Wine analysis of fining agents chemistry

Science, Chemistry

CHEMISTRY-WINE MAKING| Investigating the effectiveness of common fining agents on homemade fruit wine withrespectto Turbidity, Sediment level, Ph and Alcohol content. | | Year 12 Chemistry| Extended Experimental InvestigationA comparison of chemical flocculation agents| Mario Mitov| Mrs Cullen 2011| | Contents: 1. 0- Abstract 2. 0- Introduction/Background 3. 0- Aim 4. 0- Hypothesis 5. 0- Safety analysis 6. 0- Equipment and Materials 7. 0- Procedures/Methods . 1- Initial wine making procedure 8. 2- Addition of clearing agents including ratio conversion 8. 3- PH testing 8. 4- Vinometer testing 8. 5- Hydrometer testing 8. 6- Turbidity testing (tube) 8. 7- Sediment level measurement 8. 8- Electric conductivity (EC) testing 8. 9- EC conversion to TDS 8. 10- Alcohol titration method 8. 11- Alcohol titration calculations (refer to journal) 8. 0- Results/ Data Analysis 9. 12- Graph1. PH over time 9. 13- Graph2. Alcohol %v/v over time 9. 14- Graph3. Sediment level over time 9. 15- Graph4. Difference in sediment level over time 9. 6- Graph5. Turbidity over time 9. 17- Graph6. Electrical conductivity over time 9. 0- Discussion 10. 0- Conclusion 11. 0- Appendices 12. 0- References 13. 0- Special Acknowledgments 1. 0-Abstract: The construction of this EEI was conducted in accordance to the term 2 context (Wine: an artful process). This report is intended to present the experimental and analytical aspects of winechemistrywith focus on fining agents. By testing these fining agents on wine samples, their overall effectiveness will be observed and discussed with respect to pH, Turbidity, Sediment level etc.

This will help draw a valid conclusion as to the fining agent that has the greatest clarification effects on the tested wines and to what extent. 2. 0- Introduction: The ancient process of winemaking has captivated the human

civilisation for thousands of years. Archaeological findings have shown that the earliest production of wine can be traced back to 8000BC in the region of modern day Georgia, Iran and Armenia (Merveonur, M. 2011). Since then the art of vinification has been continually evident throughout history with its significance highlighted in many of the world's greatest cultures.

Take for example the ancient Egyptians of third millennia BC that used wine for sacred ceremonies or the ancient Greeks that traditionally conducted symposiums (social parties) by drinking wine in large groups (Biers, W. 1980). Throughout the ages wine has undoubtabley been valued for its significance in society, cultureand even religion, with its importance still prevalent in the modern world. In Australia, the exportation of wine currently contributes an astounding $6 billion dollars to the nation's economy (Adams, P. 2005).

In fact, Australia is recognised as the fourth largest exporter of wine in the world. The country's eight constitutive states all commercially produce wine of high quality with vineyards occupying approximately 160, 000 hectares throughout Australia (Wine Australia, 2010). States such as Victoria and South Australia are internationally renowned for producing highly exquisite wines that undergo the finest vinification. The process of producing wine is one that involves multitudinous techniques and requires a thorough scientific understanding referred to as Oenology.

Oenology is the modern study of winemaking encompassing everything from the initial fruit growth to the extensive chemistry behind the entire process (Boulton, R. 1996). Vinification ultimately consists of many biochemical processes that must be carefully monitored and controlled to ensure

success. The primary chemical process involved in any wine is the initial fermentation which is responsible for the formation of ethanol. During this stage, common fruit carbohydrates such as glucose and fructose are converted to alcohol through anaerobic yeast respiration (Fugelsang, C. 1997).

Ultimately, the yeast which are facultative fungal organisms provide enzymes that break down sugar molecules while releasing Ethanol and Carbon Dioxide as by-products through the exothermic reaction: C6H1206 ---> 2CH3CH2OH + 2CO2 + 115kj/mol. Winemakers will usually stop the fermentation process once a desired alcohol level is achieved however the fermentation can naturally stop after reaching a certain level of alcohol that subsequently becomes toxic to the yeast thus killing them (Smith, D. 2009). Once fermentation is over, many wines may display signs of turbidity or cloudiness.

This is due to suspended particulates such as proteins, tannins, phenols or dead yeast cells that cause haziness in wine when not removed. These particles can precipitate out of the wine naturally under the influence of gravity however this is a lengthy process and those that want fast results rely on chemical clarification. Chemicals for the purpose of wine clarification are known as fining or flocculation agents and are widely used in the wine industry. These agents are commonly derived from Earths, Proteins, Carbons, Synthetic polymers, Polysaccharides and other substances (Zoecklein, B. 988). It is extremely important that a commercial wine be cleared of any suspended impurities so that it appeals to the consumer. Fining agents generally clarify the wine in three different methods including

electrostatic attraction, absorption or enzymatic break down (Main, M. 1995). The most common method however is through electrostatic attraction as this is displayed by the majority of fining agents. Theoretically the suspended particles in wine all carry a corresponding electrical charge therefore by dding a fining agent of the opposite charge the particles adsorb to the surface of the fining molecule forming heavy clumps (refer to appendix figure 1). The clumps rapidly sink to the bottom due to their high density relative to that of the wine (Guerra, B. 2008). Ultimately, three fining agents were selected as test subjects for the experiment that was to be conducted. These agents include: Bentonite which is aluminium phyllosilicate clay with a negative charge, Isinglass; a collagen derived from fish that carries a positive charge and Kieselsol/gelatine which is a fining agent utilizing both a positive and negative charge. 3. 0- Aim:

The general aim of this scientific investigation is to assess the efficiency and clarification capabilities of three difference fining agents including; Bentonite, Isinglass and Kieselsol/Gelatine. The results obtained from the wines treated with fining agents will be contrasted to those obtained from the wine without the presence of a fining agent. This will indicate any trends in the data as to which fining agent is most efficient and if there is a great difference between the rate of clarification of the wines subject to chemical fining as opposed to the wine subject to natural clarification under the influence of gravity alone.

By testing parameters such as Ph, Turbidity, Sediment level, Alcohol content and Electrical conductivity some relationships between the results obtained and variables will be analysed to justify the efficiency of the flocculation

agents. 4. 0- Hypothesis: It can be hypothesised that all three fining agents will have noticeably different effects on the wine clarity. The ‘ Kwik Clear’ agent is predicted to exhibit the greatest impact on clarification as it comprises of both Kieselsol and Gelatine which are agents of opposite charges.

This will hypothetically create more lees as the negative and positive charges together will in turn attract more suspended particulates of either electrostatic charge. On the other hand, the negatively charged Bentonite agent is also expected to demonstrate immense fining capacity due to its great molecular surface area that will prompt the adsorption of high amounts of positively charged particles. The Isinglass agent is ultimately expected to perform less desirably as it is by nature a delicate, positively charged agent with weak fining abilities due to it fragile molecular structure.

Finally, the wine without the presence of fining agents (the control), will undoubtabley show the least satisfactory results in terms of clarification. Some sedimentation may occur due to the natural impact of gravity however this will be limited and substantially less in comparison to the wine treated with fining agents. 5. 0- Safety Analysis Due to the topic of this assessment (wine making), the laboratory has been used extensively for nearly all aspects of this assignment including the creation of wine and testing. Safety precautions must therefore be exercised together with strict lab etiquette to prevent dangers arising.

Students must remember at all times to abide by the rules which include: 1. Conducting one’s self sensibly and responsibly at all time especially when handling chemicals of potent nature. 2. Nofoodor drinks to be brought into

the laboratory to prevent the possibility of contamination with harmful substances. 3. Long hair must be firmly tied back to reduce the risk of exposure to corrosive chemicals (or the risk of contaminating the wine). 4. Safety goggles, aprons, lab coats and gloves should be worn at all times to minimise the risk of body exposure to chemicals especially the eyes. . All experimental instructions must be read and understood precisely before conducting the experiment. 6. Sitting on top of the laboratory benches is prohibited. 7. Sitting on chairs while performing chemical experiments is forbidden. 8. The labels of chemicals utilized must always be read so that one is aware of the risks and nature of the particular chemical. 9. Extreme caution must be taken when handling glass apparatus to ensure no breakage. 10. Any breakage or chemical spills must be immediately reported to theteacherso that the risk may be quickly contained. 11.

Carrying chemicals or glass across the room is not advisable and carries a great degree of risk. 12. Running whilst in the premises is strictly forbidden 13. All students must wear closed in shoes to protect feet from spills that may run down the benches. 14. All individuals must be aware of the nearest fire exits and have an understanding of how to use the fire blanket in case of emergency. 15. Any skin contact with chemicals must be quickly reported to the teacher and subsequently washed under running water for 20 minutes or the eye bath when contact has occurred with the eyes. 16.

Any instructions given by the teacher must be followed without hesitation. Note: During the course of the wine making, student are required to perform titrations which require the handling of some extremely noxious chemicals including carcinogens, potent acids and allergens that must by all means be

handled with immense care. Many chemicals also have the tendency to cause problems with the respiratory system when inhaled thus any chemical must be distanced from the nose or mouth. 6. 0- Equipment and Materials. Initial wine making: Supplied by school 1. 5L Demijohn with stopper (fermenter) 2.

Air-Lock 3. Plastic bucket 4. Wine making yeast 5. Tartaric acid 0. 5tsp 6. Nutrient (Diammonium Sulfate, Ammonium Sulfate or Ammonia Phosphate) 7. Sodium Metabisulphite wash solution (0. 5tsp in 0. 5L of water) 8. Campden tablets 9. Pectinase 10. Funnel 11. Sleeve 12. Plastic champagne cork 13. Plastic tubing 14. Balloon 15. Cottonwool 16. Hydrometer 17. Vinometer 18. 100mg Vitamin C tablets as preservatives Initial wine making: Supplied by student 1. Tea towel 2. 6-8 very ripe bananas 3. 150g Sultanas 4. 8kg very ripe fruit (oranges for this group) 5. 3 tea bags 6. 7x 750mL Wine bottles

Adding/preparing the fining agents: 1. Bentonite (Brewcraft) (solid form) 2. Isinglass (Brewcraft) (liquid form) 3. Kieselsol/Gelatine (Kwik Clear) (liquid form) 4. 2x small beakers 5. 1x 0. 1mL increment glass pipette and 1x medium 1mL increment glass pipette. 6. Electronic scales 7. Plastic milkshake cup (to blend Bentonite) 8. Electric blender 9. Distilled water Testing the wine for all parameters: 1. PH meter 2. Hydrometer 3. Vinometer 4. Turbidity tube 5. EC meter 6. Ruler or tape measure (for sediment measurement) 7. Wine samples 8. 4x beakers 250mL 9. 1x large measuring cylinder 10.

Distilled water 11. Disinfectant solution (Sodium percarbonate) 12. Funnel 13. Gloves Performing the titration for alcohol concentration: 1. 10mL wine

sample 2. 60mL of Potassium Dichromate 3. 100mL Standard sodium thiosulphate solution (0. 1M) 4. 30mL of 40% sulphuric acid 5. 6 g Potassium Iodide 6. 250mL distilled water 7. starch indicator (starch solution, freshly made) 8. 10mL pipette 9. 2 ? 20mL pipettes 10. pipette filter 11. 250mL volumetric ? ask 12. 3 ? 250mL conical ? asks with stoppers 13. 10mL measuring cylinder 14. small funnel 15. burette and stand 16. white tile 17. hot water bath 8. thermometer 19. spatula 20. electronic balance 21. safety glasses 7. 0- Procedures/Methods 7. 1- Initial wine making procedure: 1. Pour sachets of yeast into a 750mL bottle with the addition of 2 tbsp sugar, 0. 5 tbsp acid and half-fill the bottle with water placing a balloon over the neck when finished. 2. Fill the bottle with water when the balloon has popped up. Replace the balloon afterwards. 3. Once the balloon ‘ pops up’ a second time, put the bottle in the fridge. 4. Wash bucket, knife, working surface and all fruit with sodium metabisulphite solution. Avoid rinsing fruit! 5.

Remove any really bad parts off the fruit. (it’s not a problem if the fruits are mildly squashed or discoloured). 6. Chop the fruit into approximately six pieces each and place into bucket. 7. Add/mix the following into the fruit: 0. 75 tbsp acid, 0. 75 tbsp nutrients, 0. 75 tbsp Pectinase or 7. 5 drops Pectinol, 7. 5mL Sodium metabisulphite, 150g sultanas, 6-8 bananas, 3 tea bags and 7. 5L water. 8. Leave to rest for 24 hours. 9. Once 24 hours have passed, ass half of the bottle of yeast from step 3 and 750g sugar to the bucket. Stir well, then put a plate on top of the fruit. This will keep it in the liquid.

The whole thing should be covered with a damp towel. 10. Add an additional 1. 5 tbsp of sugar to the yeast bottle and fill with water. Replace the balloon in the process. 11. Begin stirring the bucket with fruit once a day for 3-4 days

consecutively. 12. The funnel, strainer and fermenter must be washed with sodium metabisulphite solution for adequate disinfection. 13. Transfer the liquid contents of the bucket into the fermenter using the funnel and discard any solids in the process. 14. Any remaining liquid must be placed in bottles, stoppered with cottonwool in the neck and placed in the fridge. 5. Use sodium metabisulphite to fill the airlock to half of each chamber. 16. After a week has passed, use PVC tubing to transfer the liquid at the top into the bucket, ensuring the lees (sediment) is left at the bottom during siphoning. 17. Discard the lees, rinse out the fermenter and then replace the liquid back into the fermenter. Use bottles from step 14 to top up the fermenter as needed. Taste and add 150 g of sugar if it is too dry. Also add 5mg of Campden tablets. (Warning: Campden sulphur allergies) 18.

Repeat step 16-17 (which is a process called racking) about once a week for 3-4 weeks. If sugar added, a record must be kept of the exact quantity. 19. When clearing from the top of the wine has initiated, the airlock chambers are evening out and the hydrometer reading is between 1005 and 995, repeat step 16. 20. Taste the samples. If a sweet wine is desired, put 100-150g of sugar in a saucepan and cover with wine fermenter. Heats until the sugar has dissolved and then gradually add this to the wine until the desired balance of sweetness is obtained. 21.

Add 10ml of Sodium metabisulphite and 1000mg of orange flavoured vitamin C (as a preservative). 22. Wash bottles and cork in the solution of sodium metabisulphite and shake out but don’t rinse. 23. Put wine into bottles and cork storing it in a cool dark place. **(refer to journal for information about the key ingredients of the wine making process) 7. 2- Addition of fining

agents including ratio calculation: Isinglass Note: The wine used for each fining agent was racked moments before adding the agents. This ensured that any sediment formation with the fining agents would be easily noticeable and recordable. . Read the instructions on the back of the Brewcraft Isinglass bottle. These instructions stipulate that 10mL are mixed in 25L of wine with the 10mL firstly mixed in a 250mL aliquot before being added to the bulk. 2. The wine bottles used in this experiment contained 750mL of wine therefore calculations were performed to find how much Isinglass must be added to 750mL. 3. Firstly divide 10mL by 25, 000mLwhich equal 4x10-4. 4. Secondly, multiply 4x10-4 by 750mL which equals 0. 3mL (this is the amount of Isinglass to be used for 750mL of wine). 5.

To find how much of the 750mL of wine must be used to initially pre mix the isinglass before adding it to the bulk, the following was calculated: 25, 000mL divided by 250mL equals 100; 750mL divided by 100 is equal to 7. 5. Therefore 7. 5mL of wine must be mixed with 0. 3mL of Isinglass before adding it to the 750mL bulk. 6. Extract 7. 5mL of wine using a small disinfected pipette placing this aliquot in a small beaker. 7. Extract 0. 3mL of isinglass with a small disinfected pipette and place it in the beaker with the 7. 5mL aliquot. 8. Mix the fining agent in the aliquot of wine for a few minutes making sure to swirl it as well. . Using a funnel, replace the mixed aliquot back into the 750mL bulk. 10. Label the bottle, indicating the fining agent used and the dosage. 11. Place cottonwool in the neck and return bottle to the fridge. **(refer to journal for information regarding Isinglass) 7. 2- Addition of fining agents including ratio calculation: Kieselsol/Gelatine (Kwik Clear) 1. Read the instructions on the side of the Kwik Clear box. These

instructions specify that 2mL of Bottle A (containing Kieselsol) must be added per gallon of wine. 2mL of Bottle B (containing Gelatine) must also be added per gallon. . Simple calculations were conducted to find how much of Bottle A and B is to be use for the 750mL bottles. Firstly 1 Galloon equals 4. 5 litres therefore 4500mL divided by 750mL equals 6. 3. 2mL divided by 6 equals 0. 33mL hence, 0. 33mL of both Bottle A and B is to be mixed into 750mL of wine. 4. Premix is not required for Kwik Clear therefore the fining agents can be directly added to the 750mL bulk. 5. Using a small disinfected pipette, take 0. 33mL of Bottle A and place it directly into the 750mL wine. 6. Stir well, then wait approximately 30 minutes before adding Bottle B. . Using a small disinfected pipette, extract 0. 33mL of Bottle B and place it into the bulk. 8. Mix well then label the bottle to include the name of the fining agent in use and the dosage. 9. Stopper the bottle with cottonwool and return it to the fridge until ready for testing. **(refer to journal for basic information about Kwik clear (Kieselsol/ Gelatine fining) 7. 3- pH testing: 1. Put on gloves and gather all necessary equipment. 2. Thoroughly disinfect the four beakers, the tip of the pH meter and the funnel with sodium percarbonate solution. 3.

Run distilled water over the tip of the pH meter until a neutral ph of between 6. 5-7. 5 is reached (this will ensure that the pH meter is configured properly). 4. Carefully pour an adequate sample of wine in a beaker. 5. Slowly dip the tip of the pH meter and leave it in the wine until the pH value on the monitor becomes constant. 6. Record the value and return the sample of wine to its corresponding bottle using a funnel (make sure the sample is

returned from the bottle it was taken from as it would be very undesirable to accidently mix the agents. This would corrupt the whole experiment). 7.

Repeat steps 2-6 for all the wines. **(refer to journal for basic information regarding Ph-(power of hydrogen) 7. 4- Vinometer testing: 1. Put on gloves and gather all necessary equipment. 2. Make sure to disinfect the four beakers, the vinometer and the funnel with sodium percarbonate solution. 3. The vinometer testing requires handling of the wine with hands therefore the hands must be disinfected prior to testing or if gloves are worn, make sure the gloves are disinfected as well especially if used in prior tests. 4. While holding the vinometer over a beaker, pour wine into it so that the wine covers the entire vinometer. . Apply pressure using the thumb on the vinometer opening as though pushing the wine in (this will ensure any bubble formations are removed). 6. Once confident that no bubbles are evident flip the vinometer upside down allowing the wine to fall into the beaker. 7. Bring the vinometer up to eye level and read the scale after the fluid has settled (the level is sometimes hard to see as it is very thin). 8. Record the value; this is usually in %v/v alcohol. 9. Repeat steps 2-8 for all the wines. **(refer to journal for basic information regarding Vinometer (% alcohol concentration). . 5- Hydrometer testing: 1- Put on gloves and gather all necessary equipment. 2. Wash all four beakers, the hydrometer, the measuring cylinder and the funnel with sodium percarbonate solution. 3. Carefully fill the cylinder to about 80% with a sample of wine. 4. Gently lower the hydrometer into the wine making sure it does not touch the bottom or the side of the cylinder. 5. Record where the surface of the wine contacts the hydrometer. 6. Repeat step 2-5 for each wine ***(Note: The Hydrometer

testing was done incorrectly thus giving inaccurate results throughout each trial.

This mistake was only noticed after testing was concluded therefore there was no way of repeating the results. All results obtained a hydrometer reading of 1. 030 which indicates there has been a general mistake with the procedure. The group decided not to use the Hydrometer due to this flaw)*** 7. 6- Turbidity testing (with tube): 1. Place gloves on both hands and gather all necessary equipment. 2. Wash the turbidity tube and funnel in the disinfectant sodium percarbonate solution. 3. Carefully pour wine into the turbidity tube by small increments at a time. 4.

Look for when the black lines at the bottom of the tube become slightly visible just enough to see the lines. 5. Record the value at which the lines are slightly visible (this is the turbidity in NTU). 6. Repeat steps 2-5 for each wine. **(refer to journal for information about Turbidity) 7. 7 Sediment level (with ruler): 1. Carefully hold the wine bottle against and angle of strong light until the lees is visible (making sure not to disturb the sediment greatly). 2. Use a ruler to measure the sediment level from the bottom of the bottle to the point at which it finishes. . Record the results to the nearest mm. 4. Repeat step 1-3 for each wine (Note: Sediment measurement should be tested first before anything else so that the lees is not disturbed from movement 7. 8 Electric Conductivity (EC meter): 1. Put on gloves and gather all necessary equipment. 2. Thoroughly disinfect the four beakers, the tip of the EC meter and the funnel with sodium percarbonate solution. 3. Run distilled water over the tip of the EC meter until a reading of 0 is obtained (this will ensure that the EC meter is configured properly). 4.

Carefully pour an adequate sample of wine in a beaker. 5. Slowly dip the tip of the EC meter and leave it in the wine until the Electrical conductivity value on the monitor becomes constant. 6. Record the value and return the sample of wine to its corresponding bottle using a funnel (make sure the sample is returned from the bottle it was taken from as it would be very undesirable to accidently mix the agents. This would corrupt the whole experiment). 7. Repeat steps 2-6 for all the wines. **(refer to journal for information about EC (Electric Conductivity) 7. EC Conversion to TDS using mathematical formula: 1. The formula stipulates that TDS (ppm or mg/L) = F x EC where F is a factor of 0. 6 and EC is the electrical conductivity in Microsiemens per centimetre. 2. The results taken by the EC meter in class are given in millisiemens therefore to covert to microsiemens simply multiply by 1000. 3. Take the number in microsiemens and multiply it by a factor of 0. 6 as stated by the formula. 4. This is the measurement in TDS (ppm). 7. 10 Alcohol Titration method: 1. Place a 10mL aliquot of wine in a 250mL volumetric flask (using a small pipette). 2.

Distilled water is then used to fill the volume up to the 250mL mark. 3. Take a 20mLaliquot from the 250mL solution and place it in a conical flask. 4. Step 3 must be repeated twice so that there are three flasks to use in 3 separate trials. 5. To every flask a 20mL aliquot of 0. 04 Molar Potassium Dichromate is added. 6. 10mL of 40% sulphuric acid is added to every flask with the aid of a measuring cylinder and the teacher (the teacher will have to do this step due to the potency of the acid). 7. Each flask should be stoppered loosely and heated in a water bath at 45-50*c. (water bath must not exceed 50*c). . Once 10 minutes have passed, remove the flasks and add 2 g of Potassium

Iodine to all flasks. 9. Fill the burette with Thiosulphate solution (0. 1 Molar). 10. Begin the titration, titrating the contents of the flask with the 0. 1M Thiosulphate solution. When the brown colour of the titrated solution becomes green add 1-2mL Starch. The equivalence point is noticed when the solution turns from blue to light green. 11. Record the result of the titres. 7. 11 Alcohol titration calculations: **(Refer to journal for titration calculations) 8. 0- Results and Data Analysis: 8. 1- graph 1 - PH over time:

The above graph displays the pH obtained for each wine over the 5 trials conducted. Initially before adding the clearing agents, the wine was tested to be at pH 4 as indicated at ‘ trial 0’. After the clearing agents were added the pH remained at 4 however the Isinglass fined wine increased to pH 4. 3. Standard wine must be kept at a pH range between 3. 2 and 4. 1 therefore anything over 4. 1 raises alarms and action must be taken to lower the pH using acidic substances. Ultimately 1g of Tartaric acid was added to all wines during trial 1 and subsequently resulted in the decrease in pH observed from trial 1-3.

Trial 3 was the point at which the pH of all four wines was recorded at a constant 3. 2 indicating that the Tartaric acid was successful at lowering the pH by almost 1 for all four wines. From trial 3-5 the pH of all the wines is seen to fluctuate once again this is due to the fact that the effects of the tartaric acid have ceased therefore the pH system increases and decreases accordingly as it tries to find a new state of equilibrium. This trend can be related to Le Chateliers principle which states that “ If a system at equilibrium is disturbed, then the system adjusts itself so as to minimise the disturbance.

At the end of trial 5 all four wines fell between a stable pH range however following the low pH of trial 3, Isinglass fluctuated to pH 3. 7 as seen in trial 5. This raises the possibility that the pH of Isinglass may have continued to raise. 8. 2- Graph 2 - Alcohol %v/v over time The above is the comparison of alcohol content for all the wines over the 5 trials conducted. All wines clearly tend to show a percentage of alcohol between 4-6% throughout the trials with the overall average being 4. 2%. At the end of trial 5, all four wines recorded a stable 4%v/v alcohol however this is unusual considering that wines are typically 7-14%v/v.

The 11%v/v recorded for the control at trial 3 is believed to be due to error as there is no justifiable reason for such an instantaneously high fluctuation. It is seen that all wines containing fining agents recorded an alcohol level that was similar to each other; this was ultimately expected as fining agents do not typically affect alcohol level. These readings from the vinometer can ultimately be relied upon as alternative alcohol testing through the more accurate titration method showed that the alcohol level was at approximately 5% which is the most common value presented throughout the vinometer testing. . 3- Graph 3- Sediment level over time Displayed above is the graphical analysis of the sediment level recorded over time for each wine. There is a clear indication that the wine finned with Bentonite produced the most lees followed by the Gelatine/Kieselsol and then the Isinglass which accumulated the least amount of lees out of the three clearing agents. Overall however, the control (wine without fining agent) showed a very limited accumulation of sediment therefore indicating that sedimentation rate is slower without a presence of a clearing agent.

Interestingly the Bentonite also recorded instant sedimentation of 1. 5cm for the first trial which is more than twice as much as that of the Gelatine/Kieselsol and three times more than the Isinglass and control. The control also displayed the slowest rate of accumulation of lees with a 0. 1cm increase occurring once every 2 trials. Some results show a drop in sediment level; this should ultimately be ignored as the measurement of sediment encompasses an uncertainty of (+- 0. 5cm) therefore the recorded drop in sediment may just be an indication of no change. . 4 - Graph 4- Difference in sediment level over time: The above graph has been constructed to aid in the interpretation of the previous (Graph 3). This displays the difference in sediment level for each wine from trial to trial. Ultimately the total increase of sediment for Bentonite over the 5 trials was +1. 9cm (assuming that the – 0. 1 is treated as a no increase rather than a decrease). The Kieselsol/Gelatine recorded a total increase of +1. 6cm. Isinglass obtained a total of +0. 8 and the Control showed a +0. 7 total increase over the 5 trial periods. 8. - Graph 5- Turbidity (NTU) over time: The above ‘ turbidity over time’ graph ultimately displays the extent at which the wines were cleared. The results obtained for turbidity show the same trend that is seen in the sediment accumulation graph in terms of the fining agent performance. Bentonite was most effective at clearing the wine, bringing the turbidity down from 600 to 250NTU followed by Kieselsol/Gelatine which was successful in a turbidity reduction from 600 to 300 NTU. Isinglass again presents itself as the weakest clearing agent only clearing the wine from 600 to approximately 450NTU.

The Control indicated only a slight decrease in turbidity of 50NTU in total. Notice that major reduction in turbidity for all four wines ceases from trial 3 onwards, this trend was also recorded in the sediment graph. Another trend that is seen in this graph and the sediment accumulation graph alike is that Bentonite again appears to have the greatest initial impact out of all the fining agents. *The lack in results for trial 1 is due to not having the turbidity tube available at that time. 8. 6- Graph 6- Electric Conductivity over time:

Electrical conductivity was tested purely because it can give an indication of TDS through a conversion factor. Again what is immediately noticed is that on the 5th trials, Bentonite had the greatest overall drop in EC/TDS. Followed by Kieselsol/Gelatine then Isinglass. The control showed the least overall reduction of EC/TDS and this is a trend seen in the previous Turbidity and Sediment graphs. (Refer to Journal for raw data tables and the conversion from EC to TDS) 9. 0- Discussion: Through the analysis of the results obtained, many justifications can be drawn as to the fining agent that had the greatest effect on clarification.

By graphing the primary trends in turbidity and sediment level, the initial hypothesis was ultimately rendered incorrect to some extent. It was hypothesised that the Kieselsol/Gelatine would have the greatest fining ability due to the agent being composed of both positive and negative charges. This however was not the case when the results were analysed. Ultimately the Bentonite was recorded to have the greatest fining ability followed by the Kieselsol/Gelatine and then the Isinglass which as predicted, presented itself as the weakest of the three fining agents.

The Bentonite agent visibly resulted in the most sediment accumulation, recording a total of 1. 8cm of lees and a turbidity reduction of 350NTU in total. Interestingly, Bentonite also exhibited a very fast rate of efficiency with an accumulation of 1. 5cm of sediment in the very first trial (Graph 3). This was more than double the sediment level of Kieselsol/Gelatine and triple the amount recorded for Isinglass. Similar trends in rapid efficiency are seen in (Graph 5) where Bentonite is noticed to reduce turbidity by half in the very first trial.

These superior clearing abilities stem from the complex molecular composition of the substance. Bentonite is comprised of a multitude of small silicate platelets that are separated by a layer of water molecules (Zoecklein, B. 1988). During the initial Bentonite hydration performed before adding it to the wine, these platelets repel each other and subsequently pop apart. As this occurs, the platelets rearrange and immense swelling takes place which results in an enormous surface area (refer to appendix figure 2).

It is this enlarged surface area that grants Bentonite the ability to absorb a greater amount of suspended matter. Further evidence shows that there are more molecular properties that can justify the dominant fining capabilities of the Bentonite agent. Foremost, Bentonite is renowned for its high cation exchange capacity (binding ability) (Catarino, S. 2007). Typically there is a tremendously fast reaction time between positively charged protein particulates and the negatively charge Bentonite.

It is common for three-quarters of proteins to react with Bentonite within the first minute of contact (Zoecklein, B. 1988). Protein contents from an initial 50-100mg/L can be cleared to less than 10mg/L in most circumstances

therefore this high clearing capacity is another reason why such elevated amounts of sediment deposits were recorded during testing. Another aspect to consider is the relationship between ethanol concentration and Bentonite. Scientific research has suggested that there is a proportional relationship between ethanol concentration and the efficiency of the Bentonite fining.

This is due primarily to the fact that ethanol separates the silicate layers within the structure therefore allowing larger particles to adsorb to the surface (Harberton, J. 2009). Basically out of the three fining agents, Bentonite is the only one that exhibits this relationship with alcohol thus its flocculation ability is advantaged when placed in an ethanol rich solution such as the wine. What can further be associated with the Bentonite's vast flocculation ability is that the agent is not exclusively of a negative charge.

Interestingly, the molecular platelet edges are comprised of a slight positive charge (AMCOL, 2005) (refer to Appendix figure 4). This gives the Bentonite an additional aptitude to bind to some negatively charged particles therefore providing a wider range of clearing potential. When looking back on the results obtained, the hypothesis was validated to the extent that the wine without a fining agent (the control) showed least efficiency in clarification. As seen in Graph 3 (sediment level over time), the control not only accumulated very small amounts of sediment but also did this at a very slow rate.

An increase of 0. 1cm is noticed only once every two trials whereas the fining agents show a substantial increase nearly every single trial. The same goes for the turbidity reduction (Graph 5) where the control is seen to take approximately three trial periods to attain a decrease of a mere 50NTU. Although the control showed signs of clearing due to gravity, it is

substantially slower than that of the fining agents. A scientific principle set out by physicist George Stokes in 1851, aids in the conceptualisation of why the fining agents are more efficient in sedimentation.

Stokes' law states that the rate of sedimentation of a spherical particle is directly proportional to the difference in density of the particle and the liquid, the acceleration due to gravity and the radius of the particle (Stoyanov, P. 1980). By the application of this law, it stands to reason that a fining agent causes swifter sedimentation as it increases the radius of the particles through electrostatic binding and this in turn causes the particles to have a greater density ultimately increasing the rate of clarification It was also initially hypothesised that Isinglass would perform least efficiently out of the three fining agent.

This was confirmed through the data analysis which showed that Isinglass produced three time less sediment than Bentonite and only reduced turbidity by 150NTU unlike Kieselsol/Gelatine which reduced turbidity by 300NTU and Bentonite which resulted in a 350NTU reduction. Isinglass was ultimately not anticipated to have great fining abilities due to its gentle nature which renders it more suitable for wine polishing rather than heavy clearing (Chorniak, J. 2007). According to external scientific sources, the overall fining rate of Isinglass stands at a low 0. 2 - 0. 1g/L-1 therefore adding justification as to the low sediment yields recorded for Isinglass in this chemistry experiment. There is however another biochemical issue that may have attributed to the underperformance of the Isinglass agent. Collagens such as isinglass are typically dependant on temperature for chemical stability. At high temperature most collagens begin to decompose on a molecular scale.

Isinglass starts to denature at a relatively low temperature of approximately 10°C.

This denaturing process results in a reduction of molecular weight which consequently weakens the fining ability (Hornsey, I. 2007). Throughout the course of this experiment, the wines tested were exposed to temperature of over 18°C hence leading to the presumption that this may have contributed to isinglass' lack in performance. When analysing the data to a greater extent, there was a very crucial trend that was established which may have affected the performance of all the clearing agents. pH is ultimately seen to have an inversely proportion relationship with the binding ability of the fining agents.

As pH increases, the strength of the electrostatic attract between the fining agent and particles is subsequently decreased. This principle is extensively evident for all three fining agents tested in this experiment. Notice that after trial 3 (graph 5) the turbidity no longer shows improvements for both Bentonite and Isinglass and only exhibits a minimal improvement for Kieselsol/Gelatine. This is also notice for the sediment level (graph 3) which shows only very slight increases in sediment accumulation after trial 3.

This reduced performance after trial 3 may be due to the fact that the pH of all wines began to fluctuate from trial 3 onwards (refer to graph 1). Overall it is seen that the clearing agents performed best when the pH was reducing during trial 1 and 2 and started to perform poorly when the pH began to fluctuate from trial 3. To comprehend this phenomenon it is important to understand the concept of the Isoelectric point (pl). Basically all proteins or

other suspended particles have a certain pH at which the particles carry no net charge.

If the pH of a solution is too high or near the isoelectric point, the particulates will not be sufficient in electrostatic attraction because at a high pH, the molecules carry an equal number of positive and negative charges resulting in neutralisation (Zoecklein, B. 1988). This is also true for the fining agents which lose electrostatic charge when there is even a slight increase in pH. Recent studies that tested the net charge of isinglass in different pH medium show that even a small increase of 0. 5 pH resulted in the reduction of approximately half the net charge of the isinglass agent (Ward, I. 000) (refer to appendix figure 3). This evidence adds credibility to the justification that the rise in pH may have indeed contributed to the trend noticed in all the fining agents. Alternatively however there is the possibility that the fining agents simply reached their capacity and began decreasing in efficiency after trial 3 indicating that more fining must be added. Overall through the in-depth interpretation of the data; the performance of the fining agents can be justified and links can be draws as to the agent that performed the best and worst (the Bentonite and Isinglass respectively).

However although this experiment was successful in giving reasonably good results it is undoubtabley encompassed by multitudinous amounts of human error and uncertainty that may render it flawed to some extent. The major flaw to this experiment is that sediment was not siphoned after every trial. The group initially chose not to siphon the lees so that results could be easily recorded. However what was not realised is that every time the wine was poured out for testing, the deposit of sediment was resuspended into the

wine therefore may have resulted in highly inaccurate results especially for turbidity.

Another associated human error is that members from the group at some stages touched the wine with bare hands that were not disinfected especially during Vinometer testing. This holds a great risk that the wine may have become contaminated thus predisposed to bacterial manifestation which would render the experiment invalid. Gloves were only used after the 2nd trial period once the group noticed the risk of contamination. Other possible sources of contamination include the fact that some equipment were not washed with sodium metabisulphite before making contact with the wine, this again posses high risk of bacterial growth.

At one stage there was speculation whether the wine had indeed contracted a bacterial strain. This is due to the hazy/cloudy appearance of the wine despite the extensive fining that was performed. Although turbidity was slightly reduced by the fining agent, the wine still seemed awfully turbid. This could be a sign of a bacterial colony which causes a liquid to appear murky and is ultimately untreatable by the fining process. When expert opinion was consulted about the wine, it was concluded that the turbid appearance was not due to bacteria.

If the wine had been infected, it would have had a very ‘ off’ taste which was not indicated during the weekly wine tasting. What must be noted is that because the wine in this group was created with oranges, it will never have a very clear appearance due to the thickness and dark colouration of the orange contents. Common wines made from grapes are naturally very clear due to the transparency of the grape juice by nature. It was therefore

presumed that the turbid look of the wine was due to the natural composition of the oranges it was made from.

If this experiment was to be performed again, many improvements must be made to ensure more accurate and valid results. It is recommended that more emphasis must be placed into contamination control and management during the winemaking and wine testing period. This will ensure no foreign material spoil the validity of the experiment. Also there was major uncertainty in the equipment used. Firstly the ruler used to measure sediment carries a +/- 0. 5cm uncertainty which is major especially when measuring something so small such as sedimentation deposit.

Maybe a more accurate measuring tool with a smaller scale can be used in the future for the lees measurement. Furthermore all testing including pH, Turbidity, Vinometer etc were only done once every trial period whereas it is advisable to always test a sample 3 times in a row to ensure consistency in the results. This was not done in this experiment as time restraints did not allow for repeated testing. If these simple improvements can be implemented for future testing, the results will be more accurate which will in turn provide more credibility to the findings. 10- Conclusion:

The process of wine making and testing was a long and arduous task that involved many procedures. Although there were some minor flaws involved with the testing, the experiment was considered an overall success. The aim from the start was to see which flocculation agent would have the greatest effect on wine clarification and through extensive testing the results clearly indicated the superior fining agent. The Bentonite was undoubtabley the

most powerful flocculation chemical and resulted in the greatest reduction in turbidity and also the greatest increase in sedimentation.

The trends and patterns in the data were evaluated and scientific justifications were made as to why each fining agent performed to the extent that was witnessed. Ultimately relationships between variables were established as explanations that coincide with the performance of the fining agents. Wine pH, alcohol level and temperature are concluded to have a drastic effect on the flocculation abilities of the fining agents. These variables must be monitored and adjusted accordingly as the efficiency of the clearing agents are found to be very dependent upon their values.

For future reference, pH is seen to affect the electrostatic bonding abilities of the substances, alcohol levels have a proportional relationship to the fining abilities of Bentonite and temperature (either to low or to high) affects the efficiency of the chemical fining process especially that of the Isinglass. With this in mind it can be concluded that the fining agents did help in the clarification of the wine however were affected by many variables that must be taken into account if this experiment was to be performed in future. By Mario Mitov 11- Appendix: {FIGURE 1}.

Below: Diagram of the electrostatic flocculation process exhibited by most fining agents. {FIGURE 2}, Below: A depiction of the Bentonite surface area expansion that is initiated during the hydration stage. This is one of the main reasons why Bentonite is such a powerful flocculation agent. From left to right; Water molecules between the silicate layers, Layers then pop apart during hydration, the platelets rearrange and swelling takes place, once rearrangement has occurred the surface area is greater than before

hydration. Diagram sourced from (Zoecklein, B. 1988) FIGURE 3} Below: Scientific studies conducted for isinglass that shows an increase in pH results in electric charge depletion thus weakening the fining agent. {FIGURE 4}, Below: A depiction of the Bentonite molecular structure, Notice that the edges are +ve (positively charged) and the inner area is predominantly of a negative charge (-ve). Because Bentonite has slightly positive charged edges it is advantaged in its fining ability. Image sourced from http://www. amcoldetergents. com/Resources/How%20Bentonite%20Softens%20Through %20the%20Wash. pdf 12- References: Books (alphabetical order): Biers, W.

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