

INTEGRATED RESEARCH IN OENOLOGY*

The creation of the Oenology degree at the University of Tarragona meant a considerable challenge for the staff involved. The initial period of consolidation of the teaching function was followed by research development, the other high priority of the Faculty of Oenology. The integration of personnel from different scientific and technological backgrounds allowed an excellent complementarity and synergy for the development of competitive research projects. Currently, more than 40 researchers are involved in oenological scientific and technological research, and these researchers make up the Oenology Unit, a part of the Centre de Referència en Tecnologia dels Aliments (Reference Centre for Food Technology), a virtual research institute created by the Generalitat of Catalonia.

Origin and development

The School of Oenology, created in 1988, meant the development of an experience in teaching and research in the discipline of oenology. The need for quality teaching in a field that was not yet recognised by an official title in Spain was a challenge for the group of researchers in the Faculty of Chemistry who were involved. Initially, the focus was on teaching and a curriculum development for oenologists that would meet the needs of the rapidly growing wine sector. Although this initial goal was achieved, the final accreditation of the 1996 Baccalaureate of Science (BSc), in Oenology changed the old School of Oenology into the present Faculty of Oenology.

The academic restructuring and teaching delivery required an additional effort in fundamental oenological research. Thus, the personnel involved with the Oenology School during this time had to develop research programmes within the wine sector. This required strong scientific disciplinary interaction among the scientists as they faced different research projects. Also, new winery facilities, experimental and sensory evaluation laboratories, were established to be used both for the formation of oenology students and for research.

In 1994, the Generalitat of Catalonia decided to develop the «Reference Centres for Research» within their «Re-

search Plan». The «Reference Centres for Research» were designed as virtual centres which aggregated some already existing facilities in public research institutions. One of those Reference Centres was the Centre de Referència en Tecnologia dels Aliments (CeRTA, Reference Centre for Food Technology), which had 7 Research Units. Among these was the Oenology Unit, grouping the researchers involved in developing the different research activities globally related to wine. This global approach allowed the development of research streams ranging from vineyard training and grape production, to wine fermentation and development, quality improvement factors which related to the health and nutritional aspects of wine consumption.

The experimental facilities

The development of Oenology teaching obliged the Faculty to complete the facilities with vineyards and a cellar. The municipality of Tarragona provided the Faculty of Oenology with a 7 hectare plot. In this plot, a 5 hectare vineyard was then developed. The vineyard was designed to fulfil both teaching and research activities. On the one hand, 17 different varieties were planted to allow the students to follow the development of representative grape varieties. On the other hand, several experimental plantations were undertaken to analyze the plantation framework, water stress, and the effect of water supplementation on grape production and wine quality. In addition, an ampelographic collection of 67 local varieties are kept to test the oenological potential and projected future usage.

The experimental plots grow around a newly built 700 m² winery (1995). The winery has a capacity for processing up to 60 000 kg of grapes which normally yield about 45 000 litres of wine. This production is mostly used for teaching purposes (wine made by the students as part of their curricula) yet a reasonable amount is diverted into research. The winery has a microvinification facility with hundred-litre vats at a controlled temperature. These comprise an excellent research complement to the industrial fermentations regularly produced in the cellar.

Research laboratories in analytical chemistry, sensorial analysis, molecular biology, microorganisms and plant biochemistry, microbiology and food chemistry make up the final set up of research resources linked to the Faculty of Oenology. For sensorial analysis a tasting room for up to 40

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people was built, with all the facilities regarding lighting and isolation. A refrigerated wine storage room connecting to the tasting room was also built.

The human factor: research groups

The research groups of the Department of Biochemistry and Biotechnology are the most numerous within the Oenology Unit. The research groups in Oenology from this department are identified as Oenological Biochemistry and Oenological Biotechnology.

The Oenological Biochemistry group deals with research subjects in viticulture, fermentation and effects of wine components on human health. In viticulture, applied research is mostly carried out in the experimental plots and the water availability to the grapevine is analyzed in terms of plant growth and grape ripening. Soil structure and nutrient uptake are strongly related to water availability and are studied as related to leaf metabolism. Among the relevant factors for wine quality in the ripening process, polyphenol synthesis is a specific area of analysis [1]. Finally, cloning and sequencing *Vitis* genome and its expression completes the viticulture research interests.

«Stuck» and «sluggish» fermentations are probably among the problems of greatest import in modern oenology [2]. A biochemical approach to this problem is taken by members of the oenological biochemistry group in analysing some of the most relevant factors, such as lipid metabolism and the composition of the plasma membrane, membrane carrier proteins, and nutrient uptake [3] on yeast exposed to different stress factors such as ethanol, copper, organic pesticides, etc [4, 5]. Due to the relevance of sparkling wine in our immediate region, projects dealing with wine protein characterisation related to foam formation and stability are undertaken [6,7]. The influence of different winemaking techniques on red colour extraction and stabilisation are also analysed [8 – 11].

There is considerable evidence to suggest that small amounts of alcoholic beverages reduce the risk of vascular disease and total mortality in middle and old age. But it is not clear whether wine is more protective than other alcoholic beverages because it contains phenolic compounds. The group has developed an animal experimental model which can distinguish the effects of alcohol from the effects of the non-alcoholic components present in wine [12], so as to determine whether wine has effects other than those of alcohol on cholesterol metabolism and oxidative status [13]. Due to the complexity of *in vivo* studies; flavonoids, specially pro-cyanidins, effects on cholesterol metabolism, fat reserves [14] and oxidative stress are analyzed in cell lines. Also, the distribution and metabolism of flavonoids are a subject of intensive research in this group.

The oenological biotechnology group has focused their research interest in the application of novel molecular biology techniques to the different fields of oenology. Some of the work was done in the field of viticulture by analysing stress

factors in the growth and adaptation of grapevines [15, 16], which led to the sequencing of some membrane proteins [17]. Knowledge of these may be of industrial interest and they are now protected by a registered patent [18]. However, the main interest has been focused on industrial microorganisms. The group has incorporated and developed molecular biology techniques to identify different yeast species [19], lactic acid [20] and acetic acid bacteria [21, 22]. These techniques have been successfully applied to analyze yeast population dynamics in different environmental conditions during wine making. Thus, analysis of different winery environments [23, 24], and oenological practices [25] have been performed or are being performed on yeast strain development [26]. Also, the selection of yeast strains and their use as fermentation starters has been carried out, already yielding a commercial presentation [27].

Molecular analysis has also been applied to lactic acid bacteria both for identification [20] and metabolic assessment as response to different environmental situations. The influence of compounds present in wine on kinetics of malolactic fermentation and physiology of malolactic bacteria have been studied, such as copper and other pesticides [28, 29], fatty acids, sulphur dioxide, and phenolic compounds [30]. The presence of bacteriophages in *Oenococcus oeni* and its relation to lysogeny and stuck malolactic fermentation has also been studied [31]. Additionally, several strains of malolactic bacteria have been isolated for use as starters [32].

The Analytical Chemistry of Wine and Aliments Research group which is a part of the Department of Analytical and Organic Chemistry, has been very active in developing analytical techniques for aroma and pesticide analysis in wine using innovative and conventional methods [4, 33-40]. Method validation for wine and must analysis has been carried out as well as assessment of the implementation of quality systems in different food analysis laboratories.

The Food Technology research group of the Department of Chemical Engineering has developed research activities in membrane separation techniques in wine and beer production, characterisation of membrane fouling during microfiltration and new processes to prevent and control this [41-43]. Also, the use of continuous processes for stabilisation and decoloration as well as the effects of must clarification on alcoholic fermentation has been the focus of its research [44].

However, none of these actions will be effective if the professional sector is not involved. Several research projects are underway, although the researchers and the research groups do not have the time to devote to administration and the amount of face-to-face contact development required. Thus, an oenologist acts as a conduit of knowledge, in order to best utilize the Faculty's human resources and match them to the needs of the wine sector while promoting and maintaining contact within the sector.

All the Faculty's researchers are very active in a wide variety of knowledge transfer activities, such as conferences, seminars and meetings. A yearly symposium is held in Vilafranca del Penedès which presents the most relevant ad-

Table 1. The research groups in the Oenology Unit of the Centre for Reference for Food Technology

<i>Department</i>	<i>Research Group</i>	<i>Permanent Members</i>	<i>Graduate Students</i>
Biochemistry and Biotechnology	Oenological Biochemistry	Lluís Arola Fernando Zamora Joan Miquel Canals Montserrat Nadal Francesca Fort Cinta Bladé Josepa Salvadó Anna Ardèvol Isabel Baiges	Montse Pinent Noemí Ferrer Guillem Vanrell Pedro Cabanillas Miriam Lampreave Josep Valls Bernardino García Francesc Puiggrós
	Oenological Biotechnology	Albert Mas José M. Guillamón Nicolas Rozes Albert Bordons Magda Constantí Montserrat Poblet	Maria Jesús Torija Angel González Gemma Beltran Maite Novo Ramon Carreté Gerrie Garcia Cristina Reguant
Analytical and Organic Chemistry	Wine and Food Analytical Chemistry	Josep Guasch Olga Busto	Montse Mestres M. Pilar Martí Cristina Sala M. Dolors Jornet Eva Brull Marc Rius Nuria Martorell Noemí Carrasco
Chemical Engineering	Food Technology	Montserrat Ferrando Carne Güell Francisco López	Vesselina Pachova Justyna Warczok

vances in oenological research done by the group researchers. The conferences are collected in a publication series, *Oenology Today*, published by the Oenology Unit of the Reference Centre for Food Technology

References

- [1] Nadal, M., Arola, Ll. Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis* 34 (3), 151-154, 1995.
- [2] Zamora, F. Los problemas de fermentación. In «Temas actuales en la microbiología enológica», Ed. Ayuntamiento de Haro, pp 51-79, 1994. ISBN: 84-7359-467-3.
- [3] Zamora, F., Fort, F., Fuguet, J., Bordons, A., Arola, Ll. Influence de certains facteurs lors de la fermentation alcoolique sur la prise de glucose par les levures. In «CEnologie 95», Ed. A. Lonvaud-Funel, Lavoisier, Paris, 1995, pp 167-171. ISBN: 2-7430-0083-X.
- [4] Sala, C., Fort, F., Busto, O., Zamora, F., Arola, Ll., Guasch, J. Fate of some common pesticides during vinification process *J. Agr. Food Chem.* 44, 3668-3671, 1996
- [5] Fort, F., Sala, C., Busto, O., Arola, Ll., Bordons, A., Guasch, J., Zamora, F. Presenza di pesticidi in vinificazione ed inibizione della fermentazione alcolica. *Vignevini*, 7/8, 42-44, 1999.
- [6] Canals, J.M., Arola, Ll., Zamora, F. Protein fraction analysis of white wine by FPLC. *Am. J. Enol. Vitic.*, 49, 383-388, 1998.
- [7] Zamora, F., Luengo, G., Margalef, P., Magriña, M., Arola, Ll. Efecto del sangrado sobre el color y la composición en compuestos fenólicos del vino tinto. *Rev. Esp. Cien. Tecnol. Alimen.*, 34, 663-671, 1994
- [8] Vivas, N., Zamora, F., Glories, Y. Etude des phénomènes d'oxydoréduction dans les vins. Mise au point d'une méthode rapide de mesure du potentiel d'oxydoréduction *J. Int. Sci. Vigne Vin*, 26, 271-285, 1992
- [9] Vivas, N., Zamora, F., Glories, Y. Incidence de certains facteurs sur la consommation de l'oxygène et sur le potentiel d'oxydoréduction dans les vins. *J. Int. Sci. Vigne Vin*, 27, 23-34, 1993
- [10] Vivas, N., Glories, Y., Bertrand, A., Zamora, F. Principe et méthode de mesure du potentiel d'oxydoréduction dans les vins. *Bull. O.I.V.*, 785-786, 618-633, 1996
- [11] Vivas, N., Saint-Cricq de Gaujeac, N., Zamora, F. Experimental principe to evaluate the degree of oxidation or reduction balance in wines. *J. Sci. Tech. Tonnelerie*, 5, 65-76, 1999.
- [12] Arola Ll., Roig R., Cascón E., Brunet M.J., Fornós N., Sabaté M., Raga X., Batista J., Salvadó M.J., Bladé C. Model for voluntary wine and alcohol consumption in rats. *Physiol. Behav.* 1997, 62: 353-357.

- [13] Roig R., Cascón E., Arola Ll., Bladé C., Salvadó M.J. Moderate red wine consumption protects the rat against oxidation in vivo. *Life Sci.* 1999, 64: 1517-1524.
- [14] Ardévol, A., Bladé, C., Salvadó, M.J, Arola Ll. Changes in lipolysis and hormone-sensitive lipase expression caused by procyanidins in 3T3-L1 adipocytes. *Int J. Obesity*, 24: 319-324, 2000.
- [15] Romeu, A., Mas, A. Effects of copper exposure in tissue cultured *Vitis vinifera*. *J. Agr. Food Chem*, 47, 2519-2522, 1999
- [16] Llorens, N., Arola, Ll., Bladé C., Mas A. Effects of copper exposure upon nitrogen metabolism in tissue cultured *Vitis vinifera*. *Plant Sci.* in press.
- [17] Baiges, I., Schaeffner, A.R., Mas, A. Sequences of *Vitis berlandieri* x *Vitis rupestris* putative aquaporins: PIP 1-1 (Accession number AF141643), PIP1-2 (Accession number AF141898), PIP 1-3 (Accession number AF141899), PIP 2-1 (Accession number AF141642), PIP 2-2 (Accession number AF141900), TIP 1 (Accession number AF271661), TIP 2 (Accession number AF271662), TIP 3(Accession number AF271660).
- [18] Baiges, I., Schaeffner, A.R., Mas, A. Nukleinsäuren und hieraus abgeleitete Oligonukleotide zur spezifischen Amplifikation und zum spezifischen Nachweis von Aquaporin-Genen aus *Vitis vinifera*. Patent register number 10011480.6 (Germany). 2000
- [19] Guillamón, J.M., Barrio, E., Huerta, T., Querol, A. Rapid characterization of four species of the *Saccharomyces sensu stricto* complex according to mitochondrial DNA patterns. *Int. J. Syst. Bacteriol.*, 44, 708-714, 1994
- [20] Zapparoli, G., Reguant, C., Bordons, A., Torriani, S., Dellaglio, F. Genomic DNA fingerprinting of *Oenococcus oeni* strains by pulsed-field gel electrophoresis and randomly PCR amplified polymorphic DNA. *Curr. Microbiol.*, in press.
- [21] Poblet, M., Rozés, N., Guillamón, J.M., Mas, A. Identification of acetic acid bacteria by restriction fragment length polymorphism analysis of a PCR-amplified fragment of the gene coding for 16S rRNA. *Lett. Appl. Microbiol.*, 30, 1-7, 2000
- [22] Ruiz, A. Poblet, M. Mas, A. Guillamón JM. Identification of acetic acid bacteria by RFLP of the PCR-amplified 16S rDNA and 16S-23S rDNA intergenic spacer. *Int. J. Syst. Evol. Microbiol.*, in press
- [23] Constantí, M., Poblet Icart, M., Arola, Ll., Mas, A., Guillamón, J.M. Analysis of yeast populations during alcoholic fermentation of wine in a newly established winery. *Am. J. Enol. Vitic.*, 48, 339-344, 1997
- [24] Torija, M.J., Ruiz, A., Martí, M., Beltran, G., Llauradó, J., Poblet, M., Rozès, N., Guillamón, J.M., Mas, A. Étude de l'évolution des levures et bactéries acétiques par l'utilisation de techniques de biologie moléculaire lors de fermentations spontanées et inoculées. *Oenologie* 99, A. Lonvaud ed, Tech&Doc, 2000, pp 399-402.
- [25] Constantí, M., Reguant, C., Poblet, M., Zamora, F., Mas, A., Guillamón, J.M. Molecular analysis of yeast population dynamics: Effect of sulphur dioxide and the inoculum in must fermentation. *Int. J. Food Microbiol.*, 41, 169-175, 1998
- [26] Llauradó, J., Constantí, M., Rozés, N., Mas, A., Velázquez, R., Bobet, R. Fermentaciones a bajas temperaturas (13C): Efectos de las cepas de levaduras y la adición de nutrientes. *Alimentación. Equipos y Tecnología*, XIX, 87-92, 2000
- [27] Torija, M.J., Rozès, N., Guillamón, J.M., Mas, A. *Saccharomyces cerevisiae*. Patent register CECT 11462. Date: 1/11/99. Commercial presentation RV1 (Lallemand Inc).
- [28] Bordons, A., Masqué, M.C., Vidal, M.T. Isolation and selection of malolactic bacteria and effect of pesticides. The management of malolactic fermentation and quality of wine. *Les entretiens scientifiques Lallemand*, 51-56, Verona, 1998
- [29] Vidal, M.T., Constantí, M., Bordons, A. Effeto del rame e dei pesticidi sulla fermentazione malolattica. *Vignevini*, 7/8, 50-53, 1999
- [30] Reguant, C., Bordons, A., Arola, L., Rozès, R. Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *J. Appl. Microbiol.*, 88, 1065-1071, 2000
- [31] Poblet, M., Bordons, A., Lonvaud-Funel, A. Lysogeny of *Oenococcus oeni* and study of their induced bacteriophages. *Curr. Microbiol.*, 36, 365-369, 1998
- [32] Masqué, M.C., Bordons, A. Isolation and selection of malolactic bacteria from southern Catalan wines. *J. Wine Res.*, 7, 91-101, 1996.
- [33] Busto, O., Guasch, J., Borrull, F. Biogenic amines in wine: A review of analytical methods. *J. Int. Sci. Vigne Vin*, 30, 85-101, 1996.
- [34] Busto, O., Guasch, J., Borrull, F. Determination of biogenic amines in wine after pre-column derivatisation with 6-aminoquinolyl-n-hydroxysuccinimidyl-carbamate. *J. Chromatogr.*, 737, 205-213, 1996.
- [35] Busto, O., Miracle, M., Guasch, J., Borrull, F. Solid phase extraction of biogenic amines from wine before chromatographic analysis of their AQC-derivatives. *J. Liq. Chromatogr.*, 20, 743-755, 1997
- [36] Sala, C., Busto, O., Guasch, J. A quick capillary gas chromatographic method for determining usual pesticides in must and wines. *Chromatographia*, 44, 320-324, 1997
- [37] Mestres, M., Busto, O., Guasch, J. Chromatographic analysis of volatile sulphur compounds in wines using the static headspace technique with flame photometric detection. *J. Chromatogr.*, 773, 261-269, 1997
- [38] Mestres, M., Busto, O., Guasch, J. Headspace solid phase microextraction analysis of volatile sulphides and disulphides in wine aroma. *J. Chromatogr.*, 808, 211-218, 1998
- [39] Mestres, M., Sala, C., Martí, M.P., Busto, O., Guasch, J. Headspace solid phase microextraction of volatile sulphides and disulphides using carboxenpolydimethylsiloxane fibers in the analysis of wine aroma. *J. Chromatogr.*, 835, 137-144, 1999

- [40] Mestres, M., Martí, MP., Busto, O., Guasch, J. Simultaneous analysis of thiols, sulphides and disulphides in wine by headspace solid phase microextraction gas chromatography. *J. Chromatogr.*, 808, 211-218, 1999.
- [41] Güell, C., Davis, R.H. Membrane fouling during microfiltration of protein mixtures. *J. Membrane Sci.*, 119, 269, 1996.
- [42] Czejak, P., López, F., Güell, C. Characterization of membrane fouling caused by protein fraction in wine. *Am. J. Enol. Vitic.*, 48, 388, 1997.
- [43] Güell, C., Czejak, P., Davis, R.H. Microfiltration of protein mixtures and the effect of yeast on membrane fouling. *J. Membrane Sci.*, 155, 113-122, 1999.
- [44] Ferrando, M., Güell, C., López, F. Industrial wine making: Comparison of must clarification treatments. *J. Agr. Food Chem.*, 46, 1523-1528, 1998
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