SUMMARY

The volatile substances found in wines are an important, though not entirely clarified, field of study in Romanian viticultural technology.

Aroma - a 15th or 16th century addition to the Romanian language via religious terminology from the Greek αρωματικό or the Slavic *aromatŭ*; *aromatic,* adj., from the French *aromatique*; *a aromatiza,* vb.; *aromeală,* s.f. (înv., temptation, sleepy daze, easy sleep); *a aromi,* vb. (to seduce, to tempt, to doze off, to go to sleep) highlights the main characteristic feature of this class of wines with a pleasant smell.

Unfortunately, the local Romanian aromatic wines - Tămâioasă românească, Busuioacă de Bohotin – have not enjoyed much success lately due to the prevalent international consumer trend in favour of dry wines. Aromatic wines tend to be sweet as the sugars emphasize the volatile substances, which explains why consumers tend to avoid them while also ignoring their originality. At present, many wine-makers try to win consumers back by offering dry aromatic wines. Basically, this means an identical technology, except that the wine is dry. The remanent sugars are in a very small amount, which is why a way of optimizing the technology has to be found that makes it possible for the aromas to persist without any support from the glucides.

The volatile substances are influenced by the specific climatic conditions and, above all, by the processing technology, the maceration procedure in particular. The aromatic substances that lend personality, specificity and authenticity to the wines are extracted from the skins (the terpenic compounds) during the maceration process and begin to figure prominently during fermentation and/or ageing (alcohols, acids, esters).

The wines obtained from the local aromatic variety Tămâioasă românească and from Muscat Ottonel, a cosmopolitan variety that has been studied comparatively in order to identify its value, ought to be turned to good account and analyzed with a view to expanding their olfactory spectrum, and launched again on the market so as to improve the range of available aromatic wines.

The doctoral thesis is structured in seven chapters, of which the first three present the aromatic grape and wines varieties, as well as the present state of research with respect to general production technologies of aromatic wines, while also discussing analysis methods used in aroma determination. The next four chapters introduce data referring to the institutional frame for the research, the vegetal material that was used, the working protocol and the analysis techniques we used, as well as the results, their interpretation and, not least important, the general conclusions.

Chapter I (pp. 33-41), provides up-to-date information on the present state of research

concerning the aromatic grape and wine varieties under study (Muscat Ottonel and Tămâioasă românească). Their origin and ampelographic characteristics are presented together with their agrobiological and technological value as demonstrated in various viticultural areas in Romania. In the same chapter, the compounds that are responsible for wine aroma, especially the primary aroma given by the compounds found the the berry's skin, are introduced to the reader.

Chapter II (pp. 42-59) enumerates and discusses the production technologies used for aromatic wines, presenting different aspects concerning common maceration techniques (classical skin contact maceration, maceration with enzymatic products and yeasts) compared to new technologies (ultrasound maceration, microwave maceration, criomaceration), and highlighting both their advantages and disadvantages.

Chapter III (pp. 60-72) provides general and specific data referring to the analysis of volatile compounds, the equipment in use, the principles and the working method of the gas-chromatographer as well as the extraction method of aroma compounds from the wines.

Chapter IV (pp. 74-76) introduces the organisational and institutional frame within which the research for the doctoral thesis took place.

Chapter V (pp. 77-86) underlines the main research objectives, the most modern analysis methods protocols, as recognized by the OIV, and the maceration technologies applied to the aromatic wines, and the research issues raised by the experimental samples under study.

Chapter VI (pp. 87-190) presents the results that have been obtained concerning the physical-chemical and compositional characteristics of the wines as well as original data about the identification of volatile compounds in the wines we analysed.

The doctoral thesis ends with the general conclusions (chapter VII, pp. 195-198) that synthethise the main contributions of the dissertation and the implications of the results we obtained as concerns the optimisation of the production technologies of aromatic wines in the Cotnari and Iaşi vineyards.

Our research focuses pre-eminently on the first part of the vinification process, that is, maceration, while also analysing in parallel both classical trends and more modern approaches such as ultrasound, microwaves, criomaceration.

The main goal of our study is getting relevant data concerning ways of improving the technology of aromatic wines from local and cosmopolitan varieties when applying various maceration-fermentation treatments under the pedo-climatic conditions of Iaşi and Cotnari vineyards.

The main objectives of our study are as follows:

- The physical-chemical analysis of the samples from various maceration methods: criomaceration, ultrasound maceration, microwave maceration, enzyme maceration and

classical skin contact maceration;

- Establishing the degree of influence of the maceration method on the dynamics of the compounds directly responsible for the aroma of the wines obtained from the Muscat Ottonel and Tămâioasă românească varieties; the terpenic compounds have been primarily considered for analysis as they figure prominently following the maceration process;
- Identifying the aromatic compounds in the wines obtained from Muscat Ottonel and Tămâioasă românească as a result of various maceration approaches.

Harvesting was carried out manually in plastic buckets and the grapes were taken to the Oenology Laboratory of the Faculty of Horticulture Iaşi for processing. Processing was carried out in accordance with the general technological flux for aromatic wines, but the emphasis was laid on the maceration phase which was performed both classically, i.e. skin contact maceration and via modern methods described below.

Variant V0. Control sample

Muscat Ottonel and Tămâioasă românească grapes were processed by observing the stages of the general technological flux for white non-aromatic wines. As such, after crushing and de-stemming, the must was pressed directly, without any contact with the grape skins. Fermentation occurred as a result of the activity of the indigenous yeasts. The wines were racked, conditioned, filtered and bottled.

Variant V1. Enzymatic maceration

Maceration with/by means of enzymes was performed by using two commercial enzymatic products, ZYMARÔME G® and ZYMOCLAIRE Muscat®.

Three aromatic wines-specific yeasts were also used (FERMOL AROMATIC®, FERMACTIVE MUSCAT® and FERMOL GRAND ROUGE NATURE®) and non-aromatic wines-specific yeasts (FERMACTIVE AP®) were added in the control sample.

The enzyme-treated must was pressed with a hydraulic press and then moved to glass containers to complete alcoholic fermentation by utilising yeasts in the way already described. Following fermentation, the wines were racked, conditioned, filtered and bottled.

Variant V2. Classical maceration

The de-stemmed and crushed grapes underwent classical maceration, i.e., 12 and 48 hours respectively, following which fermentation took place for two weeks at low temperatures, 15^{o}C maximum. The must was pressed with a hydraulic press and then moved to glass containers to complete alcoholic fermentation with Fermactive Ap® yeast. The wines were racked, conditioned, filtered and bottled.

Variant V3. Microwave maceration

Microwave maceration was performed by means of the microwave oven from the

Oenology Laboratory. The must was radiated at 350W and 650 W. The must was pressed with a hydraulic press and then moved to glass containers to complete alcoholic fermentation with Fermactive Ap® yeast. The wines were racked, conditioned, filtered and bottled.

Variant V4. Ultrasound maceration

Ultrasound maceration was carried out with the help of the ultrasound bath from the Oenology Laboratory (45 kHz frequency, 160W power). The must samples were treated for 5, 10, and 15 minutes, respectively. The must was pressed with a hydraulic press and then moved to glass containers to complete alcoholic fermentation with Fermactive Ap® yeast. The wines were racked, conditioned, filtered and bottled.

Variant V5. Criomaceration

The grape samples were stored in the freezer at ca. –20 °C and then crushed while still frozen. The must was pressed with a hydraulic press and then moved to glass containers to complete alcoholic fermentation with Fermactive Ap® yeast. The wines were racked, conditioned, filtered and bottled.

The analysis of the wines' compositional characteristics was carried out over the period January-February 2007/2008 in the Oenology Laboratory of the Faculty of Horticulture from the University of Agricultural Sciences and Veterinary Medicine „Ion Ionescu de la Brad" Iaşi. The physical-chemical analyses/tests were carried out strictly in keeping with the methods stipulated by international standards and specialised literature.

The following physical-chemical analyses/tests were carried out for the kinds of wines we obtained: volumic mass, alcoholic concentration, total acidity, volatile acidity, reductive sugars, non-reductive extract.

In addition to the general tests/analyses, we also completed the aromatic profile of the wines processed through various maceration technologies with the help of gas-chromotography and spectrophotometry. By comparing the compounds identified and the areas of the resulting peaks, we managed to establish to what extent climatic conditions and maceration technologies influence the participation ratios of the volatile substances for each variety under study.

The samples obtained through the process described above were subjected to the SPE extraction by means of LiChrolut EN/RP-18 (40-120 µm) 100 mg and RP (40-63 µm) 200 mg, 6mL Standard PP and LiChrolut EN (40-120 µm) 500 mg, 6 mL Standard PP cartridges.

20 mL wine samples were passed through a C^{18} bed SPE cartridge. The adsorbant bed was first conditioned with 10 mL dichlormethane, 10 mL metanol and 10 mL aquaeous solution of ethanol 13% v/v. The adsorbant bed was dried up by means of a 20-minute forced air jet. The compounds retained in the adsorbant layer were then recovered by percolating the bed with 2 mL diclormethane. The resulting extract was sealed hermetically and then injected into the Shimadzu

GC-2010 gas-chromatograph coupled with a QP2010 Plus mass spectrometer.

1000 μL extract were injected splitless into the chromatographic pipe. The duration of the analysis/test was 72 minutes for each wine sample. The aroma compounds were determined by means of the NIST 08, Wiley 08 and SZTERP spectrum library. The acceptable resemblance percentage was fixed to no less than 70%. The area of the representative peak for each compound is considered to be a direct proportion of the amount of the respective substance in the wine sample.

Our study represents a step forward towards the characterisation of aromatic wines obtained from autochthonous and cosmopolitan grape varieties, i.e., Tămâioasă românească and Muscat Ottonel, respectively. The study of the effects of various maceration technologies on wine aromatic compounds constitutes a novel field of research, both nationally and internationally.

It has been found that the annual climatic conditions have an important bearing on the aromatic compounds accumulation in grapes and wine. The amounts of volatile compounds, expressed in units of peak areas, have been higher in the control wines (no maceration applied) obtained from 2007 Tămâioasă românească and Muscat Ottonel grapes compared to 2008, a rainy year with low temperatures during the maturation period. In addition, the amount of sugar in the grapes (a well-known aroma modulator) was also higher in 2007.

In the specific circumstances of the experimental years, the wines obtained from the Tămâioasă românească grape variety display a higher amount of aromatic substances (expressed in units of peak areas) than the Muscat Ottonel variety. The 2007 Tămâioasă românească wines display almost double linalool, 10 times higher hotirenol and 4 times higher geranic acid concentrations than the Muscat Ottonel wines.

The aromatic profiles of the wines obtained from the two grape varieties are generally similar, although a number of differences do exist. More specifically, 3,7-dimethyl-6-octen-1-diol, 2,6-dimethyl-7-octen-2,6-diol, limonen and dihydromyrcenol have been identified only in the variants processed from Tămâioasă românească grapes, while epoxylinalol and terpendiol have been recorded only in the Muscat Ottonel sample.

The maceration techniques applied for primary wine-making have a different impact on certain classes of odour compounds. Of the terpenic compounds present in the samples under study, linalool and hotrienol deserve special mention.

The Muscat Ottonel and Tămâioasă românească wine samples processed through criomaceration and enzymatic maceration display the highest amounts of linalool. As regards the Muscat Ottonel wines, ultrasound maceration impacted negatively on linalool, its concentration in the control sample being almost twice higher than in the 10-minute ultrasound sample and

four times higher than in the 15-minute ultrasound sample. Linalool was missing altogether in the wine sample obtained through 5-minute skin contact maceration in the ultrasound bath.

As to the Tămâioasă românească wines, ultrasound maceration had a positive impact on linalool, its concentration in the control sample being lower than in the 10-minute ultrasound maceration sample. Similarly, linalool concentration was found to have increased in the 2008 wines treated with ultrasound for 15 minutes compared to the control sample.

For the Muscat Ottonel wines obtained through classical 48-hour skin contact maceration, more terpenic compounds have accumulated linalool in particular, than in both the 12-hour macerated variant and the control sample. However, from the economic point of view, the differences are not significant.

The 48-hour classical maceration applied in the wine-making of Tămâioasă românească did not result in higher numbers of compounds being extracted, while – unlike the sample obtained through 12-hour skin contact maceration – epoxylinalool and nerol disappeared. The linalool amount was not visibly modified.

In the case of wine variants obtained by processing Muscat Ottonel and Tămâioasă românească grapes, of all the samples studied, the 350W microwave maceration extracted the highest amount of linalool, whereas 650W radiation of must was shown to obtain the lowest amount of linalool.

Muscat Ottonel wines obtained through 5-minute ultrasound treatment of must, the criomaceration wine variant and the two wine variants treated with Zymarome G® and with the Fermactive Muscat® and Fermol Aromatic® yeasts, contain the highest amounts of hotrienol.

Tămâioasă românească wines obtained through the 650W radiation of must and those processed through enzymatic maceration with Zymoclaire Muscat® and with Fermactive Ap® yeast display the highest content of hotrienol.

In samples treated with the yeasts Fermol Grand Rouge Nature® and Fermactive Muscat® for both wine variants, hotrienol was undetectable. In the case of wines treated with the Zymoclaire M and Zymarome G enzymes and the yeasts mentioned above, the hotrienol content is lower than in the control sample.

With regard to the skin contact maceration of Muscat Ottonel and Tămâioasă românească wines, mention must be made of the medium and long-term maceration variants, 12 and 48-hour maceration, respectively, for which the hotrienol amounts are higher compared to the control sample, where hotrienol is to be found in lower amounts.

The maceration techniques applied for primary vinification do not influence the variation of acids, alcohols and esters with sensory print.

The Muscat Ottonel control sample, the variant obtained through enzymatic maceration

with Zymarome G® and Grand Rouge Nature® yeast and the variant obtained with the Zymoclare M® enzyme and Fermol Aromatic® yeast display the highest amounts of hexanoic acid, a compound with a scent of goat and barnyard animals. The remaining variants display very small amounts of hexanoic acid.

With regard to the Tămâioasă românească wines, the highest content of hexanoic acid has been recorded in the case of the Zymoclaire M® and Fermactive Ap® variant, as well as for the criomaceration variant.

For the remaining experimental variants, the hexanoic acid is present in much lower amounts, whereas in the sample processed through Zymoclaire M® enzyme usage and Fermol Aromatic® yeast usage, the hexanoic acid reached undetectable levels.

The alcohols identified in the highest amount are phenylethylalcohol and 4-hidroxy-benzenethanol.

Enzymatic macerations, ultrasound maceration and simple skin contact maceration are shown to lead to an increase in the amount of isobutylic alcohol, a compound with a strong aroma.

Isobutylic alcohol has been identified in high amounts in the Tămâioasă românească wines processed via ultrasound (10 minutes), 48-hour simple skin contact maceration, and microwave maceration of grapes (350W). For the enzymatic maceration variants, the amount of isobutylic alcohol is lower.

As concerns Muscat Ottonel wines, the variant obtained through Zymarome G® enzymatic maceration and Fermol Aromatic® yeast added for fermentation contained the highest amount of isobutylic alcohol. This compound is to be found in only small amounts in the criomacerated samples. Simple skin contact macerations do not result in an increase in isobutylic alcohol, as opposed to ultrasound macerations.

Phenylethylalcohol, a compound smelling of roses, readily identified in both varieties, has been found in maximum amounts in the Tămâioasă românească sample obtained through enzymatic maceration with Zymoclaire M® and Fermactive Ap® yeast, and for Muscat Ottonel, in the variant obtained with Zymarome G® and the Fermol Grand Rouge Nature® yeast. The lowest amounts have been recorded for the variants processed through ultrasound maceration.

Classical skin contact maceration is shown to lead to the formation of a reduced number of esters, whereas criomaceration favours an increase in the number of esters. As regards ultrasound maceration, the esters identified in the wine samples decrease both in number and in amount.

The variants processed through maceration with enzymatic products, classical skin contact maceration, and criomaceration display higher amounts of isoamyl acetate, a compound

with a strong banana aroma.

The identification of terpenes in the Muscat Ottonel and Tămâioasă românească control samples, where no maceration procedure was applied, clearly demonstrates the existence of a number of compounds that are responsible for the aroma in the pulp of the grape berry in accordance with previous studies.

The results of this study represent a first stage in the development of a possible future modulation of wine sensorial characteristics starting from the primary wine-making maceration phase. The production of wines with a more flowery note (crio-maceration) or fruitier hue (enzymatic maceration), while also minimising the unpleasant odours given off by fatty acids (microwave maceration) is thus facilitated.