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			x

Indicate (X) client(s) to whom this final report is submitted.
Replace any of these with other relevant clients if required.

FINAL REPORT
FOR 2012

PROGRAMME & PROJECT LEADER INFORMATION

	Programme leader	Project leader
Title, initials, surname	Dr. Hélène Nieuwoudt	Dr Hélène Nieuwoudt
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PROJECT INFORMATION

Project number	IWBT-B 08/11
Project title	Bioprocess monitoring of fermentations with infrared spectroscopy
Project Keywords	Alcoholic fermentation, malolactic fermentation, Infrared spectroscopy

Industry programme	**CFPA**	
	Deciduous	
	DFTS	
	Winetech	Microbiology committee
	Other	

Fruit kind(s)	Wine
Start date (dd/mm/yyyy)	01/01/2008
End date (dd/mm/yyyy)	31/12/2011

(Note: adjust footer – insert the project number no, researcher and research institution)

FINAL REPORT

(Completion of points 1-5 is compulsory)

1. Executive summary

Give an executive summary of the *total* project in no more than 250 words

This project developed strategies to do rapid, low cost monitoring of alcoholic and malolactic fermentation using infrared spectroscopy and chemometric modelling techniques. Technologies for three different infrared spectrometers, two in mid-infrared and one in near-infrared regions, were developed, since these technologies are all three used by the SA wine industry and in research applivcations. A portfolio of calibration models for quantification of important quality parameters in grape juice, fermenting must; both during alcoholic (AF) and malolactic fermentation (MLF) were established. **Three main approaches** were used; **firstly** a **quantitative** approach where exact chemical values can be quantified based on the infrared spectra; s**econdly** a **qualitative** approach where the spectra obtained from actively fermenting must, were modelled to obtain the metabolic status of the bioprocess and to establish trends characteristic of a batch of juice (in large scale industrial fermentations), or characteristic of the inoculation strategy followed, in the case of MLF. A **third strategy combined quantitative and qualitative models** to interpret the fermentation trends or abnormalities, in terms of the major chemical compounds. Off-line and on-line monitoring was done. Quantitative and qualitative models were combined to be able to interpret the fermentation trend in terms of the major chemical compounds approached was used to interpret the infrared **Four** postgraduate students (three MSc and one PHD), were trained or are currently still being trained in infrared spectroscopy and chemometric modelling, of whom three are already employed by the wine industry do to these tasks.

2. Problem identification and objectives

State the problem being addressed and the ultimate aim of the project.

Wine production involves a succession of processes that are catalysed by microbes and it can therefore be considered as a true bioprocess. Real-time monitoring of alcoholic fermentation (AF) and malolactic fermentation (MLF) is of utmost importance not only due to logistic considerations that include tank space being made available at the earliest opportunity during the harvest season, but also due to the inherent risks of microbial spoilage and problematic fermentations characteristic of each stage. Frequently, timely identifications of problems during each stage of the fermentation can lead to rectification, or at least containment of the impact of the problem through informed decisions that are based on real-time analysis. MLF is a complex biological process catalysed by several lactic acid bacteria (LAB). Although monitoring of both AF and MLF are complex processes, MLF monitoring can be particularly cumbersome and requires complex chemical analyses, including the quantification of organic acids as well as selected volatile and non-volatile compounds. In oenological terms the end-point of MLF is marked by that stage of the process where the malic acid concentration falls below ~0.3 g/L. The onset of MLF is frequently unpredictable and the progress slow which result in significant variation in the duration from batch to batch. This complicates the simultaneous monitoring of several fermentation vessels and also implies that large numbers of chemical analyses must be done.

Speed is an essential requirement of all the critical stages of process monitoring, including analytical measurements, data processing and data interpretation. This implies that in some instances quantification of the full range of relevant compounds is not an absolute requirement. Nevertheless, quantitative data provide valuable additional information necessary to interpret observed trends during the fermentation process. Real-time process monitoring benefits optimally from a fully automated set-up, however several fundamental aspects, including analytical instrument calibration, optimisation of data processing and establishment of effective data interpretation techniques must first be developed before the strategy can be automated to monitor several fermentation vessels simultaneously.

The aim of this project is to develop the fundamental aspects required for effective AF and MLF monitoring. The strategy described in this proposal combines the use of a rapid analytical technique, Fourier transform near infrared spectroscopy (FTNIR) to generate the instrumental measurements, with chemometric techniques to process and interpret the data. FTNIR is a well established analytical technique for on-line and at-line monitoring of biotechnological fermentation processes, but its application for monitoring AF and MLF during wine production has not been evaluated.

The following aims describe two complementary strategies necessary for the development of the fundamental aspects related to this project. These strategies are: (i) quantification of important fermentation parameters to monitor AF and MLF using FTNIR spectroscopy and, (ii) monitoring the progress of MLF through the development of multivariate statistical process control charts based on FTNIR spectroscopy.

3. Workplan (materials & methods)

List trial sites, treatments, experimental layout and statistical detail, sampling detail, cold storage and examination stages and parameters.

Milestone 1: Develop rapid multi-component quantification strategies for important major chemical compounds necessary to monitor AF and MLF using FTNIR spectroscopy

Task 1: Establish quantitative FTNIR spectroscopy calibrations for major chemical parameters including pH, tartaric-, malic-, lactic-, succinic-, citric- and acetic acid, glucose, fructose, glycerol and ethanol. Experimental red and white wines will be produced and sampled during AF and MLF to generate FTNIR spectra and analyse the samples with established reference methods including HPLC and CE.

Establishment of quantitative calibrations will be extended to include both near infrared (FTNIR) spectra, as set out in the original project description, as well as mid infrared (FTMIR) spectra. Samples will therefore be scanned using two different spectrometers, each set up for generating spectra in a different region of the electromagnetic spectrum. This additional task is motivated by the fact that several wine analytical laboratories and industrial cellars in South Africa are using FTMIR spectrometers and the usefulness of the technology developed in this study will therefore be enhanced by including FTMIR spectra as well. The timeline for completion of the workplan will not be affected by this addition. The tasks related to milestone 1 are thus redefined as follows:

Task 1: Optimisation and development of a portfolio of rapid multi-component analytical strategies for quantification of major organic acids, sugars (glucose and fructose), ethanol and glycerol in fermenting must and wine.

Task 2. Establishment of quantitative calibrations using FTMIR spectra generated on amongst other, the WineScan FTMIR spectrometer and using the reference data generated in Task 1.

Task 3. Establishment of quantitative calibrations using FTNIR spectra generated on the Multi-Purpose Analyzer (MPA) FTNIR spectrometer and using the reference data generated in Task 1.

Milestone 2: Establish correlations between qualitative information in FTNIR spectra with quantitative chemical data

Task 1: This project focuses only on MLF. The monitoring of several chemical compounds present at relatively low concentrations is important to follow the metabolism of LAB during MLF. This project is aimed to evaluate whether significant information relating to the levels of these minor, but important compounds, is indeed captured in the FTNIR and FTMIR spectra. The objective is therefore not quantitation but rather a qualitative focus. It will be of extreme importance to establish to which extent the analytical strategy using FTNIR and FTMIR can capture important chemical changes that occur during MLF. These minor compounds will be analysed with GC-FID and possibly GC-MS.

Milestone 3: Monitoring the progress of AF and MLF through the development of multivariate statistical process control charts based on FTNIR and FTMIR spectra

The tasks related to this phase of the work will involve the monitoring of several fermentation vessels (tanks and barrels) for red and white cultivars to establish the time trends for the progress of these processes. Physical and chemical parameters such as temperature and pH, will be used to induce abnormally induced fermentations. The next task will be an investigation as to the best strategy to align, in terms of time or stage of progress, the duration of the different batches, in order to plot the progress of all the fermentations that are monitored simultaneously. These strategies will then be used to establish multivariate statistical process control charts for the various fermentations.

Establishment of multivariate statistical control charts will be extended to include both near infrared (FTNIR) spectra, as set out in the original project description, as well as mid infrared (FTMIR) spectra. As already set out under Milestone 1 task 1, this additional task is motivated by the fact that several wine analytical laboratories and industrial cellars in South Africa are using FTMIR spectrometers and the usefulness of the technology developed in this study will therefore be enhanced by including FTMIR spectra as well.

Task 1: Monitoring the progress of MLF using FTMIR and FTNIR spectra of samples taken at the start, during and at the end of MLF fermentation.

Task 2. Monitoring the progress of alcoholic fermentation using FTMIR and FTNIR spectra of samples taken at the start, during and at the end of alcoholic fermentation.

Milestone 4: On-line FTNIR monitoring of alcoholic fermentation in must with nitrogen supplementation: Volatile composition and sensory properties of Shiraz wines

Task 1: Monitoring the progress of alcoholic fermentation, using on-line FTNIR in Shiraz juice with nitrogen supplementation

Task 2: Assessing the impact of nitrogen supplementation on major volatile compounds, H_2S and volatile thiol (mercaptan) content.

Task 3: Assessment of the sensory properties of the Shiraz wines.

Note: These tasks could not be executed due to logistical problems and an amendment was proposed.

Milestone 4 amendment:

Bioprocess monitoring and trend identification in alcoholic and malolactic fermentation in Shiraz, with FT-MIR and FT-NIR spectroscopy and chemometric modelling.

Milestone 5: Bioprocess monitoring using attenuated total reflection spectroscopy (FT-MIR ATR). This is newly released technology that is mobile, less expensive than the conventional large spectrometers currently in the market, and versatile in that both liquids and solids can be analysed.

Task 1: Quantification of important grape quality parameters, glucose, fructose, malic acid, pH, titratable acidity, nitrogen content and phenolic composition in grape juice
Task 2: Quantification of the before mentioned compounds in fermenting must
Task 3: Quantification of wine parameters using FT-MIR ATR

4. Results and discussion

State results obtained and list any benefits to the industry. Include a short discussion if applicable to your results. This final discussion must cover ALL accumulated results from the start of the project, but please limit it to *essential* information.

Project and Results	Achievements and industry benefits
Develop a portfolio of rapid multi-component quantification strategies for important major chemical compounds in wine, grape juice and fermenting must, necessary to monitor AF and MLF using FTMIR and FTNIR spectroscopy (Milestone 1, 2). Comments April 2012: Based on unsatisfactory accuracies obtained with infrared spectroscopy, for some parameters, including malic acid in red wine, the Konelab automated enzyme-linked robot instrument was acquired by IWBT-DVO in 2011. This instrument was calibrated for several wine compounds to date and done in collaboration with CAF, US (not funded by Winetech).	Achievements: 1. Quantitative calibration models for sugars (glucose and fructose), ethanol, organic acids (n=7), pH, TA, VA, in grape juice and actively fermenting red and white must, as well as during MLF were made. Models for YAN in fermenting grape must and glycerol in wine were also developed. In total 16 different cultivars, and more than 250 samples included in calibration models. 2. Accuracies and precisions obtained with infrared spectroscopy for different parameters were evaluated and alternative rapid analytical methods, like the enzyme robot, were tested and implemented. These alternative reference methods can now be used for establishment of infrared calibration models. Industry benefits: 1. These developments make a significant expansion to applications of the IR technology widely used in the SA wine industry. In addition, extensive capacity for high-throughput screening methods for related DVO-IWBT and ARC Winetech funded research projects was built. 2. Service for analysis of compounds on infrared instrumentation and enzyme robot analysis is offered by the chemical analytical laboratory of DVO-IWBT, to the industry.
Milestone 3. Monitoring the progress of AF and MLF through the development of multivariate statistical process control (MSPC) charts based on FTMIR and FTNIR spectra.	Achievements: 1. Monitoring the progress of alcoholic fermentation in normal and abnormal fermentations has been

	done, using FTIR spectra obtained from sampling of 21 industrial fermentation tanks (red and white wines). MSPC charts were used to establish the normal trends and to clearly identify abnormal trends. Quantitative interpretation of the causes of the problem fermentations was done based on the calibration models established in milestone 1 and 2. Industry benefits: This strategy provides a rapid, and information rich strategy, for monitoring and interpreting fermentation progress and the status of the processes.
Milestone 4: On-line FTNIR monitoring of alcoholic fermentation in must with nitrogen supplementation: Volatile composition and sensory properties of Shiraz. **Amended milestone 4:** **Bioprocess monitoring and trend identification in alcoholic and malolactic fermentation in Shiraz, with FT-MIR and FT-NIR spectroscopy and chemometric modelling.**	Achievements: Amended milestone 4. 1. Different LAB bacteria were used to elaborate Shiraz wines, using sequential and co-inoculation strategies and the fermentations were monitored by FT-MIR and FT-NIR. Chemometric modelling using infrared spectra alone, yielded models to interpret the fermentation trends. The stage of completion of alcoholic and malolactic fermentation could be modelled and clear differences related to inoculation strategy used were captured. 2. This experimental strategy was also used with Chardonnay fermentations, in which case on-line FT-NIR fermentations were also done. Industry benefits: 1. A strategy for monitoring alcoholic and malolactic fermentation without chemical analysis was developed. This approach was expanded to do real time monitoring where the actual process states are graphically shown as they evolve. This strategy potentially offers much more information than occasional sampling followed by wet chemical tests, and the volumes of wet chemical tests required are drastically reduced.
Milestone 5: Bioprocess monitoring using attenuated total reflection spectroscopy (FT-MIR ATR). This is newly released (2010) technology that is mobile, less expensive than the conventional large spectrometers currently in the market, and versatile in that both liquids and solids can be	Achievements: 1. Calibrations for major parameters (n=10) in fermenting must and wine were developed using FTIR-MIR ATR. 2. Grape juice calibrations, ammonia, alpha amino nitrogen, glucose, fructose, pH, TA, Brix, gluconic acid, malic acid were developed, using >300 samples and including 20 cultivars (red and white). Industry benefits: 1. All calibration models are available for transfer to industry. ATR calibrations have been transferred to

analysed	SA wine labs, where requested. 2.Thes technologies developed in this project were transferred to industry through application of the methods in routine analysis of (i) experimental samples in other concurrently running Winetech funded projects of IWBT-DVO, (ii) ARC Infruitec projects and, (iii) wines of private producing cellars.
	Industry benefit of trained students Four students skilled in bioprocess monitoring and infrared spectroscopy are being trained, or have completed training in this project. Two MSc students currently hold senior laboratory positions at private cellars (Namaqua Cellar and DGB) and are actively involved with fermentation process monitoring using infrared spectroscopy. The PhD student has also been employed by industry (Waterkloof winery) and is actively involved with fermentation and wine quality monitoring.

5. Accumulated outputs

List ALL the outputs from the start of the project.
The year of each output must also be indicated.

Research outputs

- Establishment of quantitative calibrations in fermenting must for FT-MIR spectrometers (7 parameters and 16 cultivars used: 2008 and 2009
- Establishment of **off-line** qualitative strategies to monitor industrial scale fermentations. Use of infrared (FT-MIR) spectra control charts and to development of models to detect fermentations with abnormal behaviour: 2009
- Establishment of quantitative calibrations for two types of spectrometers (FT-MIR and FT-MIR ATR) in freshly pressed grape juice: fermenting must (10 parameters and 16 cultivars used: 2009, 2010 and 2011
- Strategy for **at-line** monitoring of Shiraz and Chardonnay fermentations elaborated with different LAB using FT-MIR and FT-NIR established; chemometric models identify process states and metabolic status of fermentations created: 2010 and 2011
- Strategy for **on-line** monitoring of Chardonnay fermentations elaborated with different LAB, using FT-NIR 2010 and 2011

Presentations/Conference proceedings: 17

- National: 9
- International: 7
- Workshops: 1

Publications
Scientific publications (peer reviewed): 6

- Published (2011 and 2009): 2
- Under preparation for submission in 2012/2013: 4

Technology development, products and patents

Indicate the commercial potential of this project (intellectual property rights or a commercial product(s)).

- CE method was developed as analytical tool for fast and accurate quantification of organic acids in wine Contribution dr A. Buica, (not funded by Winetech).
- Quantitative FTMIR calibrations for glucose, fructose, ethanol, pH, VA, TA in fermenting must were developed.
- Preliminary statistical control charts for monitoring normal AF were established.
- CE methods were developed for fast and accurate quantification of organic acids in grape juice, synthetic must and fermenting must.
- Quantitative FTMIR calibrations for FAN, tartaric-, citric-, succinic-, acetic-, lactic-, malic- and gluconic acid were developed.
- A model for normal Colombar fermentations during vintages 2008 and 2009 was established. Statistical control charts for monitoring abnormal AF in Colombar were constructed.
- Statistical control charts were developed for normal Cabernet Sauvignon, Chardonnay and Shiraz fermentations. Abnormal fermentations due to nitrogen deficiency were detected using the models established for normal batches.
- Quantitative FTNIR calibrations for FAN, glucose, fructose, organic acids, glycerol and ethanol are currently being developed.

Human resources development/training

Indicate the number and level (e.g. MSc, PhD, post doc) of students/support personnel that were trained as well as their cost to industry through this project. Add in more lines if necessary.

	Student level (BSc, MSc, PhD, Post doc)	Cost to project (R)
1.	Cynthia Magermann - MSc	0
2.	Marinda Kruger von Eck – PhD. Due for graduation 2013	15,666
3.	Ansunette Hoon – MSc. Due for graduation 2012	0
4.	Jessica Garlick – MSc. Due for graduation 2012	20000
5.		

Publications (popular, press releases, semi-scientific, scientific)

Scientific

1. André de Villiers, Phillipus Alberts; Andreas G Tredoux, Héléne H Niewoudt. **2011**. Analytical techniques for wine analysis: An African perspective. *Analytica Chimica Acta*. doi:10.1016/j.aca.2011.11.064

2. Louw, L., K. Roux., A, Tredoux, T. Oliver, T. Naes, H. Nieuwoudt & P. van Rensburg. **2009**. Characterization of selected South African young cultivar wines using FTMIR spectroscopy, gas chromatography, and multivariate data analysis. *J. Agric. Food Chem.*, 57 (7): 2623-2632.

3. Nieuwoudt *et al.*, Monitoring of evolution of aroma compounds in Chardonnay wines elaborated with different lactic acid bacteria and inoculation strategies, using FT-IR spectroscopy and GC-MS (preliminary title, manuscript under preparation. This output is also listed in Winetech report IWBT-B 08-10: Bioprocess monitoring FINAL-REPORT 2012).

4. Garlick, J., M. du Toit, M., H. Nieuwoudt. Fermentation monitoring and trend identification in Shiraz wine fermentations with FT-IR spectroscopy and chemometric modelling (preliminary title, manuscript under preparation).

5. Hoon, A., H. Nieuwoudt. Use of FTIR-ATR spectroscopy to rapidly determine sugars, organic acids, pH and TA in freshly pressed wine grapes (preliminary title, manuscript under preparation).

6. Kruger van Eck, M., C., Aldrich, H. Nieuwoudt. Pattern analysis of process fermentation data: An oenological application (preliminary title, manuscript under preparation).

Presentations/papers delivered

1. Garlick, J., M. du Toit & H. Nieuwoudt. **2012**. Bioprocess monitoring and trend identification in wine fermentations with FT-IR spectroscopy and chemometric modelling. Macrowine 2012, Bordeaux, France.

2. Nieuwoudt, H. **2011.** Bioprocess monitoring of alcoholic wine fermentation using at-line NIR and MIR spectroscopy. 2011. 15th International Conference on Near-Infra Red Spectroscopy, Cape Town, South Africa. (13-20 May).

3. Hélène Nieuwoudt. **2011**. Bioprocess monitoring of alcoholic wine fermentation using on-line NIR infrared spectroscopy. 34th World Congress of Vine and Wine, Porto, Portugal.

4. Hélène Nieuwoudt. **2010**. Real-time bioprocess monitoring of alcoholic fermentation: application of Fourier transform infrared (FTIR) spectroscopy. Cape Biotech Conference, 24-26 March 2010, Somerset West.

5. Hélène H. Nieuwoudt, Johan Moolman, Liezel Coetzee, Ansunette Hoon, Neil Oosthuizen, Pieter Norval. **2010**. High throughput screening of wine metabolites using Fourier transform infrared spectroscopy in attenuated total reflection mode (FTIR-ATR). Cape Biotech Conference, 24-26 March 2010, Somerset West.

6. Nieuwoudt, H. **2010**. Real-time bioprocess monitoring of alcoholic fermentation: application of Fourier transform mid infrared (FTIR) spectroscopy. Cape Biotech Conference, 2010, Lord Charles Hotel, 24-26 March, Somerset West.

7. Hoon, A. & H. Nieuwoudt. **2010**. Monitoring grape quality with Fourier transform infrared spectroscopy in an industrial cellar. 32nd SASEV Congress, Lord Charles Hotel, 18-19 November, Somerset West. POSTER.

8. Hoon, A. & H. Nieuwoudt. **2010**. Quality control of Liqueur production using Fourier transform near- and mid infrared spectroscopy in an industrial cellar. 32nd SASEV Congress, Lord Charles Hotel, 18-19 November Somerset West. POSTER.

9. Magerman, C. & H. Nieuwoudt. **2010**. Real-time bioprocess monitoring of alcoholic fermentation: application of Fourier transform mid infrared (FTIR) spectroscopy. Cape Biotech Conference, 2010, Lord Charles Hotel, 24-26 March, Somerset West.

10. Cynthia Magerman, Marinda Swanepoel and Hélène Nieuwoudt. **2009**. Going real-time: IR spectroscopy makes a difference to fermentation monitoring**.** 4th International SASEV Conference, 28-30 July 2009, Cape Town, South Africa. POSTER

11. Hélène H. Nieuwoudt, Astrid Buica, Cynthia Magerman, Johan Moolman, Liezel Coetzee, Neil Oosthuizen and Pieter Norval. **2009**. Small and smart: Quantitative analysis in the wine laboratory with FTIR-ATR spectroscopy. 4th International SASEV Conference, 28-30 July 2009, Cape Town, South Africa. POSTER

12. Buica A., H. Nieuwoudt, F. Bauer. **2009**. A simple CE method for monitoring organic acids at different stages of the winemaking process. 4th International SASEV Conference, 28-30 July 2009, Cape Town, South Africa. POSTER

13. Hélène Nieuwoudt. **2009**. What is the state of the infrared spectroscopy art in South Africa? 2009. 4th International SASEV Conference, 28-30 July 2009, Cape Town, South Africa. WORKSHOP

14. Marinda Swanepoel. **2009**. Monitoring alcohol and malolactic fermentation on-line. 4th International SASEV Conference, 28-30 July 2009, Cape Town, South Africa.

15. C.M. Magerman and H.H. Nieuwoudt. **2008**. Evaluation of Fourier transform infrared (FT-IR) spectroscopy for the determination of major chemical parameters in fermenting grape must. 31st SASEV Conference, 11-14 Nov 2008, Somerset West, South Africa. POSTER

16. Marinda Swanepoel. **2008**. Process Analytics in winemaking. 1st International South African Chemometrics Society Symposium*,* 30 November-5 December 2008, Stellenbosch, South Africa. PRESENTATION

17. C.M. Magerman and H.H. Nieuwoudt. **2008**. Evaluation of Fourier transform infrared (FT-IR) spectroscopy for the determination of major chemical parameters in fermenting grape must. 31st SASEV Conference, 11-14 Nov 2008, Somerset West, South Africa. POSTER

4. Total cost summary of project

	Year	CFPA	Deciduous	DFTS	Winetech	THRIP	Other	TOTAL
Total cost in real terms for year 1	2008				R 200,000	R 95,000		**R 295,000**
Total cost in real terms for year 2	2009				R 210,000	R 105,000		**R 315,000**
Total cost in real terms for year 3	2010				R 210,000	R 105,000		**R 315,000**
Total cost in real terms for year 4	2011				R 100,000	R 50,000		**R 150,000**
Total cost in real terms for year 5	2012				R 0	R 0		**R 0**
TOTAL					**R 720,000**	**R 355,000**		**R 1,075,000**