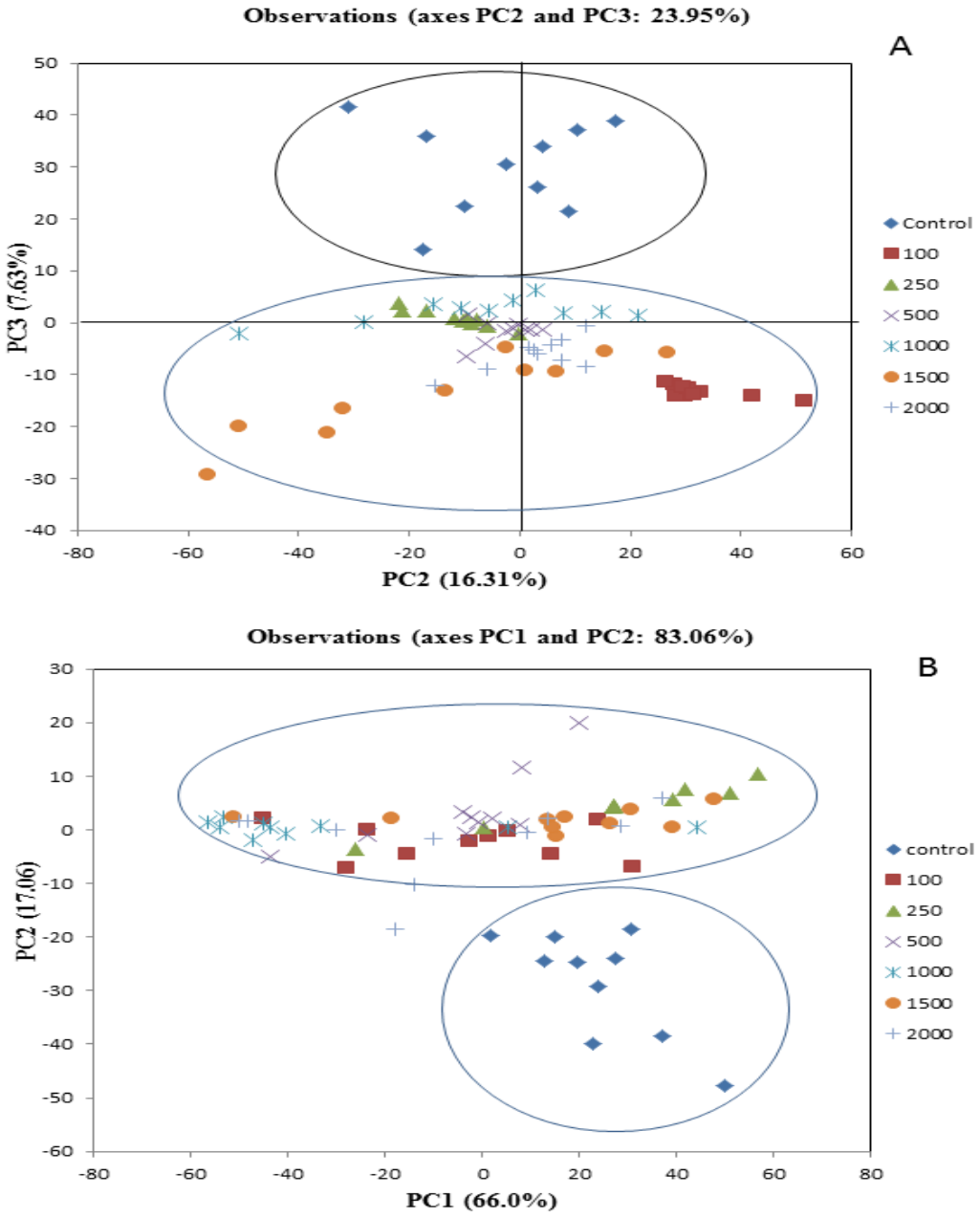


Table 19. LDA analysis for apple samples

	Control	100	250	500	1000	1500	2000	Total	% correct
Apples									
Red delicious									
Control	1							1	100
100		2						2	100
250									
500									
1000					1			1	100
1500						1		1	100
2000							1	1	100
Royal Gala									
Control	1								100
100		1							100
250									
500									100
1000					1				
1500						2			100
2000							1		100

The major m/z ions responsible for obtained discrimination in LDA models were identified as 59, 69, 73, 74, and 126 for apple samples. Majority of work related to the use of HS-MS as e-nose is reported on classification of wines and m/z fragments responsible for the reported variations are 115, 127, 129, 143 and 145 (190). Since, no reports are available for discrimination of irradiation treatments using ms-e_nose, direct correlation with existing reports was not possible.

These results demonstrate that differences in control and irradiated samples could be successfully obtained in complex mass spectrum and revealed by chemometrics. The complex mass spectral fingerprints of samples contained information enabling discrimination of samples based on radiation treatment which was verified by mathematical models of PCA and LDA. Unsupervised technique (PCA) segregated

control and irradiated samples but no dose wise segregation was achieved. However, use of supervised technique (LDA) aided in separation of samples based on different doses.

3.4.4 Conclusions

GC/MS analysis of ether fraction from XAD followed by chemometrics resulted in complete segregation of control and irradiated samples while segregation between control and irradiated samples could not be obtained in direct probe analysis of steam distilled oil. Analysis of XAD ether extract further revealed that observed differences in radiation processed samples might be due to presence of free fatty acids. However, methodology with XAD ether extract involved column purification requiring solvents, concentration of solvents followed by GC/MS and chemometrics. Thus, to make method simple and rapid head space extraction by SPME and direct infusion of extract into mass spectrometer followed by chemometrics was attempted. This also resulted in complete segregation of control and irradiated samples. Use of supervised technique such as LDA further resulted in complete segregation based on absorbed dose. Wide applicability of developed methodology was successfully demonstrated on apple samples.

Nevertheless, it should be emphasized that the method detailed herein is not based on radiation specific markers, hence it should be used only as rapid screening technique. Moreover, chemometric models could be affected by different volatile extraction matrices, kind of fruits, varieties, species, maturation, pollution, stress and phytosanitary treatments. Therefore, in actual scenario building models with large number of data while accounting for above factors could result in more robust models. However, the technique detailed herein could be of great value for screening samples suspected to be irradiated,

but, positive results should be confirmed using a standardized method to specifically prove a radiation treatment.

Most of the reports regarding use of mass spectra and chemometrics as an electronic nose employ head space sampler coupled with mass spectrometer as a tool for generating mass spectral data. However, in present study GC/MS with transfer line was used as a tool for obtaining complex mass fragmentation pattern for samples. GC/MS is standard equipment present in majority of analytical laboratories and could thus be a cheaper alternative to the costly HS-MS based e-nose. However, further studies using more number of samples will be required to validate the method before it is to be widely adopted by food irradiation industry. Nevertheless, present study demonstrated that the technique of combining instrumental methods with suitable chemometrics procedures could provide useful tool to rapidly screen samples for radiation treatment.

Chapter 4: *Conclusions*

4. Conclusions and future work direction

Present study was performed with a view to enhance aroma quality of grape products such as wine using radiation processing. Other aims were possible inactivation of spoilage microorganisms in wine using radiation processing and development of rapid detection method for radiated food products using total mass spectrum and chemometrics.

Procedures for isolation, identification and quantification of aroma glycosides were optimized. SPE extraction of grape juice followed by pectinase hydrolysis and subsequent identification by GC/MS was most suitable for identification of aroma glycosides. Radiation processing of grapes resulted in significant changes in content of bound flavor precursors. Both increased extraction and radiation induced degradation in aroma glycosides was observed. Tertiary and aromatic alcohols were found to be more susceptible for radiation induced hydrolysis as compared to primary alcohols. Maximum change in glycosidic precursors due to radiation processing was observed in the Cabernet Sauvignon variety while, least changes were observed in Shiraz variety. In free aroma constituents, most remarkable change was observed in content of n-hexanal and trans-2-hexenal. Radiation induced degradation of galactolipids resulting in increased content of linolenic acid might be reason for observed increase in trans-2-hexenal. Linolenic acid is oxidized to these C6 aldehydes by enzymes of lipooxygenase pathway. A radiation induced oxidation of 1-hexanol to hexanal is also suggested. A higher content of α-terpineol, linalool, heptanol, 1-octen3-ol, and 2-hexen-1-ol was observed in radiation processed samples as compared to control. These compounds have floral odors and can play an important role in wine aroma quality.

Radiation processing of grapes also resulted in wines with better red color, higher color intensity and anthocyanin content as compared to the controls. Wines prepared from irradiated grapes also demonstrated higher total phenolic and antioxidant content. Highest color intensity, anthocyanin content, phenolic and antioxidant content were observed in wines prepared with grapes subjected to a dose of 1500 Gy. There were no major changes in aroma profile of wine samples prepared with irradiated grapes. It was observed in descriptive sensory analysis of wines that irradiated samples (1500 Gy) had more red color and higher fruity and berry odor as compared to control. Higher fruitiness observed in wines prepared from radiation processed (1.5 kGy) is due to the presence of β-damascone, and increased content of 1-heptanol, 2-ethyl hexanol, 1-octanol. . However, no radiation induced off flavor or taste was observed by sensory panel.

When wines were directly subjected to radiation processing for possible inactivation of spoilage microorganisms, no effect was observed on color, total anthocyanins, total antioxidants and total phenolics up to a dose of 1.5 kGy. However, beyond a dose of 1.5 kGy, very less, but significant decrease in colour intensity was observed. Sensory analysis of wine revealed no differences between control and radiation processed (1.5 kGy) wine. D10 values of all microorganisms tested, demonstrated a reduction of up to 50 percent for irradiation in wine as compared to irradiation in saline. A dose of 1.5 kGy, whch was found to be amenable for wine irradiation can eliminate 99.9 percent of all spoilage microorganisms tested in present study.

Head space- mass spectrometry (HS-MS) was successfully demonstrated as a tool for obtaining complex mass spectrum and its use as e-nose. HS-MS was used as a tool to obtain total mass spectra of control and irradiated grape and apple samples. Analysis of mass spectra of samples by chemometrics resulted in successful classification of grape and apple samples according to irradiation treatment given. Although, present methodology resulted in complete classification of

irradiated samples, the technique is based on complex mass spectrum rather than any radiation specific marker. This methodology should thus be recommended as a rapid screening test and positive samples should be verified by more appropriate chemical methods. Nevertheless, these results suggest possibility of using HS-MS based e_nose for rapid screening of irradiated samples.

Finally, as compared to other physical methods such as PEF and HHP, application of radiation processing on a large industrial scale is feasible and could be a practical approach to obtain wines with better antioxidant and organoleptic characteristics.

Future work directions

Use of radiation processing to inactivate natural microflora present on grapes could possibly be attempted. Presently, SO_2 is widely used in industry to inhibit natural microflora present on grapes before start of fermentation. However, recently there are several concerns about possible health effects of SO_2. Since in present study feasibility of radiation processing (1.5 kGy) of grapes for obtaining wines with improved color and antioxidants was demonstrated. Therefore, dose of 1.5 kGy could be used for inactivation of natural micro-flora of grapes and this could possibly lead to preparation of low SO_2 wines.

A rapid screening method for identification of radiation treatment based on total mass spectrum of volatile head space and chemometrics was suggested in thus study. Efficacy of this method was successfully demonstrated on grapes and apples. In future studies, developing more robust statistical models with higher number replicates and more variety of food products could possibly be attempted.