Industry allocated project number

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

FINAL REPORT
2013

Programme & Project Leader Information

	Research Organisation Programme leader	Project leader
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Project Information

Research Organisation Project number	ZYG 001
Project title	An investigation into the occurrence of *Zygosaccharomyces* spp. during winemaking. Improving detection, quality control and hygiene with grape concentrate producers

Fruit kind(s)			
Start date (mm/yyyy)	Nov 2011	**End date** (mm/yyyy)	July 2013

Project keywords	

Approved by Research Organisation Programme leader (tick box) | X |

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

Executive Summary

Since 2011, an increasing number of wineries have experienced problems with quality defects such as re-fermentations, turbidity in finished wines and swollen and exploding bags. This was especially experienced with wines containing higher residual sugar. The responsibility of the highly resistant, osmotolerant *Zygosaccharomyces* spp. for many of these incidences is of a major concern for the industry. Since the restriction on the use of natamycin is probably the main cause for these problems, it is important to assess the current situation in order to improve quality control procedures, suggest accurate detection methods, critical control points and cellar hygiene practices. These all play a critical role to control microbial problems during winemaking.

Following a meeting called by the WSB (Wine and Spirits Board) and Winetech on 10 October 2011, an official letter of participation was sent out 21 October 2011 to possible members to confirm their participation to this project. On 7 December an Audit Questionnaire was sent out for completion by middle January 2012. By February 2012, several members have committed towards project participation and the project started. The industry partners that have agreed to partake in this project were Ashton Cellar, Namaqua Wines, Lutzville Wines, DGB, Robertson Winery, Distell, Roodezant, Orange River Wines, Simonsvlei, BBK, Van Loveren, Mooiuitsig (Bonnievale) and Paarl Valley Bottling.
These included the main concentrate and sweet must producers as well as major wineries that actively use these products. Over the course of this project all the members were contacted for inputs, technical discussions, consultations and required to allow for Audits to be performed. The latter included questionnaires and on-site inspections. These investigations were all conducted in attempt to assess the risks around re-fermentations and the incidences of *Zygosaccharomyces* spp. at wineries that use concentrate and sweet must.

During this project there were several challenges and issues which complicated this industry dependant investigation. This included the return of information on time, incorrect information, miscommunication at wineries, unavailability of personnel, and time constraints of normal production schedules. These caused significant delays. However, from the initial feedback of the Audit questionnaires it was evident that there are multiple factors and focus points that are to be considered in this investigation and it was decided to concentrate on hygiene, quality control (QC) and lab procedures as the immediate concerns to establish problematic trends.
Winery visits, Consultations and Audits were performed over a 12 month and included Laboratory QC evaluations. By April 2013 there were still information outstanding and the data that have been gathered up to that time have been used for compiling this report and to formulate findings and recommendations.

From the companies that participated and sent in samples during the lab QC evaluation the presence of different *Zygosaccharomyces* spp. were detected in predominantly sweet must and grape concentrate but seldom in grape juice. *Z. bailii* was the main species isolated in all cases. However, in grape concentrates from certain producers *Z. rouxii* was consistently more predominant. In many cases of spoiled wines, several other spoilage wine yeasts were isolated together with *Zygosaccharomyces.* In swollen bags and products with re-fermentations *Saccharomyces* spp. were commonly also found and indicated that the problems relevant for *Zygosaccharomyces* spp. also pertain to other yeasts. There were also some bottlers who experienced no significant contamination problems at all. It was clear that the changes in the production processes of one of the major concentrate producers increased the quality of their concentrates significantly with lower incidences of microbial contaminations. However, clearly the responsible storage and use of these concentrates still needs serious attention.

The fact that the problems could not solely be attributed to the *Zygosaccharomyces* spp. questioned certain winemaking practices as a whole. Following the investigations in this project, the main findings derived from the results from different participants indicated significant shortfalls in several areas:

- QC procedures
- Cellar Hygiene
- Filtration practices
- Wine preservation
- Tanker hygiene practices
- Pasteurization of concentrate
- Head-space management of concentrate

All of these are regulated by financial pressures and driven by cost implications that directly compromise the final quality. Specific guidelines and recommendations to rectify the findings have been compiled and will be distributed to industry in attempt to lower the incidences and problems where *Zygosaccharomyces* spp. and other yeast spp. are implicated.

Problem identification and objectives

Microbiological problems of the spoilage yeast, *Zygosaccharomyces* spp., have become an industry concern following quality defects such as re-fermentations, swollen bags and turbidity in final products. It is believed that the phase-out of the use of natamycin in winemaking and the ineffectiveness of alternative preservatives such as DMDC (dimethyl dicarbonate) and potassium sorbate against these yeasts holds relevancy to the problems.
In addition, there is a general concern regarding the implementation of standard hygienic practices in cellars, as well as questions regarding the effectiveness of sterile filtration on the microbial spores. *Zygosaccharomyces* spp. shows high tolerance towards the winemaking environment due to its high alcohol tolerance and high SO_2 tolerance. For this reason off-dry wines (wines with higher residual sugar) and lower alcohol wines are more likely affected and increasing number of wineries is experiencing problems.

The outcome of the project is to improve the current quality control procedures for producers including accurate detection methods, identification of critical control points and improving hygiene practices. A standard protocol of these procedures, how to control and manage *Zygosaccharomyces* spp., will be made available to industry.
As starting point, the focus will be on grape concentrate producers and specific wineries (those that have experienced problems and are willing to participate). It was speculated that the root of *Zygosaccharomyces* contamination is grape concentrate.

Workplan (materials and methods)

1. Establish participants for project by contacting potential clients currently involved or experiencing problems. Concentrate and Sweetmust producers must be included.

   List Established

2. Multiple visits and consultations to specific producers and selected wineries

   Completed

3. Sampling and analysis of several finished /defected products and concentrate.

   Completed what was submitted

   a. The level of contamination will be determined. **Completed**

   b. A representative sample of microbial contaminants will be identified to determine the extent of *Zygosaccharomyces* present. **Completed**

   c. Participants allowed to send in 30 samples (**Participants did not all submit their quota samples).**

   d. Details, QC reports and Full traceability of each problematic sample will be investigated. **Partial completion due to feedback**

4. Performing multiple QC and hygiene Audits to identify problem areas and critical control points

   a. Thales Wine Cellar Services will visit participants to perform Hygiene Audits and establish critical control points. **Completed with limitations.**

   b. Focus will include QC points just prior to bottling, product transport and logistics, bottling lines and post bottling. **Completed with limitations.**

5. Evaluating individual hygiene and sanitation regimes of participants.

   a. ThalesWCS will review Hygiene protocols (products and procedures) of participants. **Completed - Partial information obtained.**

   b. Advises and recommendations and alternative trials will be provided to participants for improvements. **Recommendations will be made by Thales Wine Cellar Services extending past this project – Specific hygiene protocols have been established at some wineries, although many still need to revise and implement recommendations.**

   c. Evaluating individual QC and microbial detection procedures of participants. **Started in June 2012. Completed with partial participation.**

d. IWBT will summarize current QC methods of microbial detection and identification of different participants. **Completed. To be communicated to the individual participants that was actively involved.**

6. Evaluation of winemaking preservatives on the yeast survival to establish a potential treatment option for the finished product.

   Investigation largely depended on the feedback from Participants

   a. Writing of reports and consultation with producers to evaluate different treatments/procedures in concentrate/sweet must production. **Partial information obtained from some participants**

   b. Practices currently in place to be evaluated would include the following (also combinations of): **Partial information obtained from some participants**
      i. Potassium Sorbate
      ii. Velcorin.
      iii. UV (Surepure technology)
      iv. Crossflow (0.2 uM)
      v. SO2
      vi. Flash pasteurization
      vii. Natamycin

7. Development of improved Microbiological methods and DNA identification for use by producers.

   a. IWBT will investigate improved methods of rapid identification and detection **Protocol has been established**

8. IWBT will investigate improved detection methods for spores. **NOT feasible in this project scope due to the technical difficulties involved in studying the formation of spores.**

9. Establish functional and effective protocols of control by combining the information and procedures gathered from industry
   a. Technology transfer to wineries as project progresses
   b. Critical control point checklist and QC tests provided to cellar laboratories.
   c. Guideline of Hygiene Protocols and Practices provided to Cellars

Results and Discussion

POINT 1 - Establish participants for project

Thirteen industry partners initially agreed to partake in the assessment which included large wine producers, the main grape concentrate and juice producers and bottling companies. Nine companies contributed with samples, of which only two submitted 30 samples plus during the QC Tests.
The list of participants included **Ashton Cellar, Namaqua Wines, Lutzville Wines, DGB, Robertson Winery, Distell, Roodezant, Orange River Wines, Simonsvlei, BBK, Van Loveren, Mooiuitsig (Bonnievale) and Paarl Valley Bottling.**
Not all of these participants actively contributed towards the project at all times.

POINT 2 - Multiple visits and consultations to specific producers and selected wineries to investigate and understand current issues, manufacturing procedures, QC procedures and hygiene protocols.

On-site consultations and visits were performed for several participants. Some received multiple visits and onsite inspections as normal production schedules and daily time-constraints did not allow for full time participation from personnel at all times. Specific Consultations were done with Robertson, Ashton, Lutzville, DGB, Namaqua Wines, Orange River Wines, Simonsvlei, Roodezant and Distell in order to gather valuable information to follow-up on Audit questionnaires and requests. The discussions focussed on Hygiene regimes, QC procedures, Filtration issues and general microbial problems. The wineries provided information regarding their cellar hygiene practices (Products used, concentrations), filtration practices as well as QC procedures as possible.

POINT 3 - Sampling and analysis of several finished grape concentrate products and defected bottled industry products

During the course of this project several problematic re-fermentation samples have been submitted for the isolation and detection of *Zygosaccharomyces* spp. in grape juice, sweetmust, grape concentrates and bottled wines. Some unproblematic products were also analysed.
Zygosaccharomyces spp. was detected in 39 samples that consisted of Concentrate samples (11 of 21 Tested), dry white en red wines, off-dry wines, sweetmust and off-dry sparkling wine products. **See Addendum A.**
Results showed:

- Several of the re-fermentations products contained only *Saccharomyces* spp, not *Zygosaccharomyces* yeasts
- Different *Zygosaccharomyces* spp. were detected in many grape concentrates, juices and some wines
- There were significant differences in microbial plating counts between the IWBT results and the QC labs of participants. In general, both parties detected cell counts and in cases where products where microbially clean, it was the same in most of the duplicate cases. Exceptions were found and a standardized testing method is proposed. However, the fact that the time frame in which the samples were analysed by both parties did not always coincided could have had a significant effect on the results. Another important factor that effected the different results is the use of different growth media for routine plating. For instance, as a rule QC labs use WL media for routine plating, while the IWBT has found that the use of a richer growth media (i.e.YPD) gave a more realistic picture of the microbial content of a particular wine.
- Unfortunately very few wineries submitted 30 samples, along with the 30 duplicate test results as requested. Some Wineries believed that they did not have problems and therefore did not think it was necessary to submit samples that could have served as positive controls.

POINT 4 - Performing QC and hygiene Audits to identify problem areas and critical control points

Electronic communications were sent out to all participants in the form of Audit questionnaires, QC and sampling recommendations and protocols, Cellar hygiene questionnaires along with reminders and follow-ups in this regard. Several of the Audit questionnaires forms that have been returned were incorrectly filled in, or relevant basic questions were not answered which required follow-up communications and consultations.
The information obtained regarding hygiene and practices were at times not accurate following follow-up discussions with other personnel, however conclusions could me made from which recommendations have been formulated and problematic areas where identified.

From the Audits, Consultations and on-site inspections it was clear that there is a serious lack of critical control points at wineries that need to be addressed. In most cases the final product is the primary critical control point in the process chain which makes it extremely difficult to trace where problems originated due to the lack of control points further upstream.
At the concentrate plants it is clear that the quality control and product storage is a major problem that has been identified. The predominant issue is the head space management of semi-filled concentrate tanks. Due to the cold storage temperatures (0-4^{0}C) the headspace allows for condensation which in turn results in dilution of the top layer of concentrate. The top layer consequently is more prone to re-fermentation problems. The headspace management has therefore been identified as a critical area that needs to be managed. In addition it was evident that the sterilization of containers and the conditions under which the concentrate and sweetmust products are handled have to be improved.

The following critical areas have been highlighted from this investigation
CONCENTRATE
- Head space management
- Sterilization of containers
- Aseptic filling
- Microbial analysis of batches in tanks, before filling to containers or loading in tankers

WINES
- Microbial analysis in final tanks before and after bottling

FILTRATION
- Microbial retainer samples after filtration to be kept to measure filtration effectiveness

IN-HOUSE LAB QC
- Microbial counts and specification to be respected and adhered to

HYGIENE
- Cleaning protocols and time frames must be respected at all times
- Concentrations and correct type of chemical products must be applied
- Overall hygiene to be improved at all participants

POINT 5 - Evaluating individual hygiene and sanitation regimes of participants

In addition to consultations and hygiene inspections participants were asked to fill in specific hygiene questionnaires about the hygiene products used for general sanitation and sterilization in the production cellars and during bottling. The main findings are:

- A variety of chemical suppliers are used - most generic food industry based products, not wine focussed.
- There is a general lack of sufficient cellar hygiene training of staff. Cellar staff in general do not have sufficient cellar hygiene training.
- Existing protocols and procedures are not always respected and followed
- In cases of production delays, hygiene protocols are typically altered/ shortened to make up for lost time.
- Wineries do not have in-house technical expertise and/or fully understand the use of cellar hygiene products.
- In an attempt to be more cost effective, wineries are willing to compromise on hygiene by using less cleaning chemicals, not implementing specific cleaning regimes and not adequately cleaning ALL necessary equipment regularly.

POINT 6 - Evaluating individual QC and microbial detection procedures of participants

An official communication was sent out in June 2012 explaining specific sampling recommendations in attempt to evaluate the wineries QC procedures. However, only a handful of participants sent in selected samples of wine, concentrate and sweetmust for microbial analysis and the detection of Zygosaccharomyces spp. to the IWBT as requested. Each participant started contributing to a quota of 30 samples, and where possible, all samples received by the IWBT were also microbiologically analysed in duplicate at the various participants' quality control labs. The duplicate procedures were done in attempt to evaluate the efficiency of the quality control laboratory practises

- From the QC evaluation, wineries use expensive media, did not perform adequate enumeration or keep results
- Most did not practise zero tolerances, kept reference of positive results, did not withdraw or stop production, but only took notice.
- Production MUST respect QC results and act upon serious cases – however due to time and cost implications processes are continued
- Microbial plating for some microbes requires incubation times that are longer than the actual incubation times implemented by the lab. These are not double checked and shorter incubation times are actually inadequate and meaningless.
- Again, due to time constraints and cost implications the actual importance of QC checks, specifically microbial stability tests, are not respected.

Frequency of detection (the results below includes results obtained from preliminary investigation of concentrates and contaminated wine that lead to the initiation of this project)

- The following four *Zygosaccharomyces* spp. that were isolated, with *Z. bailii* and *Z. rouxii* most frequently, were *Z. bisporus* and *Z. microellipsoides*
- Besides *Zygosaccharomyces* spp. some of the major spoilage yeasts detected in all the different products and identified by us were:
  Saccharomyces cerevisiae
  Pichia membranaefaciens (notorious for being preservative resistant)
  Pichia kluyveri *Candida albicans*

Candida tropicalis
Candida parapsilosis
Candida sake
Candida maltose
Candida sorbosa
Candida zeylanoides
Debaryomyces polymorphus
Torulaspora delbrueckii
Hanseniaspora/Kloeckera
Schizosaccharomyces pombe

- Detection of *Zygosaccharomyces* in *grape juice* was limited
- In *soetmos* a combination of several species were detected:
  Z. bailii, Z. rouxii, Saccharomyces cerevisiae, Pichia anomala, Kloeckera, Candida spp.
- In *concentrate* the following species were isolated:
  Z. rouxii, Candida sp., *Kloeckera, S. cerevisiae, P. fermentans, P. membranaefaciens*. It is however notable that the overall microbial loads in some of the concentrates we have analysed have significantly decreased during the course of the project. This might be due to the fact that production and hygiene regimes have been attended to resulting in lower incidences of microbial spikes.
- In some production plants it was evident that certain *Zygosaccharomyces* strains were residents and were consistently isolated from products. In one case this was particularly experienced with *Z. rouxii*.
- From bloated bagged and bottled wines the following isolates:
  Z. bailii, Z. bisporus, Z. microellipsoides, P. guilliermondi and very often *S. cerevisiae.*
- The fact that *S. cerevisiae* was the cause of many incidences of re-fermentation is pointing to inadequate cellar hygiene and filtration practices.
- Predicting the onset of *Zygosaccharomyces* growth in contaminated products (e.g. wine sweetened with contaminated concentrate) is impossible. Participants have experienced that *Zygosaccharomyces* onsets in bottled products were totally random, regardless of the amount of concentrate used and the storage periods.

POINT 7 - Evaluating preservation methods to combat the spoilage microbes associated with concentrates

Small scale evaluations of preservatives were not evaluated in the laboratory as most of the participants have already investigated different preservatives at their facilities on large scale. It is however clear that the implementation of preservatives were not followed up with critical control points and accurate microbiological evaluations. Feedbacks from participants that use Velcorin and Potassium Sorbate, or combinations thereof find these preservatives less effective as Natamycin and also apply additional methods on products that receive concentrate or sweetmust additions.
A list of preservatives and methods to manage microbiological stability currently in use at the wineries is listed below.

From this investigation it was clear that despite different manufacturing processes, storage temperatures and product clarification, pasteurization plays a critical role in minimizing the microbial problems associated with concentrate at the production site.
When flash pasteurization is applied, concentrate producers experience significantly less microbial problems and consequently the risk for buyers are reduced. Concentrate that appeared to show signs of fermentations are immediately subjected to flash pasteurization (at 95^{o}C for 30s) while it was stored in tank and the stock always received another treatment before the products are loaded for distribution.

Preservation/Method	**Participants (sites)**	
Potassium Sorbate/Sorbic acid	6 (4)	Not common practise in the production of concentrate. Used in the production of sweetmust by one producer
Velcorin	3 (3)	Used by some with mixed results
UV (Surepure technology)	1	Not common practice, used by sweetmust producer
Crossflow (0.2 uM)	6 (2)	Used by most, but not sufficient for sterile filtration
Flash pasteurization	2	Effective application at a concentrate producer
Natamycin	4 (3)	Used by one participant
Photon treatments	1	Application limited
SO2 on sweet wines at bottling	7	Differs between participants from 50 ppm to 200ppm

Information received from 7 Participants (comprising 11 different sites)

POINT 8 - Development of microbiological detection methods

A standard isolation method for *Zygosaccharomyces* from sweetmust, concentrate and wine.

Isolation from concentrates
Due to the viscosity of concentrates (and sweetmust) the isolation of *Zygosaccharomyces* should involve centrifugation of diluted concentrates and not filtration. Concentrated pellets of microorganisms can then be dissolved and diluted, preferably in 0.1% peptone water, and plated out.

Growth media
A routine plating techniques was further established for the detection of viable microbial populations in wine and concentrates. The normal incubation time of 48-72h is however not enough. Plates should be incubated for at least 8 days to detect the slower growing *Zygosaccharomyces* spp.

It was established that some expensive and sometimes complicated incubation media suggested in literature is not a prerequisite for the isolation of this yeast. It was found that although Yeast Peptone Dextrose (YPD) agar is non-selective and results in high microbial counts, it was suitable for the isolation of *Zygosaccharomyces.* The addition of 5% NaCl made the media more selective for preservative resistant yeasts, and we have observed that *Z. rouxii* and *Z. bailii* grow well after a few days of incubation.

In literature some media are suggested for the enumeration of preservative resistant yeasts (Hocking, 1996). Two common non-selective media Malt Extract agar (MEA) and Tryptone Glucose Yeast extract agar (TGY) can be made more selective for preservative resistant yeasts by adding 0.5% acetic acid, although the lower microbial counts should be compensated for if the total microbial load wants to be achieved. In our experience *Z. rouxii* grew slowly on TGYA plates, while Z. *bailii* grew very well on this media. The complicated *Z. bailii* medium (ZBM) is selective for *Z. bailii*, but counts of this yeast on ZBM are significantly lower than on TGYA. We have however experienced very weak or no growth with ZBM media. Osmophylic agar, as well as ready-made PRY (Preservative Resistant Yeast) agar was equally suitable for the isolation of *Z. bailii*.

Identification of isolates
Zygosaccharomyces colonies are identified based on RFLP-PCR analysis of the region spanning the internal transcribed spacer (ITS) region and the 5.8S rRNA gene (Guillamon *et al*., 1998; Esteve-Zarzoso *et al*., 1999).

References
Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999) Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. Int J. Syst Bacteriol. 49, 329-337.
Guillamon, J. M., Sabate, J., Barrio, E., Cano, J. and Querol, A. (1998) Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. Arch Microbiol. 169, 387-392
Hocking, A. D. (1996) Media for preservative resistant yeasts: a collaborative study. Int. J. Food Microbiol. Apr; 29(2-3):167-75.

POINT 9 - Establish functional and effective protocols of control by combining the information and procedures gathered from industry

The following list of documentations will be communicated to participants

1. Recommendations for cellar hygiene and maintenance including the correct chemical products and applications
2. Laboratory Protocols for improving the detection and enumeration of microorganisms responsible for re-fermentations.
3. List/Diagram of critical control points for sampling.
4. Recommendations to ensure Sterile filtration
5. Recommendations for Pasteurization

Milestone	Target Date	Extension Date	Date Completed	Achievement
1. Establish participants for project	Feb2012		March 2012	• Thirteen industry partners agreed to partake • Nine companies contributed with samples, only two submitted 30 samples plus during the QC Tests.
2. Multiple visits and consultations to specific producers and selected wineries to investigate and understand current issues, manufacturing procedures, QC procedures and hygiene protocols.	Dec 2012		Dec 2012	• Several consultations and discussions were performed with the participants during March 2012 untill June 2013. • Ashton, Namaqua Wines, Lutzville, DGB, Robertson, Distell, Roodezant, Simonsvlei, Orange River Wines, BBK
3. Sampling and analysis of several finished grape concentrate products and defected bottled industry products	Jun 2012		Oct 2012	• Sampling and analysis of 176 samples contributed by nine participants was achieved. The presence of *Zygosaccharomyces* spp. could be detected in 39 samples.
4. Performing multiple QC and hygiene Audits to identify problem areas and critical control points	Dec 2012		April 2013	• Identified several critical areas and processes for which recommendations could be made • Partial information gathered with some requests and information still outstanding
5. Evaluating individual hygiene and sanitation regimes of participants	Dec 2012		April 2012	• Obtained valuable information regarding different hygiene regimes, products & protocols • Identified problematic areas and critical control points
6. Evaluating individual QC and microbial detection procedures of participants	Oct 2012		Dec 2012	• Officially started in June 2012. Wineries practice different regimes, use different media, incubation time and apply results-findings differently.
7. Evaluating preservation methods to combat the spoilage microbes associated with concentrates	Dec 2012		Feb 2012	• Largely used feedback from wineries, successes, problems on actual large scale basis.
8. Development of microbiological detection methods	Jan 2012		June 2012	• The use of suitable culture media, as well as an isolation and identification protocol from wine and concentrate has been established.

9. Establish functional and effective protocols of control by combining the information and procedures gathered from industry	Dec 2012		Aug 2013	• Protocols and recommendations have continuously been made to specific participants since March 2012

Accumulated outputs

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

Conclusions

Technology development, products and patents

Indicate the commercial potential of this project, eg. Intellectual property rights or commercial product(s)

Suggestions for technology transfer

Protocols and practices for Wine Cellar Hygiene
Recommendations for improved microbial enumeration at wine laboratories

Human resources development/training

Indicate the number and level (eg. 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
1.	
2.	
3.	
4.	
5.	

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

Possibly 3 press releases

Presentations/papers delivered

Total cost summary of the project

TOTAL COST IN REAL TERMS	COST	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1	2012					137450	68725		206175
YEAR 2									
YEAR 3									
YEAR 4									
YEAR 5									
TOTAL									

Industry allocated project number

ADDENDUM A

Nr	Sample details	Date received	Date analysed	US RESULT (CFU/ml)	COMPANY RESULT (CFU/ml)	Remark	Result of Yeast Identification	Zygosaccharomyces frequency of randomly chosen sample
1	Dry white (Chenin Blanc 1) (4 June) 2	01/06/2012	13/06/2012	9.23E+04	not received		*Zygosaccharomyces bailii*	100%
	Dry white (Sauvignon Blanc 1) (4 June) 2	01/06/2012	13/06/2012	clean	not received			
	Dry white (Chenin Blanc 2) (4 June) 2	01/06/2012	13/06/2012	8.06E+04	not received		*Z. bailii*	100%
	Dry white (Sauvignon Blanc 2) (4 June) 2	01/06/2012	13/06/2012	clean	not received			
	Dry Red 2	01/06/2012	13/06/2012	2.00E+00	not received	Bacteria isolated as well	*Saccharomyces cerevisiae*	93%
	Dry Red 1	01/06/2012	13/06/2012	4.00E-01	not received	Bacteria isolated as well	*S. cerevisiae*	
	Sap 23	16/05/2012	17/05/2012	2.51E+03	1.00E+04		*Kloekera, Candida, Zygosaccharomyces*	6%
	Sap 204	16/05/2012	17/05/2012	TNTC	TNTC		*Saccharomyces*	
	Sap 202	16/05/2012	17/05/2012	1.09E+05	TNTC		*Saccharomyces*	
	Sap 706	16/05/2012	17/05/2012	1.05E+03	TNTC		*Pichia, Saccharomyces*	
	Sweet Rose bottled product	16/05/2012	17/05/2012	clean	7.00E-02			
	Sweet White bottled wine	16/05/2012	17/05/2012	clean	not received			
	Sweet Red bottled product	16/05/2012	17/05/2012	clean	3.00E-01			
	Dry Red Bulk wine	16/05/2012	17/05/2012	2.29E+04	1.30E+03		*Zygosaccharomyces*	100%
2	St. Claire Natural Sweet Rose L12 052 (5 Junie) 2	05/06/2012	11/06/2012	clean	clean			
	St. Claire Natural Sweet Rose L12 046 (5 Junie) 2	05/06/2012	11/06/2012	clean	clean			
	St. Anna Natural Sweet L12 106 (5 Junie) 2	05/06/2012	12/06/2012	2.01E+05	TNTC		*S. cerevisiae*	100%
	DG Sunkissed Natural Sweet L12 129 (5 Junie) 2	05/06/2012	12/06/2012	clean	clean			
	Boschendal Lanoy 2010 L12 145 (5 Junie) 2	05/06/2012	11/06/2012	clean	not received			
	Stellenbosch Shiraz 2011 L12 149 (5 Junie) 2	05/06/2012	12/06/2012	clean	not received			
	Concentrate L12 052 (5 Junie) 2	05/06/2012	11/06/2012	1.27E+04	Not applicable		*Z. rouxii*	100%
	Soetmos L12 052 (5 Junie) 2	05/06/2012	11/06/2012	2.40E+05	Not applicable		*S. cerevisiae; S. bayanis; S. pastorianus*	
	St Anna C14 L12 164 14 Junie 2 (500 ml)	14/06/2012	18/06/2012	2.77E+05	not received		*Z. bailii and S. cerevisiae*	64%
	St Anna A28 L12 164 14 Junie 2 (500 ml)	14/06/2012	18/06/2012	3.11E+05	not received		*S.cerevisiae*	100%
	St Anna 11h59 L12 155 14 Junie 2 (3L)	14/06/2012	18/06/2012	5.00E-01	not received		*P.membranaefaciens*	
	St Anna 8h35 L12 155 14 Junie 2 (3L)	14/06/2012	18/06/2012	2.50E+01	7.60E-02		*P.membranaefaciens*	
	St Anna 10h49 L12 155 14 Junie 2 (3L)	14/06/2012	18/06/2012	5.00E-01	2.80E-02		*P.membranaefaciens*	
	St Claire Natural Sweet Rose L12 127 07/05	15/06/2012	18/06/2012	1.31E+03	8.00E-03		*S.cerevisiae*	
	St Claire Natural Sweet Rose L12 127 07/05 (bloated bag)	15/06/2012	15/06/2012	1.81E+06	1.60E-02		*S.cerevisiae*	100%
	Soetmos E4 L12 177 (voor filtrasie met Beco filters)	26/06/2012	28/06/2012	4.98E+04	Not applicable		*Pichia*	100%

	Soetmos D3 L12 177 (na filtrasie met Beco filters)	26/06/2012	28/06/2012	4.17E+02	Not applicable		*Z. bailii, S.cerevisiae, Pichia*	3%
	C2: DG St Anna (na filtrasie met Beco pads) L12 179 28 Junie 2	28/06/2012	29/06/2012	5.79E+02	not received		*P.membranaefaciens*	41%
	B21: DG St Claire L12 179 28 Junie 2	28/06/2012	29/06/2012	2.10E+01	not received		*Z. bailii*	10%
	B26: Legacy L12 179 28 Junie 2	28/06/2012	29/06/2012	1.60E+01	9.20E-02		*P.membranaefaciens*	12%
	C19: Legacy (na filtrasie met Beco pads) L12 179 28 Junie 2	28/06/2012	29/06/2012	6.60E+01	1.28E-01		*P. membranaefaciens*	100%
	C2: DG St Claire (na filtrasie met Beco pads) L12 179 28 Junie 2	28/06/2012	29/06/2012	1.36E+03	5.12E-01		*S.cerevisiae, Pichia, Kloeckera*	17%
	B20: DG St Anna L12 179 28 Junie 2	28/06/2012	02/07/2012	6.00E+00	4.00E-01		*P.membranaefaciens*	42%
	B30: DG St Claire L12 179 28 Junie 2	28/06/2012	02/07/2012	1.20E+02	5.28E-01		*P.membranaefacien*	100%
	DG St Anna L12 191 24 Julie 2012 (2)	24/07/2012	27/07/2012	clean	clean			
	Legacy L12 186 24 Julie 2012 (2)	24/07/2012	30/07/2012	clean	clean			
	DG Sunkissed nat Sweet L12 184 24 Julie 2012 (2)	24/07/2012	27/07/2012	clean	clean			
	Tall Horse Sauv Blanc L11 199 24 Julie 2012 (2)	24/07/2012	30/07/2012	clean	not received			
	Tall Horse Pinotage L11 196 24 Julie 2012 (2)	24/07/2012	30/07/2012	clean	not received			
	DG Sunkissed Rose L12 184 24 Julie 2012 (2)	24/07/2012	27/07/2012	clean	clean			
	DG St Claire (1.5 L) L12 184 24 Julie 2012 (2)	24/07/2012	27/07/2012	clean	clean			
	DG St Claire (3L) L12 185 24 Julie 2012 (2)	24/07/2012	30/07/2012	clean	clean			
	Concentrate D9 Spinnekop	15/08/2012	16/08/2012	1.30E+01	Not applicable	Bacteria isolated as well	*Z. rouxii, Candida, P.guilliermondii*	43%
	Concentrate D9 Spinnekop	21/08/2012	22/08/2012	4.00E+00	Not applicable	Bacteria isolated as well	*Z. rouxii, Candida*	43%
	D9 Consentraat - Spinnekop 10/09/12	11/09/2012	12/09/2012	3.00E+00	Not applicable	Bacteria isolated as well	*Z. bailii*	44%
	Concentrate D9 Spinnekop 04/10	11/10/2012	15/10/2012	2.00E-01	Not applicable		*Z. rouxii*	17%
	Concentrate D9 Spinnekop	31/10/2012	31/10/2012	1.00E+01	Not applicable		*Z. rouxii*	89%
	Malibu (coconut mixed with iced tea) Peach + Mango	13/11/2012	14/11/2012	clean	clean			
	Malibu (cocnut mixed with apple and guava)	13/11/2012	14/11/2012	clean	clean			
	Nordic Watermelon Cosmo	13/11/2012	14/11/2012	clean	clean			
	Nordic Limoncello Margarita	13/11/2012	14/11/2012	clean	clean			
	Nordic Raspberry Mojito	13/11/2012	14/11/2012	clean	clean			
3	2. Natural Sweet Rose (750 ml)	05/06/2012	14/06/2012	clean	clean			
	1. Natural Sweet Rose (1L) (5 Junie)	05/06/2012	14/06/2012	clean	clean			
	5. Late Harvest B13 2 750 ml	05/06/2012	14/06/2012	4.40E+01	+++ yeast		*Z. bailii*	100%
	4. Johannisberger Semi-sweet Red 3L	05/06/2012	14/06/2012	3.00E-01	clean		*Kloeckera*	
	3. Natural Sweet Rose 3L (5 Junie) 1	05/06/2012	14/06/2012	clean	clean			
	9. Robertson Light Chenin blanc 12 Junie 2 (2012)	12/06/2012	14/06/2012	clean	clean			
	7. Robertson Light Sauvignon Blanc 12 Junie 2 (2012)	12/06/2012	14/06/2012	clean	clean			
	6. Robertson Light Merlot 12 Junie 1 (2011)	12/06/2012	14/06/2012	clean	clean			
	8. Robertson Light Pinotage Rose 12 Junie 2 (2011)	12/06/2012	14/06/2012	clean	clean			
	10. Extra Light 3 L 12 Junie 2	12/06/2012	14/06/2012	clean	clean			
	13. T669 Natural Sweet Rose: na Bulk filtrasie	01/08/2012	02/08/2012	2.94E+06	+++ yeast and bacteria		*S.cerevisiae*	100%
	12. T664 Natural Sweet Rose: na x-flow	01/08/2012	02/08/2012	5.42E+03	+++ yeast and bacteria		*Z. bailii*	100%
	11. B14 Natural Sweet Rose: voor bottelering	01/08/2012	02/08/2012	1.42E+05	+++ yeast and bacteria		*Z. bailii*	97%
	14. Natural Sweet Rose L33114 (PnP No Name) 3L	01/08/2012	02/08/2012	clean	clean			
	17. B2 (EX 624) 24/8/12 NS Rose	31/08/2012	03/09/2012	2.12E+02	clean		*Z. bailii, Saccharomyces*	29%

	19. B8 (EX 663) 24/8/12 Fat Bastard Cab	31/08/2012	03/09/2012	1.70E+02	clean		*Z. bailii, Saccharomyces*	100%
	18. B13 (EX 672) 24/8/12 Grand Cru	31/08/2012	03/09/2012	2.00E-01	+++ bacteria		*Z. bailii*	100%
	16. B14 (EX 606) 24/8/12 Dry Red	31/08/2012	03/09/2012	4.10E+01	+++ bacteria		*Z. bailii, Saccharomyces*	19%
	15. B16 (EX 664) 24/08/12 NS Rose	31/08/2012	03/09/2012	5.90E+01	+++ yeast and bacteria		*Z. bailii*	100%
	22. Robertson Winery Beaukett L31973 29/08/2012	31/08/2012	03/09/2012	clean	clean			
	20. Robertson Winery Alcohol free Sparkling L30170	31/08/2012	04/09/2012	clean	clean			
	21 .Robertson Winery Gewurtz L32047 29/08/2012	31/08/2012	04/09/2012	clean	clean			
	23. T638 NS Rose RW Bulk Filter	22/11/2012	22/11/2012	1.00E+01	TNTC yeast and bacteria		*Z. bailii, P. membraenafaciens*	69%
	24. T633 NS Red X Flow RW	22/11/2012	22/11/2012	7.00E+00	TNTC yeast and bacteria		*Z. bailii, P. membraenafaciens*	3%
	25. T627 NS White Bulk Filter RW	22/11/2012	22/11/2012	2.19E+02	TNTC yeast and bacteria		*Z. bailii*	100%
	26. T636 NS White RW X Flow	22/11/2012	22/11/2012	6.00E+00	TNTC yeast and bacteria		*Z. bailii*	100%
	27. T675 Robertson NS Rose Local X Flow	22/11/2012	22/11/2012	3.70E+04	TNTC yeast and bacteria		*S. cerevisiae*	100%
	28. B4 (Ex 636) Sparkling Wit (NS Wit) Robertson	27/11/2012	28/11/2012	4.57E+02	TNTC yeast and bacteria		*Z. bailii*	100%
	29. NS Rose Robertson B13 (ex 675)	27/11/2012	28/11/2012	1.38E+02	TNTC yeast and bacteria		*P.membranaefacien*	
	30. Sparkling White S1T2 Bottellyn Robertson	27/11/2012	28/11/2012	1.94E+03	TNTC yeast and bacteria		*Z. bailii*	93%
	31. N Sweet Rose T3T7 B1B3 Robertson	27/11/2012	28/11/2012	7.00E+00	TNTC bacteria, 3.60E-01 yeast		*P.membranaefacien*	
	32. L35330 Sparkling White (S1T2)	06/12/2012	11/12/2012	clean	clean			
	33. L35551 3L N/sweet Rose (F3T7)	06/12/2012	11/12/2012	clean	TNTC yeast and bacteria			
	34. B15 N/Sweet Red (ex 633) 24878	06/12/2012	11/12/2012	6.23E+02	TNTC yeast		*Z. bailii*	97%
	35. F1T3 N/sweet Red B1B1/2	06/12/2012	11/12/2012	2.47E+04	TNTC yeast		*S. cerevisiae*	100%
	36. L35647 N/sweet Red (F1T3)	06/12/2012	11/12/2012	clean	x			
	37. L33393 N/Sweet Rose blaas in ons store	06/12/2012	07/12/2012	8.60E+01	TNTC yeast and bacteria		*Z. bailii*	100%
	38. L34504 Chapel S. sweet (maak flokkies in mark)	06/12/2012	13/12/2012	clean	x			
	39. Ino colour verdun (0.8g/L)	06/12/2012	07/12/2012	clean	Not applicable			
4	Concentrate T39	12/06/2012	15/06/2012	clean		Only few bacteria detected		
	Concentrate T32	14/06/2012	15/06/2012	clean		Only few bacteria detected		
	Concentrate T31 ligbruin	19/06/2012	20/06/2012	9.00E+00			*Z. rouxii*	21%
	Concentrate T39 verhelderd	23/07/2012	30/07/2012	clean				
	Concentrate T38 ligbruin (natamycin+sorbic)	01/08/2012	06/08/2012	5.50E+01			*Candida and Pichia*	
	Concentrate T38 ligbruin	15/08/2012	16/08/2012	2.00E+01		Bacteria isolated as well	*Z. rouxii, Candida*	91%
	Concentrate T39 verhelderd	15/08/2012	16/08/2012	1.00E+01		Bacteria isolated as well	*Z. rouxii*	100%
	Concentrate T31 ligbruin	22/08/2012	22/08/2012	clean		Only few bacteria detected		
	Concentrate T32 verhelderd	22/08/2012	22/08/2012	5.00E+00			*Candida and Torulaspora delbrueckii*	no Zygo
	Concentrate T34 verhelderd	06/09/2012	06/09/2012	clean		Only few bacteria detected		
	Concentrate T38	23/10/2012	24/10/2012	2.05E+02			*Z. rouxii*	90%
	Concentrate T39	23/10/2012	24/10/2012	clean				
	Bruin rosyntjie konsentraat	31/10/2012	31/10/2012	clean				
	Concentrate T30	07/11/2012	08/11/2012	7.00E+00			*Z. bailii*	36%

	Concentrate T32 ligbruin	28/11/2012	29/11/2012	clean		Only few bacteria detected		
5	PMB - MER 18 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - MER 14 Junie (2)	20/06/2012	21/06/2012	2.00E-01	clean		*Saccharomyces*	100%
	PMB - MER 15 Junie (2)	20/06/2012	21/06/2012	clean	mould			
	PMB - CAB 15 Junie (2)	20/06/2012	21/06/2012	3.00E-01	mould		*Saccharomyces*	100%
	PMB - P. Blanc 14 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - CAB/MER 13 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - CAB/SHI 14 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - S/S Red 14 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - Pinotage 13 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PMB - Merlot 13 Junie (2)	20/06/2012	21/06/2012	clean	clean			
	PBM Chard 20 Junie (2)	04/07/2012	04/07/2012	clean	clean			
	PBM S Pin Rose 28 Junie (2)	04/07/2012	04/07/2012	clean	Bacteria TNTC			
	PBM PIN 28 Junie (2)	04/07/2012	04/07/2012	clean	Bacteria TNTC			
	PBM DRR 26 Junie (2)	04/07/2012	04/07/2012	clean	clean			
	PBM DRR 18 Junie (2)	04/07/2012	05/07/2012	clean	clean			
	PBM B Vonkel 26 Junie (2)	04/07/2012	05/07/2012	clean	clean			
	PBM R. Vonkel 26 Junie (2)	04/07/2012	05/07/2012	clean	clean			
	CHB/SBL 01/11/2012	06/11/2012	07/11/2012	clean	clean	Only few bacteria detected		
	S/S Red 01/11/2012	06/11/2012	07/11/2012	clean	clean	Only few bacteria detected		
	CAB/MER 13 Sept (1)	06/11/2012	07/11/2012	clean	not received	Only few bacteria detected		
	CAB/SHI 01/11/2012	06/11/2012	07/11/2012	clean	clean	Only few bacteria detected		
	CAB/MER 13 Sept (2)	06/11/2012	07/11/2012	clean	not received	Only few bacteria detected		
	MER 14 Sept (1)	06/11/2012	07/11/2012	clean	clean	Only few bacteria detected		
	Shiraz 12 Sept (1)	06/11/2012	07/11/2012	clean	not received			
	Shiraz 12 Sept (2) Bosman Shiraz WSB 00341D Lyn 1	06/11/2012	07/11/2012	clean	not received	Only few bacteria detected		
	DRR 10 Sept (1)	06/11/2012	07/11/2012	clean	yeast+bacteria TNTC			
6	Nr 2 (16/7) Bott datum Aloe Tree Chenin blanc	18/07/2012	27/07/2012	clean	clean			
	Nr 2 (14/6) Bott datum Natural Sweet Red	18/07/2012	27/07/2012	clean	not received			
	Cape Elephant Natural Sweet White S37429	25/09/2012	25/09/2012	clean	not received	Only few bacteria detected		
	Cape Elephant Natural Sweet Red S37433	25/09/2012	25/09/2012	clean	not received	Only few bacteria detected		
	Cape Elephant Ruby Cabernet S37556	25/09/2012	25/09/2012	clean	clean	Only few bacteria detected		
	Cape Elephant Natural Sweet Red	14/11/2012	20/11/2012	clean	clean	Only few bacteria detected		
	Cape Rhino Pinotage	14/11/2012	20/11/2012	clean	not received	Only few bacteria detected		
7	Nederburg Stein 2012 LN1 23 G12 13:14 (750 ml)	07/08/2012	10/08/2012	clean	clean			
	Nederburg Stein 2012 LN1 23 G12 8:30 (250 ml)	07/08/2012	10/08/2012	clean	clean			
	Nederburg Winemakers Reserve Cab Sauv LN1 26 G12 12:24	07/08/2012	10/08/2012	clean	4.00E-03			
	Nederburg Duet Shiraz/Pinotage 2012 LN1 26 G12 06:10 (750 ml)	07/08/2012	10/08/2012	clean	1.20E-02			
	Nederburg Foundation Cab/Sauv 2012 LN1 24 G12 14:20 (750 ml)	07/08/2012	10/08/2012	clean	1.20E+00	More than		
	Capenheimer 1.5L LG1 30 G12 13;04 T337	07/08/2012	10/08/2012	clean	clean			
	Autumn Harvest Crackling Rose (1.5 L) LG1 25 G12 17:00 T334	07/08/2012	13/08/2012	clean	clean	Only few bacteria detected		
	Hunters Dry (660 ml) LW1 10 G12 03:47	07/08/2012	13/08/2012	clean	clean			
	Hunters Dry (660 ml) LW1 29 G12 12:47	07/08/2012	13/08/2012	clean	clean			
	Drostdy Hof Extra Light (5L) LA2 03G12 21:44	07/08/2012	10/08/2012	clean	1.60E-01	Only few bacteria detected		
	Drostdy Hof Extra Light (5L) LA2 31G12 07:56	07/08/2012	10/08/2012	clean	clean	Only few bacteria detected		
8	T604 R Muskadel soetmos 17/10/2012	18/10/2012	19/10/2012	7.10E+04	Not applicable		*Saccharomyces and Pichia*	
	T606 W Muskadel soetmos 17/10/2012	18/10/2012	19/10/2012	4.54E+03	Not applicable		*Saccharomyces, Pichia and possibly Candida*	
	T621 Chenin Bl. Soetmos	18/10/2012	19/10/2012	1.68E+02	Not applicable		*Z. bailii*	100%

	T621 Chenin Bl. Soetmos	18/10/2012	19/10/2012	1.68E+02	Not applicable		*Z. bailii*	100%
9	A TT03 WW Light Red L14601 19/11/12 09h10 S 39952 (2L)	21/11/2012	22/11/2012	clean	1.60E-02			
	A T54 Sm. Classic Red L14598 20/11/12 8h20 S40009 (3L)	21/11/2012	22/11/2012	clean	1.00E+00			
	BT01 MM. Srood L14599 19/11/12 13h30 S39990 (750ml)	21/11/2012	22/11/2012	clean	6.00E-02			
	A T3 L14682 21/11/12 15h30 S40119 Koopmanskloof Merlot 2011	27/11/2012	28/11/2012	clean	5.60E-02			
	A T4 L14681 21/11/12 13h40 S40108 Koopmanskloof Cab Sauvignon 2011	27/11/2012	28/11/2012	4.00E+00	4.00E-03		*No Zygo*	
	A T1 L14683 21/11/12 10h55 S40093 Koopmanskloof Shiraz 2011	27/11/2012	28/11/2012	clean	clean			
	A BT03 L14680 21/11/12 8h20 Koopmanskloof Pinotage 2011	27/11/2012	28/11/2012	clean	clean			
	A BT04 22/11/12 7h53 S40131 Dry White Ocean Basket	27/11/2012	28/11/2012	clean	4.00E-03			
	A TT01 WW Dry Red L14600 22/11/12 8h20 S40137 (papsak 2L)	27/11/2012	28/11/2012	clean	TNTC			
	A TT03 WW. Merlot L14621 27/11/12 9h40 S40294	05/12/2012	06/212/2012	clean	1.40E+00			
	A T52 Solms Vastrap L12-331 27/12/12 8h30 S40285	05/12/2012	06/212/2012	clean	4.00E-03			
	A T1 29/11/12 8h57 S40362 Simonsrood	05/12/2012	06/212/2012	clean	clean			
	A PT3 HP Shz Cab L14705 29/11/12 13h15 S40387	05/12/2012	06/212/2012	clean	TNTC			
	A BT03 3/12/12 L14714 9h45 S40426 Koopmanskloof Cab/Merlot	07/12/2012	12/12/2012	clean	clean			
	A BT02 29/11/12 15h50 L14706 S40391 Koopmanskloof Cab/Shiraz	07/12/2012	12/12/2012	clean	x			
	A T54 SM. Classic Red L14424 4/12/12 14h45 S40522 (papsak) 3L	07/12/2012	12/12/2012	clean	TNTC			
	A TT04 WW Cab Merlot L14666 4/12/12 8h30 S40492 (papsak) 1L	07/12/2012	12/12/2012	clean	6.00E-01			
	A T53 SM. Bl. De Bl L14671 6/12/12 11h15 S40599 (papsak)	07/12/2012	12/12/2012	clean	4.00E-02			
	A TT03 WW. Light Rose L14701 7/12/12 8h05 S40619 (papsak)	07/12/2012	12/12/2012	clean	8.00E-01			