

Industry allocated project number

PHI allocated project number

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FINAL REPORT (2015)

1. PROGRAMME AND PROJECT LEADER INFORMATION

	Research Organisation Programme leader	ARC Research Team Manager	Project leader
Title, initials, surname	W Jonker	-	W Jonker
Present position	Manager: Liquor Products	-	Manager: Liquor Products
Organisation, department	Department of Agriculture, Forestry and Fisheries	-	Department of Agriculture, Forestry and Fisheries
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2. PROJECT INFORMATION

Research Organisation Project number	Allergen 1-2013		
Project title	Analyses of allergens in wine to establish the impact of filtration and fining of wine		
Short title	Allergens in wine		

Fruit kind(s)	Wine		
Start date (mm/yyyy)	1 January 2013	End date (mm/yyyy)	31 August 2015

Key words	Allergens, wine, filtration, fining
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Approved by Research Organisation Programme leader (tick box)

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3. EXECUTIVE SUMMARY

Objectives & Rationale

It is important for the South African wine industry to be informed regarding the levels of allergens remaining in the final bottled wine, after normal fining techniques and filtration processes have been applied. This will enable winemakers to make an informed decision whether to label for allergens, without having to analyse each batch of wine for each type of allergen. The sample set used in the study was based on actual wine being made in real time in order to assess the effect of filtration on the allergen concentration of the final wine. The principle of good manufacturing practice applied to the dosage of allergens; therefore only the amount that was needed, was added. No experimental samples were used during the study and a sliding scale of dosage was therefore not possible. The objective was to determine whether allergen labelling would still be necessary if the correct filter size was used during filtration to remove residual allergens.

Methods

The determination of allergenic fining agent protein residues in wines were done using the ELISA method, which is based on a basic microtiter plate spectrophotometer. Rida@Soft Win software was used to interpret and document the results.

Key Results

In the samples set almost all wines were fined using the following type of proteins: ovalbumin (egg white), casein and gelatin. Very few producers added lysozyme to their wines. The study focused on the analyses of allergens: ovalbumin, casein and lysozyme. Gelatin was not part of the study, as it is not an allergen. The main dosage of fining agent used by producers was between 2 and 3 g/Hl of wine. All wines were fined between 3 and 11 days after production. All wines that were analyzed directly after fining, but before filtration, showed the presence of allergens at different concentrations, except for one wine. However, all wines that were positive for the presence of allergens showed that no allergens could be detected after the wine was filtered. The size of the filters that were used for filtration was between 0.2 and 0.65µm.

Conclusion/Discussion

All wines that tested positive for the presence of allergens after fining, tested negative for the presence of allergens after filtration, suggesting that filtration, using the correct filter size, could indeed remove all residual allergens from the wine and therefore negate the need to label or test for allergens and therefore save cost.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

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EU Commission Regulation (EC) No 607/2009, that deals with the labelling requirements of allergens in wine (when the ingredients used during the production of wines are still present in the final product), has been amended by EU regulation 579/2012 on 29 June 2012. This regulation determines that wine exported to, or produced in the EU, must be labelled for allergens (terms

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concerning sulphites, milk and milk-based products and eggs and egg-based products) under the following conditions:

- All wine from the harvests of 2011 and wine of the 2012 harvest, labeled before 30 June 2012, will be exempt from mandatory egg/milk allergen labeling.
- Wine of the 2012 harvest, labeled after 30 June 2012, will not be exempt.

The presence of egg/milk allergens need however not be declared on the label if the wine has tested negative for the presence of these residual allergens using a technique with a detection limit of 0.25 mg/L.

It is important that the South African wine industry are informed regarding the levels of allergens remaining in the final bottled wine to be sold to the consumer, after normal fining techniques and filtration processes have been applied. This will save cost in terms of not being required to perform constant allergen analyses and having to re-label or redesign labels to include an allergen statement.

To analyze for these allergens on a continuous, non-targeted basis, will be prohibitively expensive. It is therefore of the utmost importance that a range of wines that have been treated with these three allergens (ovalbumin, casein or lysozyme) be analyzed, to determine the status of South African wines and to determine the impact of fining and filtration processes on the concentration and presence of these allergens.

This will enable winemakers to make an informed decision whether to label for allergens, without having to analyze each batch of wine for each type of allergen.

5. DETAILED REPORT

a. PERFORMANCE CHART (for the duration of the project)

Milestone	Target Date	Extension Date	Date completed
1. Securing funding for the project (for payment of analyses over time)	31 October 2012		1 January 2013
2. Meet with SAWIS to discuss sampling procedure and questionnaire that should accompany samples	November 2012		30 June 2013
3. Sampling of wine and completion of questionnaires	31 December 2012	June 2014	June 2014
4. Analyses of samples, interpretation and reporting of results	January to March 2013	31 July 2015	31 July 2015
5. Compilation and summary of results	April to May 2013	31 July 2015	31 July 2015
6. Drawing up and reporting conclusions to the wine industry	June 2013	31 July 2015	31 July 2015

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b) WORKPLAN (MATERIALS AND METHODS)

The determination of allergenic fining agent protein residues in wines were done using an ELISA method, which is based on a basic microtiter plate spectrophotometer with wavelength range of at least 340 to 850 nm, with optical filters for wavelengths 405, 450 and 620 nm. The following software was used to interpret and document the results :

RIDA®SOFT Win
Software for interpretation and documentation of RIDASCREEN ELISAs
RIDASCREEN FAST Casein ELISA
RIDASCREEN FAST Ei/Egg protein ELISA
RIDASCREEN FAST Lysozyme ELISA

Sampling was done with the assistance of the SAWIS inspectors. Wine samples were requested from wine cellars who wished to participate in the project. These samples must have been treated with either casein (milk powder), egg white proteins (ovalbumin) or lysozyme. The winemaker was requested to declare the amount of allergen or fining agent that was added, the type of fining/filtering techniques used and the size of the filters, e.g. 0.6 µm filter.

Samples were collected over two harvest periods. Samples were drawn at various intervals during the winemaking process: before fining, after fining, after bulk filtration, before bottling and after bottling. The sample set was red wines of the 2013 and 2014 harvests.

In addition to the voluntary sampling, a questionnaire was introduced on Wine Online for a period of one month for all certified wine submissions. Samples were collected from certified wines that were treated with allergens, which were then submitted to the laboratory for analyses. All the wines in the sample set were final bottled and labelled red and white wines, ready to be sold on the market or exported.

These above samples were analyzed for the presence of these three allergens and the results quantified for concentrations above 0.25 mg/L. The conclusions will be made available to the participating cellars and the wine industry to ensure that winemakers are informed which fining/filtering practices can lower or eliminate the presence of allergens. South African wines may also be generally more attractive to consumers, if fining can be done in such a way as to eliminate or minimize allergens from the final product.

A total of 79 samples were collected for analysis. The sampling procedures were statistically sound. The Level of detection and level of quantification for the analyses of lysozyme, ovalbumin and casein with the ELISA method were as follows:

Lysozyme: LOD = 0.02 mg/kg, LOQ = 0.05 mg/kg
 Ovalbumin: LOD = 0.27 mg/kg, LOQ = 0.5 mg/kg
 Casein: LOD = 0.24 mg/kg, LOQ = 0.5 mg/kg

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c) RESULTS AND DISCUSSION

The results found after a sampling period over 2 harvest seasons, were as follows:

TABLE 1: SAMPLES AT VARIOUS INTERVALS OF WINE PRODUCTION

Sample No.	Sample status: before fining, after fining, after filtration, after bottling	Description of Wine (vintage/cultivar/ origin)	Type of Allergen eg. Casein, Egg Albumen, Lysozyme	Amount of allergen added in g or kg (include unit)	Volume of wine treated	Size of filter used $\leq 0,45 \mu\text{m}$, $> 0,65$ and $< 1 \mu\text{m}$ etc.	How long after addition was filtering done?	Analyses result
1A V1354709	Before fining	Merlot 2013			197200L			Not detected
1B V1354710	After Fining	Merlot 2013	Albumin	3g/HL	197200L	No Filtration		9.80 ppm
1C V1354711	After Filtration	Merlot 2013			197200L	$> 0,2$	3 days	0.525 ppm
1 D V1363135	Before Bottling	Merlot 2013			197200L	0.45 after cold stabilization		Not detected
1 E	After Bottling – final dressed sample	Merlot 2013			197200L	0.45 at bottling		Not detected
2A V1363132	Before Fining	Contemporary Cab Sauv 2013			524000L			Not detected
2 B V1363133	After Fining	Contemporary Cab Sauv 2013	Albumin	2g/HL	524000L	No Filtration		2.6 ppm
2 C V1363134	After BULK filtration	Contemporary Cab Sauv 2013			524000L	$> 0,2$	3 days	Not detected
2 D V1363136	Before Bottling	Contemporary Cab Sauv 2013			524000L	0.45 after cold stabilization		Not detected

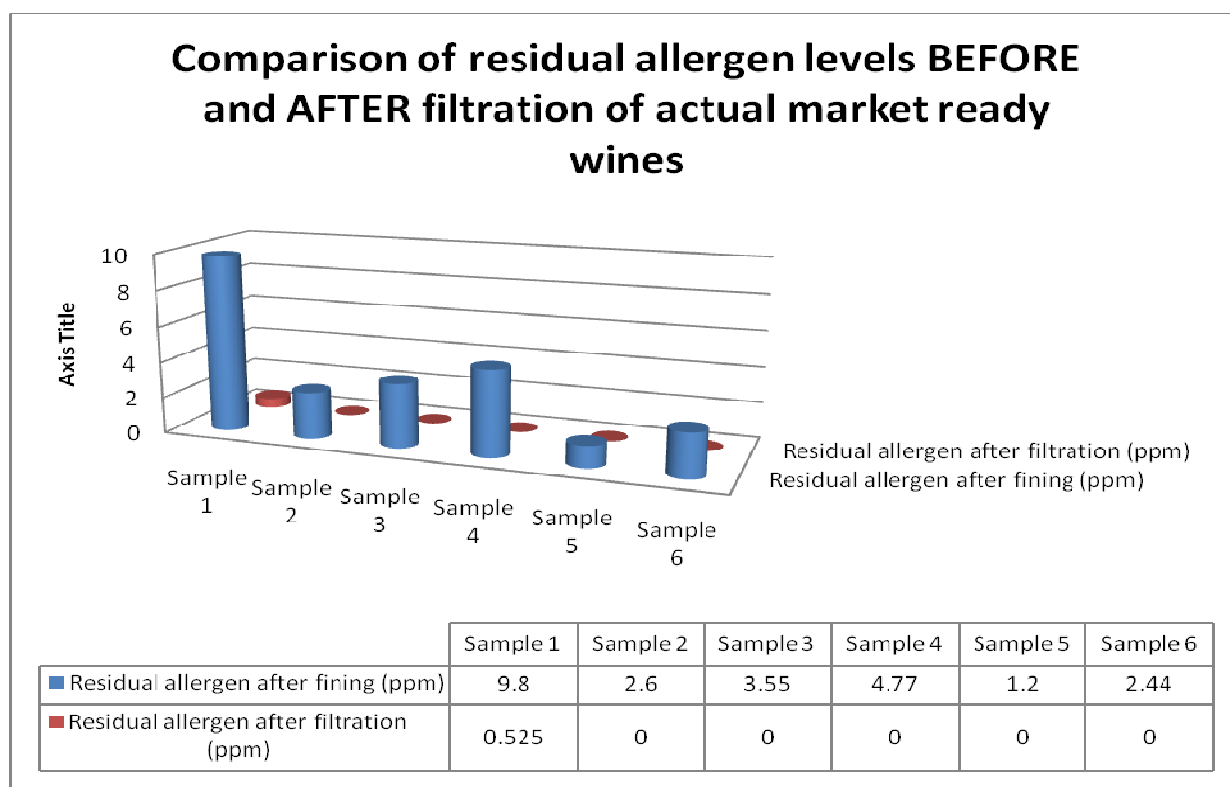
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2 E	After Bottling – final dressed sample	Contemporary Cab Sauv 2013			524000L	0.45 at bottling		Not detected
3A	Before fining	Roodeberg 2013	Albumin		2.7 miljoen L	0.65	5 days	Not detected
3B	After fining	Roodeberg 2013	Albumin	2g/HL	2.7 miljoen L		5 days	3.55 ppm
3C	After bulk filtration	Roodeberg 2013	Albumin		2.7 miljoen L	0.65	5 days	Not detected
4A	Before fining	Shiraz 2014			92000L			Not detected
4B	After fining	Shiraz 2014	Albumin	2g/HL	92000L	0.2 Darcy	11 days	4.77 ppm
4C	After filtration	Shiraz 2014			92000L	0.2 Darcy	11 days	Not detected
5A	Before fining	Cabernet Sauvignon 2014			165000L			Not detected
5B	After fining	Cabernet Sauvignon 2014	Albumin	2g/HL	165000L	0.2 Darcy	9 days	Not detected
5C	After filtration	Cabernet Sauvignon 2014			165000L	0.2 Darcy	9 days	Not detected
6A	Before fining	Pinotage WCE 2014			76661L			Not detected
6B	After fining	Pinotage WCE 2014	Albumin	2g/HL	76661L	0.2 Darcy	6 days	1.20 ppm
6C	After filtration	Pinotage WCE 2014			75500L	0.2 Darcy	3 days	Not detected
7A	Before fining	Cabernet Sauvignon WCE 2014			542000L	No filtration	3 days	Not detected
7B	After fining	Cabernet Sauvignon WCE 2014	Albumin	2g/HL	542000L	No filtration	3 days	2.44 ppm

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7C	After filtration	Cabernet Sauvignon WCE 2014			542000L	>0.2 - 0.45 after cold stabilization	3 days	Not detected
8A	After fining	2013 Pinotage	Lysozyme	10g/Hl	Total volume unknown - after fining placed in 300 L vats for ageing		No filtration	< 0.5 mg/l
9A	After fining	2013 Pinotage	Lysozyme	10g/Hl	300 L		No filtration	< 0.5 mg/l
10A	After fining	2013 Pino Noir	Lysozyme	10g/Hl	300 L		No filtration	< 0.5 mg/l
11A	After fining	2013 Pinotage	Lysozyme	10g/Hl	300 L		No filtration	< 0.5 mg/l

In the samples set all wines were treated using the following type of allergens: ovalbumin (egg white) and lysozyme. Very few producers however used lysozyme. Studies in Europe indicated that it was difficult to remove residual lysozyme from wine after treatment. Only four samples could be obtained during this period that was treated with lysozyme. The analyses of these samples however indicated the opposite result: 10g/hl of lysozyme was added to a relative smaller volume and even though the wines were not filtered, no residual lysozyme could be detected in the wine samples. A larger sample set will however have to be studied before a conclusive observation can be made.



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The average dosage of fining agent (albumin) used by producers was between 2 and 3 g/hl of wine (see [Table 1](#) for sample dosage and size of filters used). All wines were fined between three and 11 days after production. All wines were analyzed directly after fining, but before filtration, showed the presence of allergens at different concentrations, except for one wine. However, all wines that **were positive** for the presence of allergens, showed that **no residual allergen could be detected after the wine was filtered**. The size of the filters that were used for filtration was between 0.2 and 0.65µm. Only in one instance (sample 1) residual albumen was detected after filtration, but this can be attributed to a higher quantity of fining agent that was added to a smaller volume of wine. The wine was then filtered with a filter size of 0.45µm and cold stabilized before bottling, no residual fining agent could be detected after the analyses. The wine was re-analyzed after bottling and no residual albumin could be detected.

The Certified wine sample results after the online sample survey were as follows:

CERTIFIED FINAL BOTTLED AND LABELED WINE SAMPLES

YEAR	CULTIVAR(S)	VINLAB SAMPLE NO	SAWIS APP	NAME OF ALLERGEN	Analyses Result
2012	PINOTAGE	1322750	2013/11235	ALBUMEN	Not detected
2012	CABERNET SAUVIGNON/ MERLOT	1322746	2013/11263	ALBUMEN	Not detected
2012	PINOTAGE	1322728	2013/11264	ALBUMEN	Not detected
2012	CABERNET SAUVIGNON	1322741	2013/11256	Albumien	Not detected
	NON - CULTIVAR	1322742	2013/11257	Albumien	Not detected
	NON - CULTIVAR	1322727	2013/11258	Albumien	Not detected
2012	MERLOT	1322735	2013/11272	Albumien	Not detected
2011	NON - CULTIVAR	1322749	2013/11274	Albumien	Not detected
2011	NON - CULTIVAR	1322751	2013/11274	Albumien	Not detected
	NON - CULTIVAR	1325061	2013/11216	Albumien	Not detected
2012	PINOTAGE	1322743	2013/11227	Albumien	Not detected
2012	CABERNET SAUVIGNON	1322736	2013/11241	Albumin	Not detected
2012	CABERNET SAUVIGNON	1322747	2013/12122	Albumin	Not detected
2011	NON - CULTIVAR	1322745	2013/12126	Albumin	Not detected
	NON - CULTIVAR	1322744	2013/12133	Albumin	Not detected
2012	MERLOT/ CABERNET SAUVIGNON	1322730	2013/12134	Albumin	Not detected
2012	MERLOT	1322734	2013/13884	Albumin	Not detected
2012	SHIRAZ/ CABERNET SAUVIGNON	1322748	2013/13885	Albumin	Not detected
2012	CABERNET SAUVIGNON	1322729	2013/13894	Albumin	Not detected
2012	MERLOT	1322738	2013/11223	Albumin	Not detected
2011	NON - CULTIVAR	1322733	2013/11233	Albumin	Not detected
2012	SHIRAZ	1325062	2013/11218	Albumin	Not detected
2011	SHIRAZ	1322740	2012/23288	Albumin	Not detected
2011	CABERNET FRANC/ CABERNET SAUVIGNON/ PINOTAGE/ SHIRAZ	1322732	2013/5942	Albumin	Not detected
2012	CHARDONNAY	1326269	2013/12121	Casein & Gelatine	Not detected
2010	PINOTAGE/ CHARDONNAY/ PINOT NOIR	1326266	2013/9395	Casein & Gelatine	Not detected
2012	CHARDONNAY	1326270	2013/11270	Casein / Gelatin	Not detected
	NON - CULTIVAR	1326271	2013/11269	CASEIN / GELATINE	Not detected
2012	VIOGNIER/ CHARDONNAY	1326274	2013/11254	CASEIN / GELATINE	Not detected
	NON - CULTIVAR	1326277	2013/11259	CASEIN / GELATINE	Not detected
2012	CHARDONNAY	1326264	2013/11252	CASEIN / GELATINE	Not detected
2012	CHARDONNAY	1326265	2013/11252	CASEIN / GELATINE	Not detected

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2012	CHENIN BLANC	1326272	2013/11250	CASEIN / GELATINE	Not detected
2012	MERLOT	1326273	2013/11228	Casein/Gelatien	Not detected
2012	CHARDONNAY	1326275	2013/11236	Casein/Gelatine	Not detected
2012	CHARDONNAY	1326276	2013/11231	Casein/Gelatine	Not detected
				CASIEN/ EGG ALBUMEN / EVAPORATED MILK / GELATINE / LYSOZYME	
2012	SAUVIGNON BLANC	1326263	2013/9701	LYSOZYME	Not detected
2013	SAUVIGNON BLANC	1326268	2013/11265	Casien/Gelatien	Not detected
2012	MERLOT/ CABERNET SAUVIGNON/ SHIRAZ	1325060	2012/26047	egg albumen	Not detected
2012	MERLOT/ CABERNET SAUVIGNON/ SHIRAZ	1322731	2013/7965	egg albumen	Not detected
2009	MERLOT	1322739	2012/7923	egg whites	Not detected
2009	MERLOT/ CABERNET SAUVIGNON	1322737	2012/10641	egg whites	Not detected
				Potassium Caseinate	
2011	NON - CULTIVAR	1326267	2011/11223	Caseinate	Not detected

All wines in the sample set were final bottled and labelled samples that were ready to be sold in the market place or exported. A total of 43 samples were analysed. In the samples set almost all wines were fined using the following type of proteins: ovalbumin (egg white), casein and gelatin. Very few producers used lysozyme. Only one sample was treated with lysozyme and it tested negative for the presence of residual lysozyme. The size of the filters that were used for filtration before bottling was between 0.45 and 0.65µm. All bottled samples (both red and white wine) tested negative for the presence of residual albumin or casein.

d) CONCLUSIONS

All wines that tested positive for the presence of allergens after fining, tested negative for the presence of allergens after filtration. This suggests that filtration, using the correct filter size (between 0.2 and 0.65 µm), could indeed remove all residual allergens from the wine (if the dosages in this study is applied) and therefore negate the need to label or test for allergens and therefore save cost.

All certified final bottled and labeled wine in the sample set tested negative for the presence of residual albumin or casein. The levels of residual allergens were all found to be below the 0.25 mg/l detection level, above which allergen labeling becomes mandatory (EU regulation 579/2012 and OIV Resolution OIV-COMEX 12-502).

If a higher dosage of allergen was added to a smaller volume of wine, then the initial filtration after fining can be followed up by a second filtration just before bottling and cold stabilization to ensure that all residual allergens are removed.

No correlation could be found between the initial dosage of fining agent added, the volume of wine and the quantified residual allergen remaining in the wine after fining. Literature suggests that different fining agents react differently with different wines and even with the same wine.

6. ACCUMULATED OUTPUTS

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a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

- Skills in the application of the ELISA methods for the determination of allergens in wines.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Publication of findings/Possible papers:

- Impact of fining and filtration on allergens in wine
- Allergen status of South African wines

c) HUMAN RESOURCES DEVELOPMENT/TRAINING**PERSONS PARTICIPATING IN THE PROJECT (Excluding students)**

Initials & Surname	Highest Qualification	Degree/ Diploma registered for	Race (1)	Gender (2)	Institution & Department	Position (3)	Cost to Project R
P Alberts	MSc	BSc	W	M	DAFF	Coll	None
W Jonker	Hons	BSc	W	F	DAFF	PL	None
T Swart	National Diploma	Food Technology	W	F	DAFF	TA	None
H Smit	Degree	B Ed	W	F	Vinlab	TM	None
N Wilton	Degree/Diploma	B Commerce Diploma in Viticulture and Oenology	W	F	Vinlab	TA	None
B Cronje	Degree	B Com (Final year)	W	M	SAWIS	TM	None

⁽¹⁾Race
B = African, Coloured or Indian
W = White

⁽²⁾Gender
F = Female
M = Male

⁽³⁾Position
Co = Co-worker (other researcher at your institution)
Coll = Collaborator (participating researcher that does not receive funding for this project from industry)
PF = Post-doctoral fellow
PL = Project leader
RA = Research assistant
TA = Technical assistant/ technician

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d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

Down S. (2013) Allergens in wine: Protein fining agents identified.

Monaci L, Losito I, De Angelis E, Pilolli R, Visconti A. (2013) Rapid Communications in Mass Spectrometry 2013, Volume 27, pages 2009-2028: "Multi-allergen quantification of fining-related egg and milk proteins in white wines by high-resolution mass spectrometry"

OIV SECSAN 12-520 - 2014: Good fining practice guidelines for wine to be applied after the use of proteinaceous [allergenic] wine fining agents [casein and egg white].

OIV OENO SCMA 10-458: Determination of Lysozyme in wine using high performance liquid chromatography.

OENO-SCMA 12-509: OIV guidelines for validation of ELISA test kits quantification of potentially allergenic residues of fining agent proteins in wine by collaborative trial.

OIV-COMEX 12-502: Revision of the limit of detection and limit of quantification related to potentially allergenic residues of fining agent proteins in wine

e) PRESENTATIONS/PAPERS DELIVERED

None.

7. BUDGET**TOTAL COST SUMMARY OF THE PROJECT**

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
2013-2015					<u>R35 000</u>			<u>R35 000</u>

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