Chapter 1

Introduction and Literature Review
Chapter -1

Introduction and Literature Review
1. Grape

1.1 The Origin and Evolution

Grape is believed to have originated in Armenia near the Black and Caspian seas in Russia. An independent and recent origin of grapes is also traced to North America. From Armenia, grapes spread westwards to Europe and Eastwards to Iran and Afghanistan. Grape was introduced into India in 1300 AD by the Moghul invaders. Grape cultivation flourished in Baluchistan and North-West Frontier Province during the 16th century. In India, grape cultivation declined after the fall of Moghul rulers but was reintroduced in south India (Aurangabad district of Maharashtra) by Mohammed-Bin-Tughlak and since last 60 years grape is commercially cultivated in India. The old \textit{Vitis vinifera} grapes, originating in Armenia, have perfect flowers while the grapes of America, which are of recent origin, usually have imperfect flowers. It is believed that originally varieties with pure male / female flowers to varieties with various degrees of maleness / femaleness to those with perfect flowers existed and during the course of evolution only the varieties with perfect flowers have been selected.

1.2 Reproductive Biology of Grape

The flowers of cultivated grapes are usually hermaphrodite (perfect), while wild grapes are often dioecious. Flower buds just before bloom (anthesis) are covered by the interlocking petals (cap or calyptra). At anthesis the cap separates from the base of the ovary and falls off. The stamens spread out and pollen is shed and falls on to the stigma of the pistil. This is bloom and it lasts from 2-7 days depending on temperature. In grapes not all ovules are capable of fertilization and the unfertilized ovules drop off. This is known as shatter. Commercial grapes mostly belong to Euvitis section comprising of \textit{V. vinifera}, \textit{V. labrusca}, \textit{V. riparia} and \textit{V. rupestris} with the haploid chromosome number 19. In the other section, Muscadinia, the haploid chromosome number is 20. The species of grapes are quite heterozygous and seedling offspring show wide genetic variability. Seedlings vary not only in the qualities of their fruit but in vegetative vigor also. Because of these variations, seeds are not used for propagation of vines for vineyard purpose.
1.3 Area, Production and Productivity

1.3.1. India on world background

Grapes are grown commercially in 89 countries worldwide. Table 1.1 narrates the present status of area, production and yield of grapes in India with reference to world and leading countries during the year 2007-08. Total area under grape was 7.5 million hectare with total production of 66.3 million MT of fresh grapes. Spain stands first with 16.00% of total area under grape cultivation while Italy aheads with 12.85% share in world total fresh grape production. India accounts for 64.4 thousand hectare (0.86%) and 1.68 million MT (2.53%) of grape production. In terms of yield per hectare, India stands first with average fresh grape production of 26.00 MT/ha as against world average 8.84 MT/ha.

Table 1.1: Present Status of Area, Production and Yield of Grapes in India with Reference to World and other Leading Countries during the Year 2007-08

<table>
<thead>
<tr>
<th>Leading countries in the world</th>
<th>Name</th>
<th>Qty MT</th>
<th>% Share</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape area of harvest (Ha)</td>
<td>Spain</td>
<td>1,200,000</td>
<td>16.00</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td>830,000</td>
<td>11.06</td>
</tr>
<tr>
<td></td>
<td>Italy</td>
<td>770,000</td>
<td>10.26</td>
</tr>
<tr>
<td>Grape production (tonnes)</td>
<td>Italy</td>
<td>8519418</td>
<td>12.85</td>
</tr>
<tr>
<td></td>
<td>France</td>
<td>6500000</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>China</td>
<td>6250000</td>
<td>9.40</td>
</tr>
<tr>
<td>Grape yield (tonnes/ha)</td>
<td>India</td>
<td>26.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>16.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>South Africa</td>
<td>13.91</td>
<td></td>
</tr>
</tbody>
</table>

(Ref: FAOSTAT 2009, Indian Horticulture Database 2008)

Table 1.2 presents export-import of grapes and various grape products at global level vis-à-vis India during 2006-07. In spite of the highest productivity in India, it lags far behind in terms of share in export of fresh grapes (2.50%), raisins (0.04%) and wines (0.01%). With reference to global trade Chile leads in fresh grape export (19.23%) while France leads in wine export (34.88%).
Table 1.2: Export-import of Various Grape Products at Global Level vis-à-vis India during the Year 2006-07

<table>
<thead>
<tr>
<th>Product trade</th>
<th>World (MT)</th>
<th>India (MT)</th>
<th>% Share (Value)</th>
<th>Leading countries in the World</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export of grapes</td>
<td>3,416,087</td>
<td>85,563</td>
<td>2.50 (1.65)</td>
<td>Chile 823,198 24.09 (19.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Italy 417,217 12.21 (12.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>USA 290,008   8.48 (12.36)</td>
</tr>
<tr>
<td>Export of raisins</td>
<td>774,502</td>
<td>355</td>
<td>0.04 (0.03)</td>
<td>Turkey 240,743 31.08 (28.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Iran 148,035   19.11 (13.24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>USA 113,897   14.71 (21.58)</td>
</tr>
<tr>
<td>Export of wine</td>
<td>8,352,554</td>
<td>1,059</td>
<td>0.01 (0.01)</td>
<td>France 1,461,663 17.50 (34.88)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Italy 1,793,152 21.47 (18.01)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spain 1,336,762 16.00 (8.74)</td>
</tr>
<tr>
<td>Import of grapes</td>
<td>3,387,567</td>
<td>1,976</td>
<td>0.06 (0.04)</td>
<td>USA 530,889   15.67 (18.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Germany 326,546 9.64 (9.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Russia 320,677 9.47 (6.73)</td>
</tr>
<tr>
<td>Import of raisins</td>
<td>8,874</td>
<td>1,13</td>
<td>1.13 (1.34)</td>
<td>UK 117,569   16.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Germany 79,404 10.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Russia 67,910 4.19</td>
</tr>
<tr>
<td>Import of wine</td>
<td>7,808,225</td>
<td>1,815</td>
<td>0.02 (0.05)</td>
<td>Germany 1,330,423 17.04 (10.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UK 1,184,626 15.17 (18.38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>USA 782,423 10.02 (18.42)</td>
</tr>
</tbody>
</table>

(Ref: FAOSTAT 2009, Indian Horticulture Database 2008)

1.3.2 Maharashtra on India background

The figures 1a and 1b illustrate the state wise area and production of grape in India during 2007-08. The state of Maharashtra stands at leading position with 46.60 thousand hectare area with 72.36% share in total area under grape cultivation in India. Maharashtra produced 1.29 million MT fresh grapes with 76.92% share in India. Karnataka (12.46%), Tamilnadu (4.98%) and Andhra Pradesh (3.12%) were other major grape growing states.
(Ref: Indian Horticulture Database, 2008)

**Figure 1.1** State wise area (A) and production (B) of Grapes in India during the Year 2007-08.
1.3.3 Consumption Pattern

There is vast difference in consumption pattern of grapes in India as compared to other major grape growing countries of the world. The major food products made from grapes are reflected in the utilization data (USDA 2008).

Table 1.3: Consumption pattern of grapes in India.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>India</th>
<th>World</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table grapes</td>
<td>75-80%</td>
<td>10-15%</td>
</tr>
<tr>
<td>Raisins (dried grapes)</td>
<td>17-20%</td>
<td>25-30%</td>
</tr>
<tr>
<td>Wine</td>
<td>0.5%</td>
<td>50-55%</td>
</tr>
<tr>
<td>Juice, Jelly, Canned foods</td>
<td>1.5%</td>
<td>6-10%</td>
</tr>
</tbody>
</table>

Currently 75-80% production is utilized as table grapes. The major bulk is harvested in March-April, but as cold storage facilities are inadequate, there are frequent market gluts. Therefore, there is need to diversify the uses of grapes. There is great scope for development of grape wine industry in India. Looking forward to this Maharashtra and Karnataka State Governments has come up with new policies for promoting grape processing.

Grape is an important commercial fruit crop, which receives frequent application of large number of agrochemicals, e.g. pesticides etc. throughout the cropping season for management of various pests and diseases. At present, in India grape is grown over an area of 60,000 ha with an annual production of 1.2 million tonnes (FAO, 2005). Although exact figures are not available regarding the current area and production of wine grapes in India, it is estimated that around 1200 hectares with annual Production of 9.5 million liters. In Maharashtra and about 100 hectares near Bangalore in Karnataka are currently under wine grape cultivation. Indian grape is under constant scrutiny of the environment and health protection agencies worldwide, as in India, the cultivation of grapes receives frequent application of large number of pesticides and further, grape is mostly consumed as fresh fruit in intact form without any processing. The residues left on the grapes during harvest can be carried through into the wine \(^{[1]}\).
1.4 Residue Analysis Literature Review

In food analysis, the methods of analysis need to confirm and quantify as many residues as possible. The multi-analysis of pesticides is a difficult task for the chemist, due to the variety of different groups of pesticides having a broad range of physio-chemical properties, e.g. polarity, thermal stability, acidity etc. Determination of pesticide residues in food are often complicated by the presence of fats and interfering natural compounds, this is why it is often requires a time-consuming clean up and pre-concentration procedure before analysis. Due to the large number of pesticides on the world market, the development of multi-residue methods is preferred in terms of pesticide residue analysis [2]. Food matrices are complex and may be difficult to analyze and require highly selective analytical techniques to determine target compounds and to characterize unknown compounds. The use of Gas Chromatography (GC) and Liquid Chromatography (LC) with Mass Spectrometry (MS) is a well-accepted method for confirmation on the identity of pesticides at very high sensitivity, whereas Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) has become an analytical technique to determine the content of inorganic compounds in food. Although LC-MS-MS is a powerful tool for fast and selective analysis, which enables chromatography with low separation efficiency, coeluting compounds can lead to problems in the MS response due to ion suppression of the signal. So an efficient sample purification and chromatographic separation should not be under-evaluated [3].

The interest for making the analysis as quick and cheap as possible have led scientists to search for a coupling between the clean-up system and the system for the determination of analytes, and also to reduce the clean-up/concentration procedure. Slobodnik et al. [4] have used on-line coupling of solid phase extraction (SPE)-LC to MS to detect 17 pesticides in water and trace enrichment separation before LC-MS was used by Hogenboom et al. [5] for the determination of 17 pesticides in water.

The use of pesticides to control pests of crops, to eliminate parasites and insects of livestock, and to maintain hygienic conditions in production lines or during storage, has played a major role in expanding the availability of produce to the consumer because they have allowed growers and handlers to increase
production volume, extend shelf life, and improve the appearance of many foods. The consequences of their use and the realization that some foods contain residues of these compounds are of paramount importance to the consumers. Such contaminated food can readily reach the population and growing concern has been expressed as to the possible hazards for human health. Since human safety is the foremost consideration in food production, superseding even the obvious importance of economic factors, international systems of legal control have been established to prevent residue contaminated products from entering the human food supply. In this way, an important aspect of food safety is the control of pesticide residues on food.

The analysis of pesticide residues represents a basic instrument not only for the protection of human health, through risk assessment studies and prevention strategies, but also for trade and official control purposes. There is a growing interest to apply liquid chromatography mass spectrometry (LC-MS) in control of pesticide residues to ensure food safety. Social Sciences Citation Index, and Arts & Humanities Citation Index) using the keywords “Pesticides, Food,” and “Liquid Chromatography-Mass Spectrometry.” Among these articles, there are several reviews, which have been published since 2000, about some aspects of pesticide residue control by LC-MS, as applications of some of these instruments in pesticide trace analysis or as representative with other techniques used in the analysis of pesticide residues in food and drinks.

This review addresses exclusively LC-MS and is thus focused on the LC-MS analysis of pesticide residues to ensure food safety. After an introduction about the regulations that highlights the importance of these techniques to meet the official requirements on analytical performance, the different mass spectrometers used in this field of research, as well as the LCMS interfaces and the difficulties associated with quantitative LC-MS determination, are discussed. The ability to use practical data for quantifying pesticides together with the option of obtaining structural information to identify target and non-target parent compounds and metabolites is illustrated. Special attention is paid to the impact of sample preparation and chromatography on the ionization efficiency of pesticides from food. The last section is devoted to its applications from a food safety point of view. [6]
1.5 Pesticides and Health

Public concern over pesticide residues in food is increasing since it has become a significant food safety concern. Very little data are available regarding human exposure to pesticides through consumption of processed and finished food products. However, a study by Andrey and Amstutz\(^7\) showed that 61% of 83 labeled “organic” wines and 87% of 15 conventional wines found in Swiss market places contained pesticide residues. Currently, there are few studies in the United States on the presence of pesticides in wines or alcohol-based beverage products, although there are tolerances set for table and wine grapes\(^8,9\). These concerns have caused many regulatory agencies to increase their scope of analysis as well as the number of samples analyzed in their monitoring programs for risk assessment\(^9\). Cabras et al\(^10\) and Navarro et al\(^12, 13, 14\) explored into the fate of pesticides from the processed grapes through the vinification process to the final wine product. There were many multiresidue procedure for beverage alcohol products such as wine\(^\)\(^11\).

1.5.1 Regulations

Maximunm residue limits (MRLs): Currently, more than 800 pesticides (active ingredients) are sold worldwide. For many of these compounds, legal action levels (e.g., maximum residue levels (MRLs) or tolerances) in food have been established. Pesticide residues have been regulated by several legislative authorities throughout the world, all concerned with the quality, efficacy, and safety in the use of pesticides. For instance, MLRs for pesticide residues are set at European Union (EU) level for approximately 150 plant protection products and at member state level for any other unharmonized products. In an attempt to facilitate world trade while at the same time protecting the health of the consumers, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations (UN) have set a joint FAO/WHO Codex Alimentarius Commission to coordinate food standards. One of the main tasks of the Codex committee is to establish universal MRLs\(^15\).

EU has also concerned with the concordance of pertinent regulations that differ among member states to allow the free circulation in the market of food. Considerable progress has been made in establishing EU-harmonized MRLs for all
pesticide residues. New laws, such as the European Directive 91/414/EEC \cite{16} or the Food Quality Protection Act (FQPA) in the United States of America (USA) \cite{17}, have increased the standards for human health. The quality standards include the reassessment of the MRLs, which have been reduced in comparison to the previous limits \cite{18, 19, 20}. In general, the MRLs are in the range of 0.01–10 mg/kg, depending on the combination commodity and pesticide, the lowest is characteristic of banned compounds because it is considered that this would be the minimum limit of detection (LODs) achievable. A value of zero is considered below the LOD because of the slight inaccuracies in the measurement methods available.

These regulations encompass special provisions for infants and children, including additional safety factors. Specific rules on the presence of pesticide residues in infant and follow-on formulae, as well as in processed cereal-based baby food and baby food are set out in Commission Directives 1999/50/EC and 1999/39/EC \cite{21, 22}. These Directives require that baby food contains no detectable levels of pesticide residues, meaning not more than 0.01 mg/kg of pesticide residues. In addition, Directives 2003/14/EC and 2003/13/EC prohibit the use of certain very toxic pesticides in the production of any baby food, and establish levels lower than the general maximum level of 0.01 mg/kg for a few other very toxic pesticides \cite{23, 24}.

\section*{1.6 Grape and Wine Chemistry}

In the recent years, viticulture and enology play an important role for economy of many countries, and considerable efforts are devoted to improve the product quality and to match the widest approval of market. Many important industrial processes are finalized to improve organoleptic characteristics of wine: alcoholic fermentation is promoted by inoculums of selected yeast, extraction of grape components is enhanced by maceration of grape skins in controlled conditions and by addition of selected enzymes, malolactic fermentation and barrel- and bottle-aging are performed to achieve biological stability and to improve flavor and fragrance of product \cite{25}. To guarantee the quality of product, all these steps have to be monitored and verified. Community laws, as well as the single country ones, are devoted to protect the consumer health, other than the internal market from introduction of low quality products, by accurate controls of foods.
As a consequence, for exporting of wine and derivate products, quality certificates are often required, in particular with regard to the presence of contaminants such as pesticides, heavy metals, ethyl carbamate and toxins, for which legal limits are often defined. To prevent frauds and to confirm the product identity, accordance between the real product characteristics and the producer declarations (e.g., variety, geographic origin, quality and vintage) has to be verified. Some maximum limits of grape and wine contaminants are fixed by national and community regulations, and are reported in Table 1.3

**Table 1.4:** Maximum Limits of Some Grape and Wine Contaminants Fixed by Regulations of Single Countries, European Union (EU), United Nations (UN)

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Grape (mg/kg)</th>
<th>Wine (ppm)</th>
<th>grape juice (ppm)</th>
<th>Country</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>3</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>UN</td>
<td>Codex alimentarius</td>
</tr>
<tr>
<td>Diuron</td>
<td>0.05</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td>Fenoxy carb</td>
<td>0.02</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td>Folpet</td>
<td>10</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>UN</td>
<td>Codex alimentarius</td>
</tr>
<tr>
<td>Iprodione</td>
<td>10</td>
<td>2*</td>
<td>--</td>
<td>UN / Italy</td>
<td>Codex alimentarius/ DM 14.12.2004</td>
</tr>
<tr>
<td>Myclobutanil</td>
<td>1</td>
<td>0.1*</td>
<td>--</td>
<td>UN / Italy</td>
<td>Codex alimentarius/ DM 14.12.2004</td>
</tr>
<tr>
<td>Penconazole</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
<td>UN / Italy</td>
<td>Codex alimentarius/ DM 14.12.2004</td>
</tr>
<tr>
<td>Primicarb</td>
<td>0.2</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td>Procymidone</td>
<td>5</td>
<td>0.5</td>
<td>--</td>
<td>Italy</td>
<td>Codex alimentarius/ DM 14.12.2004</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td>Triadimefon</td>
<td>2</td>
<td>--</td>
<td>--</td>
<td>Italy</td>
<td>DM 14.12.2004</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>5</td>
<td>--</td>
<td>--</td>
<td>UN / Italy</td>
<td>Codex alimentarius/ DM 14.12.2004</td>
</tr>
<tr>
<td>Ochrotoxin A</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>EU</td>
<td>CE regulation n° 123/2005</td>
</tr>
<tr>
<td>Pb</td>
<td>0.2</td>
<td>0.2</td>
<td>0.05</td>
<td>EU</td>
<td>CE regulation n° 466/2001</td>
</tr>
<tr>
<td>Histamine</td>
<td>--</td>
<td>2</td>
<td></td>
<td>Germany</td>
<td>Recommended (souza et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>5</td>
<td></td>
<td>Belgium</td>
<td>Recommended (souza et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>8</td>
<td></td>
<td>France</td>
<td>Recommended (souza et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>10</td>
<td></td>
<td>Switzerland</td>
<td>Recommended (souza et al., 2005)</td>
</tr>
</tbody>
</table>

*Only for Italy.
Activity of researchers and organisms of control is devoted to develop new methods to verify the product origin\cite{26} to detect illegal additions and adulteration such as sugarbeet, cane sugar or ethanol addition and watering\cite{27}, to protect consumer health through determination of food contaminants\cite{28-31}.

GC/MS and LC/MS have also been applied to develop methods for the legal parameters control finalized to the consumer health protection and to prevent frauds, such as determination of pesticides in wine, detection of compounds formed during alcoholic fermentation by yeast and bacteria, determination of illegal additions to the wine. Also methods for determination of toxins in the wine have been proposed\cite{32}. Inductively coupled plasma-mass spectrometry (ICP/MS) is nowadays a large-used technique to determine heavy metals in wine. For these aims the knowledge of the chemical composition of grape and wine is essential.

There is a large interest regarding health and safety issues associated with the fungicides, insecticides and herbicide use, and the possible presence of their residues in processed foods and drinks. The high concern about health risks connected with pesticides, led to the development of several European Community (EC) Directives (also adopted by the Italian Legislation) stating maximum residue limits (MRLs) tolerated for each food commodity\cite{33,34}. Wine and grape are included among these commodities, in particular in wine the procymidone MRL has been defined to 0.5 mg/L, such as for cyprodinil and fludioxonil, 0.1 mg/L for myclobutanil, and 2 mg/L for iprodione; MRLs in grape have been fixed to 10 mg/kg for folpet, 5 mg/kg for vinclozolin, and 3 mg/kg for carbaryl\cite{35}.

Fungicides, insecticides, and herbicide are commonly used in viticulture. Dicarboxyimide fungicides have been widely used against *Botrytis cinerea* in vineyards. Vineyards are treated in the final stage of vegetation to prevent grape’s attack, which may occurs shortly before the harvest. Among them, vinclozolin and iprodione are currently employed in Italy\cite{36,37}. These fungicides show reduced toxicity, but 3, 5-dichloroaniline, the probable common final product of their degradation or metabolic pathway, seems to be as hazardous as other aromatic amines. Although maximum residue limits for most pesticides in wine have not been
fixed, several countries have established guidelines in the authorized use of pesticides and MRLs for the treatment of vines and grapes used in wine production. Pesticide residues are regulated at MRLs in grapes at low ppm levels. Because the vinification process lowers the level of pesticides, their contents in wines are significantly lower than in grapes. As a consequence, methods to detect pesticide residues must be very effective and sensitive. One of the first MS methods for determination of pesticides in wine reported in literature was GC/MS-EI by performing analysis with a diphenyl-dimethyl polysiloxane capillary column and the use of aldrin as internal standard (IS). Analysis of procymidone was performed by liquid extraction of sample with hexane, and SIM mode analysis recording signals at m/z 96 and 283 for procymidone, and m/z 263 and 265 for the IS. By using both electronic capture detector (ECD) and MS detector, the same limit of quantification (LOQ) of 2 mg/L was reported.

In 1996, analysis of vinclozolin and iprodione in wine by solid phase extraction (SPE) sample preparation with a porous carbon stationary phase was performed. Analytes were recovered with toluene and analyses performed by GC/MS. Thermal stability, chemical resistance, and stability over a wide pH range of carbon sorbents were evaluated. Recoveries of two fungicides in both standard solutions and spiked wine samples ranging between 80% and 97% were reported. MS analyses were carried out by ion trap detector (ITD) in both multiple ion detection (MID) and SCAN mode. By selecting the characteristic ions of each compounds, LOQs of 50 µg/L for vinclozolin (by recording signals at m/z 178, 180, 198, 200, 212, 215, 285, 287) and of 50 µg/L for iprodione (by recording signals at m/z 187, 189, 244, 247, 314, 316), were recorded with a signal to noise ratio of 3 (S/N 3). By performing analysis of a vinclozolin 0.01 mg/L standard solution in MID mode, unambiguous compound identification was obtained, by ITD sensitivity of method for iprodione resulted significantly lower than for vinclozolin.

In the same year, a GC/MS method for analysis of fungicide metalaxyl in wine by SPE sample preparation using a carbon sorbent, was performed. Recoveries greater than 92% were reported for metalaxyl standard solutions at concentration 3–100 mg/mL, whereas recoveries in spiked wines ranged from 80% to 99% depending on the concentration and the sample matrix. LOQ by GC/MS-IT
was 0.50 mg/L. Metalaxyl residue concentration in wine closely related to the interval between the last treatment of the vines and the harvest of the grapes was observed. Cabras et al. performed two different GC/MS methods by micro-extraction with acetone/hexane to determine the fungicides cyprodinil, fludioxonil, pyrimethanil, tebuconazole, azoxystrobin, fluazinam, kresoxim-methyl, mepanipyrim, and teflunofoz in grapes, must, and wine. The methods limits of detection (LODs) resulted 0.05 mg/L for cyprodinil, pyrimethanil and kresoxim-methyl, and of 0.10 mg/L for the other analytes.

To perform the routine monitoring of pesticides, in 1997 Kaufmann developed a fully automated reverse-phase SPE and GC/MS method for the simultaneous determination of 21 different pesticides in wine. By performing SIM mode analysis and monitoring the m/z species, the method showed LODs between 5 and 10 µg/L, and linearity regression coefficients greater than 0.99 (except for 4,4-dichloro-benzphenone and dicofo). Recoveries of 17 pesticides in spiked wine samples ranged from 80% to 115%. In 1998, Vitali et al. proposed a solid-phase micro-extraction (SPME) and GC/MS SIM mode method to determine seven different insecticides (lindane, parathion, carbaryl, malathion, endosulfan, methoxychlor, and methidathion), 4 fungicides (procymidone, folpet, vinclozoline, and captan) and 3 herbicides (terbuthylazine, trifluralin and phosalone) in wine.

1.7 Method Development

Pesticide residue analysis of food and environmental samples has been performed in numerous government and private laboratories throughout the world for approximately 40 years. However, the methods used for analysis of common pesticides are far from ideal. Some residue monitoring laboratories still use methods developed 30 years ago when analytical needs were less demanding, solvent usage was less of an issue, extended analysis time and manual labor were the norm, and technology was less capable than today. Modern residue monitoring programs, however, are expected to be responsive to the latest developments in agriculture and new legislation.

The introduction of new, more rapid, and effective analytical approaches, therefore, is essential for laboratories to improve overall analytical quality and
laboratory efficiency. Without question, the most efficient approach to pesticide analysis involves the use of multiclass, multiresidue methods (MRMs). The first notable MRM was the Mills method developed in the 1960s by U.S. Food and Drug Administration (FDA) chemist P.A. Mills\textsuperscript{[46]}. At that time, non-polar organochlorine insecticides (OCs) were the main focus for analysis. With the Mills method, OCs and other non-polar pesticides were extracted from non-fatty foods with acetonitrile (MeCN), which was then diluted with water, and the pesticides were partitioned into a non-polar solvent (petroleum ether). As a consequence, relatively polar pesticides, such as certain organophosphorus insecticides (OPs), were partially lost during this step.

The need to analyze more polar OPs and other pesticides in agriculture initiated the development of alternative procedures to determine compounds not extracted by the Mills method. These methods often simply modified the Mills procedure by using the initial acetonitrile extract but with different partitioning, cleanup, and determinative steps\textsuperscript{[47-49]}. In the 1970s, new methods were developed to extend the analytical polarity range to cover OCs, OPs, and organonitrogen pesticides (ONs) in a single procedure\textsuperscript{[50,51]}. These multiclass MRMs differed from the Mills approach in that acetone, rather than acetonitrile, was used for the initial extraction. However, the new methods still used non-polar solvents (dichloromethane or dichloromethane–petroleum ether) to remove the water in a liquid–liquid partitioning step.

Furthermore, NaCl was added to the water phase in both methods during the partitioning step, the amount of which had a direct effect on the polarity range covered by the methods. Becker\textsuperscript{[50]}, who developed the first MRM of this kind, added an NaCl solution to the initial extract, which only partially saturated the water phase with salt. Luke et al.\textsuperscript{[51]} and Specht and Tilkes\textsuperscript{[52]}, however, added solid NaCl to saturate the water phase, which forced more acetone into the organic layer, thus increasing its polarity and leading to higher recoveries of the polar analytes. Soon after their introductions, the Becker method became official in Germany as the DFG-S8 procedure, and the Specht method became the official DFG-S19 procedure\textsuperscript{[53]}. The Luke method, which replaced the Mills method in the FDA, became the official
PAM 302 E1 procedure \[^{[54]}\] , and several years later became AOAC Official Method 985.22\[^{[55,56]}\].

These multiclass MRMs and their many variations are still widely used by pesticide residue monitoring laboratories worldwide. Since the 1980s, environmental and health concerns related to the use of chlorinated solvents have led to the development of many new methods in which such solvents were avoided. Specht et al.\[^{[57]}\] and Anastassiades and Scherbaum\[^{[58]}\] used a mixture of cyclohexane–ethyl acetate (1 + 1) instead of dichloromethane (or dichloromethane–petroleum ether, 1 + 1) to induce partitioning. Casanova\[^{[59]}\] and Nordenmeyer and Thier\[^{[60]}\] used solid-phase extraction (SPE) to extract pesticides from diluted acetone extracts, thus completely avoiding liquid–liquid partitioning.

Luke et al.\[^{[61]}\] added a combination of fructose, MgSO\(_4\), and NaCl to separate water from acetone in the initial extract without using non-polar solvents. Schenck et al.\[^{[62]}\] also investigated the use of salts to form a water–acetone partition in extracts. Parfitt\[^{[63]}\] studied exposure of the initial Luke extracts to low temperatures to remove the water phase by freezing it out of solution. All these separation approaches have one or more disadvantages in practice because acetone is simply too miscible with water to be easily separated without using non polar solvents. MeCN is more easily and effectively separated from water than acetone\[^{[62-64]}\] upon addition of salts. In the case of MeCN, salt alone can be used to form a satisfactory separation from water, and Lee et al.\[^{[65]}\] used NaCl for this purpose rather than the nonpolar co-solvent of the Mills method. Several other multiclass MRMs have been published since then following this salting-out principle with MeCN\[^{[66-72]}\].

A third extraction solvent commonly used in multiresidue MRMs is ethyl acetate (EtAc)\[^{[73-77]}\]. EtAc has the advantage of partial immiscibility with water, which makes the addition of other nonpolar solvents to separate water from the extract unnecessary. However, a problem with EtAc is that some of the most polar pesticides do not readily partition into the EtAc phase. To increase recoveries of polar compounds, large amounts of Na\(_2\)SO\(_4\) are usually added in MRM procedures, with EtAc used to bind the water. Polar co-solvents, such as methanol and ethanol, have been used to increase the polarity of the organic phase\[^{[76]}\] . Another disadvantage associated with the use of EtAc is the extraction of a large amount of
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non polar co-extractives, such as lipids and epi-cuticular wax material, which must be removed before the determinative step.

This is commonly achieved by a time-consuming and wasteful gel-permeation chromatographic (GPC) cleanup step. During the 1990s, increased urgency to further reduce solvent usage and manual labor in analytical laboratories led to the commercial introduction of several alternative extraction approaches, including supercritical fluid extraction (SFE; )\textsuperscript{[78-81]}, matrix solid-phase dispersion (MSPD; )\textsuperscript{[82-84]}, microwave-assisted extraction (MAE; )\textsuperscript{[85]}, solid-phase micro-extraction (SPME; )\textsuperscript{[86]}, and pressurized liquid extraction (PLE), also known commercially as accelerated solvent extraction (ASE; )\textsuperscript{[87-89]}. Despite their advantages, none of the techniques have overcome critical flaws or practical limitations to enable their widespread implementation. For example, PLE and SFE, which are instrument-based techniques that perform extractions in sequential, semi-automated fashion, still require time-consuming manual steps and specialized items that require cleaning after each use; sample throughput is not optimal, and instruments and maintenance are costly.

SFE, MSPD, and SPME do not provide a wide enough analytical scope within a single procedure, and MAE and PLE do not provide enough selectivity (extracts require more cleanup than liquid-based extractions at room temperature). Also, sample size in all of these approaches may be too small. These approaches are useful for certain applications but are not sufficiently simple or effective to provide the ideal MRM. Despite the many different multiclass MRMs that have been described in the past 40 years, none has been truly streamlined to the minimum factors that achieve a fast and easy extraction while still maintaining high recoveries for a wide range of analytes and providing the selectivity and repeatability needed of a reliable procedure. Many of the advantages and opportunities provided by modern analytical techniques are not properly integrated into most current methods. The aim of this study was to develop a simple, rapid, and inexpensive multiclass MRM that provides high-quality results, but minimizes the number of analytical steps, uses few reagents in small quantities, and requires very little glassware. The main focus was to simplify the analytical process as much as possible.
during extraction and cleanup without sacrificing high recoveries even for the most difficult analytes.

**Guidelines for Method Development:**

Reliable residue analytical methods are necessary to measure the magnitude of residues in a commodity, and to enforce legal MRLs (tolerances). That reliability can be certified by two specifications. The most traditional one enforces the use of standard methods. However, as a result of advances in analytical chemistry, the concepts of routine methods and reference methods have been displaced in favor of a “criteria approach,” which establishes quality control principles, limits, and procedures for the validation of screening and confirmatory methods\[^{86-87}\]. The remarkable feature of this last tactic is its superior versatility, which allows the ready adaptation of analytical methods to technical improvements and brings the possibility to react rapidly to new emerging problems, such as, for example, in the case of analyte/matrix combinations that have not been considered so far. The analytical quality control (AQC) requirements are necessary to support the validity of data used for checking MRLs, to support enforcement actions, or to assess consumer exposure to pesticides. The objectives are (i) to ensure that false positives or false negatives are not reported, (ii) to ensure that acceptable trueness (bias) and precision are achieved, and (iii) to harmonize cost-effective AQC\[^{86-87}\]. Laboratories that carry out analysis of pesticide residues meet the requirements of a recognized accreditation scheme, complying with ISO 17025 or Good Laboratory Practices (GLPs). These approved laboratories prove their competence by regular and successful participation in adequate proficiency testing schemes, recognized, or organized by the national or community reference laboratories\[^{86}\].

Both, the guidelines on quality control procedures for pesticide residue analysis\[^{87}\] and the 2002/657/EC Commission Decision, concerning the performance of analytical methods for the determination of organic residues and contaminants in live animal and animal products\[^{88}\], state that “methods based only on chromatographic analysis without the use of molecular spectrometric detection are not suitable for use as confirmatory methods” and the latter explicitly requires a combination of either an on-line and/or off-line chromatographic separation. In this
context, laboratories in many countries rely on detection by MS for unambiguous confirmation of pesticides in food.

The guidelines on quality control procedures for pesticide residue analysis establishes the principles of results confirmation indicating that when the increased sensitivity obtained by scanning a limited mass range or by selected ion monitoring (SIM) is essential, the minimum requirement is for data from two ions of m/z >200; or three ions of m/z >100. Intensity ratios obtained from the more characteristic isotopic ions may be of particular utility. If possible, the ions selected for medium/high resolution mass spectrometry (MS) or tandem mass spectrometry (MS/MS) should be characteristic of the analyte, not common to many organic compounds.

The 2002/657/EC European Commission Decision also defines performance requirements for the different approaches acceptable in mass spectrometric detection: full mass spectra (full scans), SIM, tandem mass spectrometry (with multiples stages) (MS/MSn) techniques, such as selected reaction monitoring (SRM) and any other technique with appropriate ionization procedures. That Decision outlines a recommended approach, using identification points (IPs). For the confirmation of substances, a minimum of three or four IPs are required. According to the report, any MS technique or combination of techniques may be employed to attain the number of IPs needed for the identification of a compound. The number of IPs achieved by MS detection depends on the technique used. The relative intensities, both in full scan and in the SIM modes, of the detected ions, expressed as a percentage of the most intense ion or transition, correspond to those of the calibration standard within the tolerances. The smaller the relative ion intensity is, the greater range of variations is permitted.

When mass spectrometric determination is performed by recording the full spectra, the presence of all measured diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, and characteristic fragment ions and isotopic ions), with a relative intensity of more than 10% in the reference spectrum of the calibration standard, is mandatory. A minimum of four ions shall be present with a relative intensity of >10%, including the molecular ion if possible. When mass spectrometric determination is performed by SIM or using MS/MSn, the
molecular ion is preferably one of the selected diagnostic ions. These ions should not be exclusively originated from the same part of the molecule. The signal-to-ion ratio for each diagnostic ion is >3:1. These criteria are minimum performance criteria, that is, a laboratory can decide to be more rigorous.[88]

1.9 Antibiotic Residues

At present, the occurrences of drug residues especially antibiotics in foods and foodstuffs originating from veterinary uses have become increasingly apparent. Sulfonamides (SAs) and tetracycline (TCs) are broad spectrum antibiotics frequently used in Thailand as veterinary medicines. They are commonly used for the prevention and treatment of dairy cattle for several infectious diseases, prophylactic, or as feed additives to promote growth in farm animals.[89]

An implementation of the effective monitoring program requires specific, sensitive, and reliable analytical methods that can detect all drug residues below these regulated levels. It is possible to analyze SAs, TCs, and PYR in foods. Microbiological and immunological [90, 91] assays are commonly used for rapid screening. However, these techniques are complicated, lack sensitivity and specificity, and thus are only suitable for semi-quantitative measurements. Liquid chromatography (LC) coupled with UV [92-95], DAD and FLD [96, 97] emerged as attractive alternatives for the determination of antibiotics due to their higher selectivity, sensitivity, and precision. GC [98] and CE [99,100] methods were also reported. However, these techniques require preceding sample cleanup to achieve acceptable detection. Currently, mass spectrometry (MS) technique has gained popularity as a highly sensitive detection method for LC due to its ability to provide simultaneous and unambiguous identification and quantification of drug residue at trace levels.

From the literature review, we found that most of the published analytical procedures were specific for the detection of each class of antibiotic. These methods are varied in methodological approaches suitable for several varieties of matrices by specific instrument. To the best of our knowledge, there is no report of an analytical procedure that can separate and detect SAs, TCs, and Pyrimethamine (PYR) residues simultaneously. This is due to the differences in structural and chemical properties of
these compounds that make simultaneous separation difficult. A detection of trace level antibiotic residues in a rich matrix such as milk requires a good sample preparation and cleanup procedure. Sample preparation procedures such as liquid–liquid extraction (LLE) [101] and solid-phase extraction (SPE) are commonly employed for simultaneous extraction and cleanup. However, due to its ease of operation and environmental interest, SPE has gained increasing popularity over LLE. Common adsorbents including C8, C18, and ion-exchange have been applied for the analyses of SAs in various matrices such as milk [102,103] and animal tissues [102,103]. SPEs were also applied to the analyses of TCs in milk [104-108], TCs in animal tissues [109-112].

Recently, an availability of dual quality polymeric SPE adsorbents such as hydrophilic–lipophilic balance (HLB) made simultaneous enrichment and cleanup of analytes in biological, environmental and food matrices possible. Oasis HLB SPE cartridges were successfully applied for the analyses of SAs in honey, SAs in wastewater [113-114], TCs in milk [115-116], and TCs in water [117-118]. There was a report of simultaneous extraction and cleanup of SAs and TCs in environmental matrices with Oasis HLB SPE cartridges as well [119]. Simultaneous extraction of SAs and PYR from human plasma was also well described [120-121]. However, there is a strong need for a reliable simultaneous extraction and cleanup method for the analysis of multi-class antibiotic residues in complex matrix such as milk and muscles for routine monitoring purposes. A straightforward SPE cleanup and enrichment using Oasis HLB SPE cartridges for six sulfonamides, three tetracyclines, and pyrimethamine residues in milk was successfully developed in our laboratory recently and will be reported elsewhere [122].

In order to ensure correct identification of the compounds, a reliable and sensitive analytical method is also needed. This work describes a validation process of the developed chromatographic and detection procedure coupled with the developed simultaneous SPE sample preparation. Validation parameters tested included method detection limit (MDL), method quantitation limit (MQL), selectivity, linearity, precision, accuracy, and robustness. To enhance the quality and accreditation of the method, the validation procedure was complied with acceptable
guidelines \cite{123-124} and used statistical techniques to guarantee the solidity of the conclusion reached.

The major challenge when analyzing sediment is the extraction of the antibiotic compounds from the solid phase to the liquid phase prior to further purification. Since most antibiotics are sensitive to strong acids and bases, a weakly acidic buffer solution has an advantage in this extraction \cite{125}. For example, a McIlvaine-EDTA solution was used to extract oxytetracycline in fish farm sediment\cite{126,127}. A citric acid buffer solution, in combination with ethyl acetate, was used to extract tetracyclines in egg, poultry, fish and tissues \cite{128}. This method was also adapted to measure the concentration of tetracyclines and tylosin in fertilized soil \cite{129}. Both methods show a good recovery ratio for TCs, although weakly acidic extractants are not suitable with macrolides and ionophore polyethers.

However, the use of phosphate buffer solution might cause degradation of the analytes during the evaporation step, especially the TCs \cite{130}. Three different antibiotic groups (TCs, SAs, and MLs) were simultaneously extracted in soil and pig slurry using McIlvaine buffer solution (pH 7.0) \cite{131}, with recoveries ranging from 27–51%, 68–85%, and 47–61% for oxytetracycline, sulfachloropyridazine and tylosin, respectively, in a clay soil. However, the limit of detection was again too high (18 µg/kg–40 µg/kg for soil and 70 µg/kg–140 µg/kg for pig slurry) to measure most environmental samples. High performance liquid chromatography (HPLC) equipped with mass spectrometry (MS) or tandem mass spectrometry (MS/MS) is the most widely used method to separate and quantify antibiotic samples. HPLC/MS or MS/MS with electrospray ionization (ESI) has been used in several types of water and food matrices \cite{132,133}.

2 Grape Products

There are 16 bi-products made from grapes viz. raisin, grape juice, squash, syrup, jam, jelly, vinegar, wine, pickles, chocolates, tartaric acid, oil, cattle feed, tannin, etc. However, looking to the world scenario of different bi-products, it was necessary to consider setting up of projects for manufacturing other value added products from grapes.\cite{134}. The Government of India has announced Agri Export Zone (AEZ) for Nashik, Sangli, Pune, Solapur, Ahmednagar and Satara districts for
grapes and grape-wine parks in Maharashtra. The Government of Maharashtra had nominated Maharashtra Industrial Development Corporation (MIDC) as a ‘nodal agency’ for establishment of wine parks in Maharashtra state. Accordingly MIDC is developing Godavari Wine Park at Vinchur, Nasik and Krishna Wine Park at Palus, Sangli.

2.1 Raisin

Raisins are basically dry grapes and they are known as Kishmish, Bedana, Manuka or dry fruit. During earlier days, grapes were dried on plants only which was a very crude method resulting in wastages. Processing using some chemicals was invented but there was considerable consumer resistance. Since then a new method without use of chemicals, known as Australian method, has been introduced successfully. Sangli and Nasik districts of Maharashtra grow large quantities of grapes and many growers or gardeners are keen to supply to raisin makers due to assured market. Maharashtra is, therefore, a preferred location. Raisin is a popular dry fruit item with shelf life of around 6 months if stored properly. Apart from use as a dry fruit item, it is used in large quantities in many sweet preparations, some farsan items and desserts. Compliance under the FPO (fruit product order) and PFA (prevention of food adultration) Act is mandatory.

Raisins are popular in India since long. Apart from regular use in many preparations, a small quantity is also used in some herbal medicine preparations. Grapes from Nasik and Sangli districts of Maharashtra are famous all over the country. Grapes are perishable but raisins have a fairly long shelf life. There are stockists at all major trading centres who buy large quantities as and when required and then sell it to retailers in smaller lots. Normal demand is witnessed for raisins throughout the year and it picks up during festive and marriage seasons. Demand from individual households is not much but restaurants, star hotels, caterers and sweet-meat makers are the major consumers. These two centres of Maharashtra supply bulk quantities to western, central and north Indian states.

Raisins are made primarily by sun drying several different types of grapes. They are small and sweetly flavored with a wrinkled texture. The technique for making raisins has been known since ancient times and evidence of their production
has been found in the writings of ancient Egyptians \cite{135-137}. Currently, over 227 million kg of raisins are sold each year in the United States and the number is expected to increased because raisins are recognized as a healthy snack. Most raisins are small, dark, and wrinkled. They have a flavor similar to the grapes from which they are made, but the drying process which creates them concentrates the amount of sugar making them taste much sweeter. They are a naturally stable food and resist spoilage due to their low moisture and low pH.

Raisins are composed of important food elements such as sugars, fruit acids, and mineral salts. The sugars provide a good source for carbohydrates. Fruit acids such as folic acid and pantothenic acid, which have been shown to promote growth, are also significant components. Vitamin B6 is found in raisins and is an essential part of human nutrition. Important minerals in raisins include calcium, magnesium, and phosphorus. Additionally, iron, copper, zinc, and other nutrients are found in trace amounts in raisins. Considering the composition of raisins and the fact that they have no fat, it is no wonder that this fruit is considered a healthy snack.

The majority of grapes used for making raisins in the United States are grown in California. This area has an ideal climate for grape growing because it has plenty of sun during the summer and very mild winters. Five other countries, which produce a substantial amount of raisins include Greece, Australia, Turkey, Iran, and Afghanistan. Each of these countries have their own variety of raisin that they consistently grow.

It has been evaluated in the European Union (EU) in the framework of 91/414/EEC Council Directive \cite{138} as a new molecule (Azoxystrobin) and was approved in 1998 as an active substance of minimum purity 930 g/kg containing 25 g/kg Z isomer \cite{139}. It is a compound with no particular toxicological concerns, with an acceptable daily intake (ADI) for man of 0.1 mg/kg b.w./day. On the basis of critical good agricultural practice (GAP) in Europe, which refers to uses in Northern countries, the value of 2 mg/kg was set as the EU maximum residue level (MRL) for grapes \cite{140}. According to the current EU legislation, in the case of dried or processed products for which maximum levels are not explicitly fixed, the MRL applicable is that for the fresh product taking into account, respectively, the concentration caused
by the drying process or the concentration or dilution caused by processing \[^{141}\]. Trials carried out in Greece by the manufacturer on the Black Korinth variety of grapes have shown that residues in raisins are higher than in the fresh commodity (unpublished proprietary data). Procedures used in industrial food processing and domestic cooking frequently have dramatic effects on residue levels \[^{142-144}\]. In most cases, large decreases in residues occur, as shown by Schattenberg et al.\[^{145}\] in a study to determine the effects of normal household preparation on pesticides in many food commodities. However, residues may concentrate in processed commodities following certain procedures. The ratio of the residue in the processed commodity to that in the raw commodity is referred to as the transfer factor (TF). If this ratio is greater than 1, the residue is said to concentrate upon processing. Studies on the effect of processing and the determination of TFs have been recognized among the main priorities in the process of MRL setting \[^{146}\] and for enabling a more realistic exposure assessment and risk management \[^{147}\]. The aim of this work was to assess the magnitude of residues of azoxystrobin on fresh grapes of the typical cv. Thomson seedless (Sultana) and a seed-producing clone and on raisins produced from these after the two types of processing commonly used, i.e., sun drying alone and sun drying after treatment with a solution containing 3% K$_2$CO$_3$ and 1% ethyl oleate.

Azoxystrobin, a fungicide of the strobilurin group, has an European Union maximum residue level (MRL) of 2 mg/kg for grapes. This work aimed to assess residues on fresh and washed grapes and on raisins following processing with (i) alkali treatment and sun drying and (ii) sun drying only. QUADRIS 25% SC was applied according to good agricultural practice for two consecutive years on a typical cv. Thomson seedless and a seed-producing clone. Samples were collected 0, 15, and 21 days post application and analyzed using gas chromatography/electron capture detection; recoveries were 86 (12\% for grapes and 99 (15\% for raisins. Residues on grapes were 0.49-1.84 mg/kg, and washing removed 75\% of the residue. Residues in raisins produced from seedless grapes were 0.51-1.49 (treatment 1) and 1.42-2.08 mg/kg (treatment 2), with residue transfer factors sometimes >1, even following alkali treatment, which reduced residues considerably. To avoid trade problems, a higher MRL for raisins is necessary \[^{148}\].
2.2 Wine

2.2.1 Wine Grape and Wine Production Scenario

The state of Maharashtra is undoubtedly the leader in both production and consumption of wine in the country. It produces more than 75% of the total grape and wine production in the country. At present Maharashtra totals 7,775 to 8,000 acres area under cultivation of wine grapes and it is increasing day by day to meet the demand of new coming grape wine units. The total production of wine in India was 22.5 million liters (from 62 established wineries). Out of this 21.1 million liters wine is produced only in Maharashtra by 58 wineries. Nashik district, the wine hub of India-Maharashtra, alone produced 9.4 million liters of wine in the year 2006. Gore and Sundar estimated Indian wine consumption to be 1.1 million 9-liter cases at a value of about US$ 60 million. With an annual growth rate of 20% to 25%, consumption is projected increase to 2.0 million cases by 2011. On a per capita basis, Indians consume only 9 ml wine annually as compared to 9000 ml in U.S. The wine industry in India is in its nascent stages now; to follow quality parameters will go a long way in making wines from India as a brand in International market.

2.2.2 Consumption and Market Analysis - Indian Wine Consumption

a. Tastes, Preferences and Presentation

The tastes and preferences of the Indian population err towards still wines and more specifically, table wines. Though a market exists for champagne and sparkling wines, these varieties sell at a much lesser rate (8-10% market share). In general, slightly sweet wines and the varieties of Sauvignon Blanc, Chenin Blanc, Rieslings, and Gewürztraminer are fairly popular and also pair well with typical Indian dishes. Similarly, rose and blush have been projected as good fits for the Indian market. However, the majority of sales have stayed on traditional still red and white wines. In regards to presentation, wine producers have two different demographics in the Indian market upon which to focus: the upper class and the general consumer. While the upper class prefers the classic presentation, i.e. real cork, full bottle size, and dry red and white wines, the growing consumer class in India gravitates towards approachable wine packaging, i.e. screw caps, half bottle sizes, and sweet wines.
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For 2008, the authors of this study estimate Indian wine consumption to be 1.1 million 9-liter cases at a value of approximately US$ 60 million. With an annual growth rate of 20% to 25%, consumption in this emerging market is projected to increase to 2.0 million cases by 2011 (consumption projections, conservatively, are 4.0 million cases by 2015 and 8.0 million cases by 2020). On a per capita basis, Indians consume about 9 milliters annually (compared to 9000 milliters in the U.S.)

c. Upper class Indians as Wine Consumers

This level of growth in the Indian wine market is in large part driven by the upper class Indians, which is widely understood to be 2% of the population and therefore approximately 20-25 million people. Many of these Indians have increasing levels of disposable income and international experience and lifestyles (either through studies, travel or work) that they have brought back to their country. These changing tastes and preferences, coupled with higher levels of disposable income and the increasing availability of domestic and imported wines, have resulted in the emergence of India as a viable wine market.

d. Wine Consumption in Relation to that of Spirits and Beer

Indian alcohol consumption has traditionally focused on spirits and beer instead of wine. Annually, Indians consume 50 million cases of whiskey, 14 million cases of brandy, 25 million cases of rum, 110 million cases of beer, 200 million cases of country liquor, and 1 million cases of imported spirits (400,000 bottled imports and 600,000 bulk imports which are bottled in India). This long-standing dominance of spirits and beer as the alcohol beverages of choice among Indians has made it difficult for wine to take a place in the market; however, despite this structure wine is becoming more accepted, sought-after, and available.

e. Indian Wine Consumption by Location

The two largest and dominating markets in India are not regions, but rather the city-areas of greater Mumbai and Delhi. It is estimated that as much as 65% of total Indian wine consumption is accounted for in these two locations. This number reaches an estimated 80% when including other major cities such as Bangalore,
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Chennai, Kolkata (formerly Calcutta), Nashik, and Pune. This market dominance of Mumbai and Delhi ensures their place as the fulcrum points for any producer or distributor looking to increase sales to India. The city of Bangalore (with its high-tech industry inflows), and the State of Goa (with its high energy tourism sector), are a secondary, yet important focus for marketers as well. The cities of Kolkata, Chandigarh, Nashik and Pune are all important niche markets and should be followed and acted upon as appropriate. Chennai and Hyderabad have much potential due to the growth of their IT industry but their government policies are not yet conducive to wine sale.

f. Indian Wine Consumption - Historical and Projected (By volume)

The below figures for overall consumption are project through 2015. The market share for imports remains steady for this analysis (at approximately 15%). This market share, and its ability to increase, depends heavily on the level of protectionism enforced by the federal and state governments in India. Should certain onerous procedures and taxes be reduced (either voluntarily by India or through dispute settlement proceedings in the World Trade Organization) the import market share could rise significantly.

Table 1.5: Indian wine consumption (by volume)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>TOTAL</th>
<th>DOMESTIC</th>
<th>IMPORTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Number of 9 Lit cases)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>550,000</td>
<td>470,000</td>
<td>80,000</td>
</tr>
<tr>
<td>2005</td>
<td>620,000</td>
<td>520,000</td>
<td>100,000</td>
</tr>
<tr>
<td>2006</td>
<td>750,000</td>
<td>630,000</td>
<td>120,000</td>
</tr>
<tr>
<td>2007</td>
<td>900,000</td>
<td>750,000</td>
<td>150,000</td>
</tr>
<tr>
<td>2008</td>
<td>1100000</td>
<td>920000</td>
<td>180000</td>
</tr>
<tr>
<td>2009</td>
<td>1400000</td>
<td>1180000</td>
<td>220000</td>
</tr>
<tr>
<td>2010</td>
<td>1700000</td>
<td>1440000</td>
<td>260000</td>
</tr>
<tr>
<td>2011</td>
<td>2,000,000</td>
<td>1700000</td>
<td>300000</td>
</tr>
<tr>
<td>2015</td>
<td>4,000,000</td>
<td>3,400,000</td>
<td>600,000</td>
</tr>
</tbody>
</table>

*Note: Figures exclude regional flavoured fruit wines. Figures are consumption estimates
As elaborated on in the regulation section of this report, current government measures to protect the nascent Indian wine industry have eliminated the ability of imports to compete with domestic producers in a fair and open marketplace.

g. Wine Consumption for 2008 according to Price structure

Table 1.6: Wine consumption (by price structure)

<table>
<thead>
<tr>
<th></th>
<th>DOMESTIC</th>
<th>IMPORTED</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under $10</td>
<td>600,000</td>
<td>-</td>
<td>600,000</td>
</tr>
<tr>
<td>Under $10 to under $20</td>
<td>250,000</td>
<td>50,000</td>
<td>300,000</td>
</tr>
<tr>
<td>Under $10 to under $30</td>
<td>60,000</td>
<td>90,000</td>
<td>150,000</td>
</tr>
<tr>
<td>$30+</td>
<td>10,000</td>
<td>40,000</td>
<td>50,000</td>
</tr>
<tr>
<td>Total</td>
<td>920,000</td>
<td>180,000</td>
<td>1,100,000</td>
</tr>
</tbody>
</table>

The market share of imports for high value wines soar as cost increases into the $20+ range. Domestic wines are still unable to demand a high price (during the visit to India, the highest price domestic wine found by the authors of this report on a menu was $23). Furthermore, the protectionist policies put in place at the federal and state level in India have had their desired effect of ensuring that cheap foreign wines cannot compete against domestics below $10/bottle.

h. Wine Consumption for 2008 by Wine type (Still Wines)

Traditional red and white varietal wines have gained a foothold in the emerging Indian wine market. Rose wines have long been considered to be a good fit for the Indian consumer, as they are thought by many to fit well with the Indian cuisine and climate; however, they have consistently under-produced in the marketplace.

Table 1.7: wine consumption (by type)

<table>
<thead>
<tr>
<th></th>
<th>Table Wines (9L cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Domestic</td>
</tr>
<tr>
<td>Red</td>
<td>480,000</td>
</tr>
<tr>
<td>White</td>
<td>420,000</td>
</tr>
<tr>
<td>Rose</td>
<td>20,000</td>
</tr>
<tr>
<td>Total</td>
<td>920,000</td>
</tr>
</tbody>
</table>
The dominance of red and white still wines in the marketplace reinforces the assertion that the majority of wines sold in India are consumed by the upper 2% of the population. Not surprisingly, this upper class has focused its consumption on traditional wine types and styles.

i. Import Markets Shares by Country of Origin

Table 1.8: Import Market shares by country wise

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Country</th>
<th>Share by Country in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>France</td>
<td>45%</td>
</tr>
<tr>
<td>2.</td>
<td>Australi</td>
<td>16%</td>
</tr>
<tr>
<td>3.</td>
<td>Italy</td>
<td>11%</td>
</tr>
<tr>
<td>4.</td>
<td>US</td>
<td>5%</td>
</tr>
<tr>
<td>5.</td>
<td>S. Africa, Chile, New Zealand, Argentina and Spain</td>
<td>1-3.5</td>
</tr>
</tbody>
</table>

The United States, while low at 5%, has only one way to go up. The California lifestyle is appreciated in Indian culture. Additionally, while the majority of wines are sold by France, the middle class of Indians have a palate that is more accustomed to new-world style wines.

j. Indian importers (9L cases) top 107.

Table 1.9: List of importers in India.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Country</th>
<th>9L cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Brindco</td>
<td>51,000</td>
</tr>
<tr>
<td>2.</td>
<td>Sonarys</td>
<td>24,000</td>
</tr>
<tr>
<td>3.</td>
<td>Moet Hennessey</td>
<td>18,000</td>
</tr>
<tr>
<td>4.</td>
<td>Global Tax Free Traders</td>
<td>14,000</td>
</tr>
<tr>
<td>5.</td>
<td>Hema Connosieur Coll</td>
<td>13,000</td>
</tr>
<tr>
<td>6.</td>
<td>Pernod Ricard</td>
<td>12,000</td>
</tr>
<tr>
<td>7.</td>
<td>Sula</td>
<td>7,000</td>
</tr>
<tr>
<td>8.</td>
<td>Fine Wines&amp;More</td>
<td>6,500</td>
</tr>
<tr>
<td>9.</td>
<td>Mohan Bros</td>
<td>4,500</td>
</tr>
<tr>
<td>10.</td>
<td>T &amp;G Trading</td>
<td>4,500</td>
</tr>
</tbody>
</table>

Note: The current number of wine importers in India is estimated to be 80.
### Table 1.10: Imports of Wine by India

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>L</td>
<td>6775880</td>
<td>1252348</td>
<td>9304230</td>
<td>1563110</td>
<td>14413326</td>
<td>2833442</td>
<td></td>
<td></td>
</tr>
<tr>
<td>France</td>
<td>L</td>
<td>3058019</td>
<td>472301</td>
<td>3939683</td>
<td>683768</td>
<td>6500437</td>
<td>867057</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td>L</td>
<td>794271</td>
<td>198358</td>
<td>1123938</td>
<td>236340</td>
<td>2315211</td>
<td>804071</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>L</td>
<td>836427</td>
<td>138204</td>
<td>1275967</td>
<td>181825</td>
<td>1564735</td>
<td>254735</td>
<td></td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>L</td>
<td>416491</td>
<td>62164</td>
<td>683059</td>
<td>93898</td>
<td>693722</td>
<td>92024</td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Africa</td>
<td>L</td>
<td>105043</td>
<td>46849</td>
<td>56070</td>
<td>105043</td>
<td>46849</td>
<td>56070</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>L</td>
<td>288118</td>
<td>64447</td>
<td>250131</td>
<td>38086</td>
<td>335294</td>
<td>78997</td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Zealand</td>
<td>L</td>
<td>49367</td>
<td>7507</td>
<td>47574</td>
<td>5625</td>
<td>162757</td>
<td>27682</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>L</td>
<td>100126</td>
<td>27340</td>
<td>318751</td>
<td>35370</td>
<td>160324</td>
<td>32075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>L</td>
<td>159844</td>
<td>9994</td>
<td>84288</td>
<td>18284</td>
<td>151639</td>
<td>33389</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Those on this list, especially the top four, are well engrained in the existing market.

#### 2.2.3 Pesticides Residues during Grape to Wine Processing

The main distribution area of the grapevine is in European countries. Among these, France, Italy, and Spain control the sector on an international scale. Spain dedicates the largest surface in the world to the cultivation of the grapevine (1230 x 10^3 ha, in 2001). The major part of this surface is inscribed in 56 Appellations d’ Origine Controlles (AOC). Three of these belong to Murcia (southeastern Spain), an area of peculiar climatic characteristics that favor the development of pests and diseases. The principal parasites of the vine are the grape moth \((Lobesia botrana)\), downy mildew \((Plasmopora Viticola)\), powdery mildew \((Uncinula necator)\), and, on some occasions, gray mold \((Botrytis cinerea)\). To control these parasites, vine growers use insecticides and fungicides. This is important in maintaining grape productivity and wine quality. However, in many cases, when the dose and/or the established preharvest time for each product is not respected, hazardous residues are
left, and these become a permanent danger to the quality of the wine, the environment, and consumer health \[155-165\]. In this sense, the elaboration method, the correct winemaking processes, and the correct use of phytosanitary products are influential in the dissipation and elimination of the current residues in grapes and must. Most studies on pesticide residues deal with the transformation from vine to wine, and the results reported show, on the one hand, their fate during vinification and the influence of each technological process on the residue amount and, on the other hand, that is almost impossible not to find residues in wine, even though at very low or nondetectable levels \[166-177\].

The evolution of residual levels of four fungicides (cyprodinil, fludioxonil, pyrimethanil, and quinoxyfen) during the elaboration of three types of wine with maceration (traditional red wine, carbonic maceration red wine, and red wine of long maceration and pre-fermentation at low temperature) and two types of wine without maceration (rose and white) has been studied \[178\]. The disappearance curves of each fungicide have been analyzed during the period of each winemaking process (21 days) and during the different enological steps involved in the elaborations. The residual levels of fludioxonil reduce most quickly during the winemaking processes without maceration, whereas the decrease in levels of pyrimethanil was the slowest in practically all cases (with and without maceration). During carbonic maceration winemaking, the decay constant of cyprodinil was greater than that of the other pesticides in all assays (time and steps).

### 2.2.4 Heavy metals in Wine

Sodium arsenite is employed in viticulture as fungicide against the esca plant disease (Eutypa lata). Arsenic is a suspected carcinogen and little is known about its chronic sub-lethal effects. The concentration of As in wines mainly depends on factors such as soil composition, grape variety, climatic conditions, use of pesticides, process of vinification, and storage conditions. The Officer Internationale de la Vigne et du Vin (O.I.V.) has set the maximum limit of As in wines at 200 pg/mL, but in general it is accepted the presence of only a few pg/mL of As in uncontaminated wines \[179-182\]. Traditionally the determining of As in wine is based on the generation of volatile amines followed by reaction with silver diethyl
dithiocarbamate, and measurement of molecular absorption of the As-containing complex formed. But the method LOD, estimated at 10 pg/mL, is not sufficient for the wine analysis. Alternatively, hydride generation atomic absorption spectrometry has been used for the quantification of several hydride-forming elements in wine, including As. The technique normally requires sample decomposition, a time consuming procedure, which may result in sample contamination or analyte loss. Unfortunately, both spectral and non-spectral interferences of As are present, in particular intensity of As signals are affected by non-spectral interference of carbon containing substances. By ICP/MS signals of As may be enhanced from carbon-containing compounds.

REFERENCES


Chapter 1


