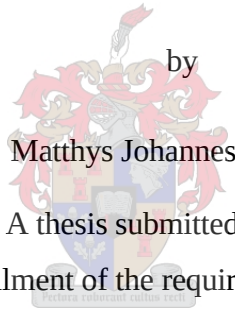
DISCOLOURING OF GRAPE JUICE CONCENTRATE: CAUSES AND POSSIBLE WAYS OF INHIBITION



by

Matthys Johannes Loedolff

A thesis submitted in partial fulfilment of the requirements for the degree of

Master of Science in Engineering
(Chemical Engineering)

Department of Process Engineering
University of Stellenbosch

Supervisor: Prof. L. Lorenzen

December 2004

DECLARATION

I hereby declare that the work done, the results obtained and the conclusions made are the work of my own. Wherever information was used and obtained from references it is so stated.

Signed on this ____ day of _____ 2004

M.J. Loedolff

SUMMARY

The grape juice concentrate (GJC) plant of the KWV at Robertson spent significant amounts of money on the decolourisation of grape juice concentrate. A chemically activated powdered activated carbon (PAC) purchased from Norit, namely CA1, was used as decolourisation product. Apart from the expenses involved, it contributed largely to the solid waste produced at this plant. A way was sought to minimise or prevent GJC discolourisation (and possibly solid waste) without increasing operating expenses.

Browning reactions in GJC are as old as the product itself. Numerous researchers have studied the origins of these reactions, the reactants and products involved, as well as the reaction kinetics of these reactions. From the work of these researchers four possible browning reaction pathways were identified, namely:

- enzymatic oxidative browning,
- non-enzymatic oxidative browning,
- non-enzymatic browning (the Maillard reaction), and
- caramelisation.

It was also identified that 5-hydroxymethylfurfural (HMF) are indicative of the browning potential of GJC. A method to analyse for HMF (quantitative and qualitative) was develop for the purposes of this study, namely positive electron-spray ionisation preceded by high-pressure liquid chromatography (HPLC) and followed by dual mass spectrometry. This method showed good repeatability and was used to analyse all samples generated during this study.

It was confirmed that the manufacturing process at this plant favours non-enzymatic browning reactions, since mild heat treatment deactivates enzymes. Further investigation indicated that the overruling browning reaction on this plant was non-enzymatic oxidative browning. It was shown that neither the presence, nor the absence of protein had any effect on the rate of formation of HMF. It was, however, confirmed that HMF formation could be attributed to high temperatures and prolonged exposure to these temperatures.

Other adsorption products were evaluated against the then current PAC (CA1), namely a steam activated PAC supplied by Norit, SA4, and a polymeric adsorbent, Polyclar V (polyvinylpolypyrrolidone/PVPP). Both SA4 and PVPP indicated superior HMF adsorption capacities. Replacing CA1 with SA4 could result in operating expenses savings and possible solid waste reduction. However, PVPP were too expensive to be considered an economically viable replacement for CA1.

Improved concentration technologies such as reverse osmosis (RO) membrane concentration followed by centrifugal evaporation (CE) or two-stage CE should be considered as possible replacement for the existing concentration technology (multi-stage falling film evaporator). This should decrease heat treatment/exposure by more than 90% and thus reduce browning significantly. An added advantage could be the reduction of solid waste, since less (if not no) decolourisation will be required.

Alternatively, juice should be stored with added sulphur dioxide (SO_2), since it was shown that this juice contained much lower HMF concentrations than diluted concentrate (stored for the same time). This should reduce heat exposure by up to 50% and thus minimise browning reactions.

OPSOMMING

Die druiwesapkonsentraat (DSK) aanleg van die KWV in Robertson het jaarliks aansienlike bedrae geld spandeer tydens die ontkleuringsproses van DSK. ‘n Chemies geaktiveerde verpoeierde koolstof (GVK) verkrygbaar van Norit, naamlik CA1, is gebruik as ontkleuringsproduk. Buiten die kostes verbonde aan hierdie produk het dit ook grootliks bygedra tot soliede afval by hierdie aanleg. Oplossings is gesoek om die verbruining/ontkleuring van DSK (en dalk ook soliede afval) te verminder (of selfs te voorkom) sonder om bedryfskostes te verhoog.

Verbruiningsreaksies in DSK bestaan al so lank soos DSK self. Verskeie navorsers het die oorsake, reaktante, produkte en reaksiekinetika van hierdie reaksies oor die jare heen bestudeer. Uit die werk van sommige van hierdie navorsers kon vier moontlike verbruiningsreaksieroetes geïdentifiseer word, naamlik:

- ensiematiese oksidatiewe verbruining,
- nie-ensiematiese oksidatiewe verbruining,
- nie-ensiematiese verbruining (die Maillard-reaksie), en
- karamelisering.

Daar was verder geïdentifiseer dat 5-hidroksiemetielfurfuraal (HMF) aanduidend is van die verbruiningspotensiaal van DSK. ‘n Analitiese metode (kwalitatief en kwantitatief) om vir HMF te analiseer is vir die doel van hierdie studie ontwikkel, naamlik positiewe elektronsproei ionisasie, voorafgegaan deur hoëdruk vloeistof chromatografie en gevolg deur dubbele massa spektrometrie. Hierdie analitiese metode het goeie herhaalbaarheid getoon en was deurgaans gebruik om monsters te analiseer gedurende hierdie studie.

Dit was bevestig dat die vervaardigingsproses by hierdie aanleg nie-ensiematiese verbruiningsreaksies begunstig, aangesien geredelike hittebehandeling ensieme deaktiveer. Verdere navorsing het getoon dat die oorheersende verbruiningsreaksies by hierdie aanleg nie-ensiematiese oksidatief van aard is. Resultate het getoon dat proteinstabiliteit geen invloed op die vormingstempo van HMF het nie. Dit was egter bevestig dat vorming van HMF direk verband hou met hoë temperature en lang blootstellingsperiodes aan hierdie temperature.

Ander adsorpsieprodukte was vergelyk met die huidige GVK (CA1), naamlik ‘n stoom geaktiveerde verpoeierde koolstof (Norit se SA4) en ‘n polimeriese adsorbant, Polyclar V (polivinielpolipirrolidoon/PVPP). Beide SA4 en PVPP het CA1 oortref wat betref HMF adsorpsie. Moontlike bedryfskostebesparings (en soliede afval verminderings) potensiaal bestaan indien CA1 vervang word met SA4. Die teenoorgestelde is egter waar vir PVPP wat bedryfskoste aangaan.

Instede van die huidige verdampinstegnologie, naamlik vallende-filmverdamping, hoort verbeterde konsentrasietegnologieë soos tru-osmose membraankonsentrasie gevolg deur sentrifugale verdamping, of, alternatiewelik, twee-stadium sentrifugale verdamping, orrweeg te word. Op hierdie wyse behoort hittebehandeling (en dus verbruining) met sowat 90% verminder te word. ‘n Moonlike addisionele voordeel is die vermindering van soliede afval aangesien minder ontkleuring nodig sal wees.

Indien die verbeterde tegnologieë te duur is moet daar gekyk word daarna om die ongekonsentreerde sap met addisionele swaweldioksied (SO_2) te stoor, aangesien veel laer HMF konsentrasies in sulke sap waargeneem is as in verdunde direkte konsentraat wat vir dieselfde typerk gestoor is.

Hittebehandeling sal op hierdie wyse met tot 50% verminder word (en dus verbruining ook).

TABLE OF CONTENTS

LIST OF FIGURES

Number *Page*

LIST OF TABLES

ACKNOWLEDGMENTS

To everybody that contributed to this thesis, thank you. I want to thank Prof. Leon Lorenzen, my study leader for guidance during this study. Also, to Dr. Thinus van der Merwe who worked long hours on the analysis that was essential to complete this study. Mr Niel Rademeyer, the technical officer on the plant this study was based on, a huge thank you for assisting me even when you didn't have the time. To Jannie Barnard, Anton Cordier, and Howard Koopman who helped constructing the experimental setup. Also thanks to Vincent, Charles and James for all the assistance.

To my parents: "Pa, Ma, dankie vir alles! Ek weet julle het baie opgeoffer in die tyd wat ek hier op Stellenbocsh deurgebring het (en voor dit ook!!). Ek is baie lief vir julle!"

To my wife: "Marli, my skat, dankie vir die ondersteuning en vir die tyd wat jy broodwinner was in die huis. Ek is oneindig lief vir jou!"

To all that showed an interest during this few years, my friends, my younger sister, and my in-laws, thank you for the support.

Special thanks to my financial sponsors, Winetech and NRF, who funded this project. It is much appreciated.

And lastly, but the most, to the Lord of lords and the King of kings, Jesus: Thank You for your strength and the abilities You have given me. I acknowledge You as Lord of my life.

1 INTRODUCTION

The process where grape juice is boiled to what is known in South Africa as "Moskonfyt" (jam), is a very old method of grape juice concentrate (GJC) manufacturing. It was the first method in which grape must or "mos" was concentrated. The original process involved the boiling of the grape juice in open pots at atmospheric pressure. This caused the boiling point to increase as the water evaporated. Due to this increase in temperature, thermal degradation of the sugars and other compounds occurred, causing the jam to lose most of its grape flavour and to obtain a "boiled" taste. "Moskonfyt" was not an acceptable GJC and other methods of concentration were developed.

1.1 BACKGROUND

The three major grape juice concentration technologies used are:

- Concentration by freezing;
- Concentration by reverse osmosis (RO);
- Concentration by distillation processes.

1.1.1 Concentration by freezing

Concentration by freezing involves the exposure of the grape juice to intense cold (down to -14^{o}C). The water fraction of the juice freezes under these conditions. The small ice crystals can then be removed by a specialized centrifuge able to separate the ice crystals from the concentrate while washing the ice with clean water to remove the remaining grape juice. What remains is the concentrated juice. This process is more energy effective than any distillation process. However, operating and capital expenses do not warrant its use for GJC manufacturing.

1.1.2 Concentration by RO

Reverse osmosis is often used to adjust sugar content in grape juice, to adjust alcohol and volatile acid levels in wines, or to concentrate wines. The juice is pumped to flow across the one side of a selectively permeable membrane. The water then selectively permeates the RO-membrane leaving a concentrated grape juice on the concentrate-side of the membrane. Applying pressure to force more water through the membrane can further increase the effect of concentration. However, there is an optimum operating point determined by product yield and operating cost (input energy, etc.). Pressures as high as 80bar(g) is required to obtain a GJC with a maximum sugar concentration of 50% (wt/v). Thus, the maximum attainable sugar concentration is approximately two thirds of the required concentration.

1.1.3 Concentration by distillation processes

The most common method of grape juice concentration is multiple effect evaporation (MEE). The plant, on which this study was conducted, made use of MEE. Juice is boiled (at approximately 70^{o}C maximum) under vacuum, causing evaporation of water. The vacuum draws the vapour off and a GJC remains as product.

1.1.4 Application of GJC

GJC is used in the following applications:

- The sweetening of table wines;
- Some countries that do not commercially produce grapes, reconstitute the GJC, and then inoculate it with yeast to produce their own wines;
- The base for fruit juices and cooled drinks (also carbonated);
- Baby foods, ice creams, and yoghurt.

1.2 PROBLEMS EXPERIENCED DURING GJC MANUFACTURING

The major single problems currently experienced during concentrate manufacturing are:

- Tartrate instability;
- Sugar crystallisation;
- Fermentation;
- Browning of the concentrate during storage.

1.2.1 Tartrate instability

Tartrates are salts that form when minerals such as Potassium and Calcium combine with natural acids (tartaric acid) in the juice. During the concentration process, water is removed, causing the tartrate concentration to increase. This increase in tartrate concentration causes tartrates to visibly precipitate. The murkiness is unacceptable to consumers and must thus be removed. Tartrate stabilisation alleviates this problem.

1.2.2 Sugar crystallisation

The crystallisation of sugar at the bottom of the tanks is a problem that already occurs when the concentrate is in cold storage (might in some instances be confused with tartrate precipitation). Sugar crystallising in the shipping containers pose a major problem. Receiving clients deem such juice as unacceptable. Tests and practice have proved that the addition of citric acid during container filling alleviates this problem, almost entirely.

1.2.3 Fermentation

Fermentation is can be prevented by good hygiene. All apparatus should be thoroughly cleaned and sterilised before and after use.

1.2.4 Browning of the concentrate during storage

During long periods of storage, GJC tend to degrade visibly by discolouring (also referred to as browning). Colour changes from a golden yellow to dark brown. Three possible browning pathways exist. They are:

- Oxidative browning (including enzymatic and non-enzymatic);
- Non-enzymatic oxidative browning;
- Caramelisation.

1.2.5 Oxidative browning

During storage of fruit juice or fruit juice concentrate some of the phenolic compounds present in the juice tend to react via an oxidation pathway (either catalysed by enzymes or an acidic environment) to form larger phenolic molecules or polyphenolics. After some time the colour turns to dark brown. This phenomenon can be seen in some bottled apple and grape juices if the colour of a rather new batch is compared with the colour of a rather old batch.

1.2.6 Non-enzymatic browning

The Maillard reaction in foodstuffs is very complex and, even today, is not fully understood. This study focuses only on two of the pathways within the entire Maillard reaction, namely the ones involving the formation of 5-hydroxymethylfurfural (HMF).

1.2.7 Caramelisation

Caramelisation does not form part of this study, since it involves very high temperatures applied for significant periods of time with little to no water. Such high temperatures are not reached during the grape juice concentration process we are focusing on.

1.2.8 In general

There are a number of products that can remove the brown colour from the juice. However, the most common method of decolourising is by contacting the juice with activated carbon for a few hours, followed by filtration to remove any suspended solids. This method is sufficient for dealing with the symptoms of the browning reaction. However, the efficiency of removing the precursors of the browning reactions remains a challenge.

1.3 SCOPE OF WORK

This study will focus on the cause and possible elimination of browning reactions. This study involved one grape juice concentration plant where browning reactions are a problem. This plant is situated in Robertson, Western Cape, South Africa, and is the property of the KWV (this plant has stopped operation during the course of this study). A detailed description of the process used in manufacturing the concentrate, is given in Chapter 2 (refer to Figure 2-1).

The browning of the concentrate was the direct implication of added expenses on this specific plant. After long periods of storage the GJC required decolourisation. When fresh juice is received at the plant it is directly concentrated to 70+ ^{o}Balling. This juice is then stored at 10^{o}C for a few months. During this storage period, the colour changes from a golden yellow to a dark brown, which is unacceptable. The concentrate has to be reworked. This involves the dilution of the concentrate, decolourisation of the juice, and re-concentration. Needless to say, it is a costly operation.

The possibility exists for the manufacturing process to be altered slightly to possibly eliminate (or scale down) the decolourisation process, or at least

reduce solid wastes generated and the raw materials required during this process.

1.4 OBJECTIVES OF STUDY

The objectives of this study can be summarised as follows:

1. *Background and literature study*. This includes assessing the conventional GJC manufacturing process searching for references focused on decolourisation products, existing decolourisation procedures, the chemistry of discolouring (browning) reactions, adsorption products, etc.
2. *Development of a method of analysis to effectively qualify and quantify 5-hydroxymethylfurfural (HMF)*. It involves the qualification and quantification of HMF, which is a product of the Maillard reaction and/or non-enzymatic oxidation. HMF is indicative of the browning potential of GJC. HMF is a precursor to browning, since it only needs to react with an amine to produce a melanoidin (Dutson & Orcutt, 1984). Melanoidins are visible as a brown colour in juice and juice concentrate.
3. *Investigating the effect of the conventional process on the concentration of HMF*. This involves the sampling of the juice after each step of the conventional concentration process, qualifying and quantifying HMF, and drawing conclusions on the results obtained. The aim is to determine which pathway of browning is the most predominant.
4. *Investigating the effect of three adsorption products on the concentration of HMF*. The aim of this objective is to compare the effects of a few products, such as polyvinylpolypyrrolidone (PVPP), a steam activated

carbon, and a chemically activated carbon, on the concentration of HMF under various process conditions. This objective will include three different experimental procedures, specifically chosen to determine the effect of temperature, time, dosage volume, and protein stability on the HMF removal efficiency of the three adsorption products. One experimental procedure will be aimed at determining the effect temperature has on the constitution of both a protein stable and unstable juice.

5. *Suggesting a possible change in the conventional process to minimise treatment, minimise waste production, and ensure a longer storage life of the product.* This objective is thus focused towards the optimisation of the existing process.
6. *Comparing the running costs of the conventional process and the suggested process.* This objective will indicate the viability in the change in process and will take into account cost and dosage volumes of the adsorption products.

2 BACKGROUND AND LITERATURE STUDY

As mentioned previously this study is focused on one specific grape juice concentration plant using one specific process to concentrate grape juice. This process will be termed: "The conventional process."

2.1 BACKGROUND ON THE CONVENTIONAL PROCESS

The conventional process consists of the following main stages. (A simplified process flow diagram is given in Figure 2-1)

2.1.1 Stage 1 - Harvesting/crushing

The grapes are harvested on the wine farms and taken to the cellar where the crushing is performed. The quality of the grapes is usually determined by the sugar content thereof. By determining the sugar content, in degrees Brix (^{o}Brix), the winemaker is able to determine whether the grapes are fit for making wine and/or juice concentrate. The minimum sugar content of the juice allowed by the concentrate manufacturer is approximately 16.5 ^{o}Brix. The temperature at which the grapes should be delivered to the cellar is 12 to 20^{o}C. The grapes are crushed and the juice is either stored at the cellar or delivered directly to the concentrate manufacturer. SO_2 is added to the juice during storage. When the juice is stored at the cellar for longer than a weekend (3 days), approximately 1200 to 1400 mg/L SO_2 is added to the juice. This juice will be called: SO_2-juice. After a few weeks/months this stock will then be delivered to the juice concentration plant, depending on the need of the clients. If the juice is taken to the concentration plant on the day of harvest, the allowed SO_2-content is between 150 and 200 mg/L. If the harvest came in on a Friday and has to remain at the cellar of harvest during the weekend, the allowed content of SO_2 is 400 mg/L. This juice will be called: fresh juice.

SO_2-juice and fresh juice are handled differently in the following stage. SO_2-juice simply continues from Stage 2 onwards. Fresh juice is incorporated into the manufacturing process by stages 2a and 2b.

2.1.2 Stage 2a - Direct concentration and storage

Fresh juice is directly concentrated to 70+^{o}Balling without undergoing any protein or tartrate stabilisation, etc. From here it is pumped to a storage facility where it is stored for up to nine months at 10^{o}C.

2.1.3 Stage 2b - Dilution

Depending on the requirements of the client, the stored concentrate is then diluted to e.g. 20^{o}Balling by adding water. From here on forward the process for both fresh and SO_2-juice is the same (stage 2).

2.1.4 Stage 2 - Desulphurisation process

The diluted fresh juice/SO_2-juice is preheated by the desulphited product stream of the stripper column to a temperature of 86^{o}C. It is then pumped to the stripper column and fed to the top plate of the column. Gas from the outlet at the top of the desulphurisation (stripping) column enters at the bottom of lime tanks (simplified) where suplhur dioxide is removed from the air. The SO_2 is removed from the juice by vapour rising to the top of the column while in contact with the falling juice. The bottoms, or residue is pumped to a reboiler where steam heats it to a temperature of 90^{o}C. The generated vapour is fed to the bottom tray of the column. The product stream (desulphited juice) is collected from the reboiler. The vapour from the stripping column is fed to a condenser, which is cooled by cooling water. The SO_2 is drawn of to lime tanks while the condensate is returned to the column to prevent juice concentration during this stage of the process. The lime tanks are kept under vacuum. The vacuum draws the SO_2 from the distillate and bubbles it through the lime solutions in the lime

tanks. A pH-meter constantly measures pH in the lime tanks giving an indication of when the lime is spent.

The product stream then preheats the feed stream and is cooled down to a temperature of 55^{o}C. From here it goes to the first concentration.

2.1.5 Stage 3 - 1st Concentration

Concentration is established by a multiple effect evaporator. This process uses a double-effect evaporator. The first effect is operated at a vacuum of -40 kPa(g) and the second effect at -85 kPa(g). The steam flows at 9 ton/hr at a temperature of 105^{o}C, while the juice exits at 18 000 L/hr. The concentration increases from 17 to 35 ^{o}Balling and the exit temperature is 55^{o}C.

2.1.6 Stage 4 - Protein stabilisation (and decolourisation)

Protein stabilisation takes place over a period of seven hours, at a temperature of approximately 55^{o}C. It is done in four tanks, two 600 hL and two 400 hL tanks. It involves the addition of calcium-bentonite and activated carbon. The bentonite is added in doses of 140 g/hL, but in the form of a 10% (mass/volume) mixture in water. The activated carbon is added to the juice in doses of 400 g/hL. A natural acid is added to the juice when the acidity is too low, while potassium carbonate is added when the acidity is too high. The solid wastes are discarded after filtration.

2.1.7 Stage 5 - Filtration

The filter used on this plant is a stainless steel sheet filter, with a mesh size of 9 micron, currently handling a juice flow of 20000 L/hr. The filtering medium is diatomaceous earth and one kilogram can filter 1250 litres of juice.

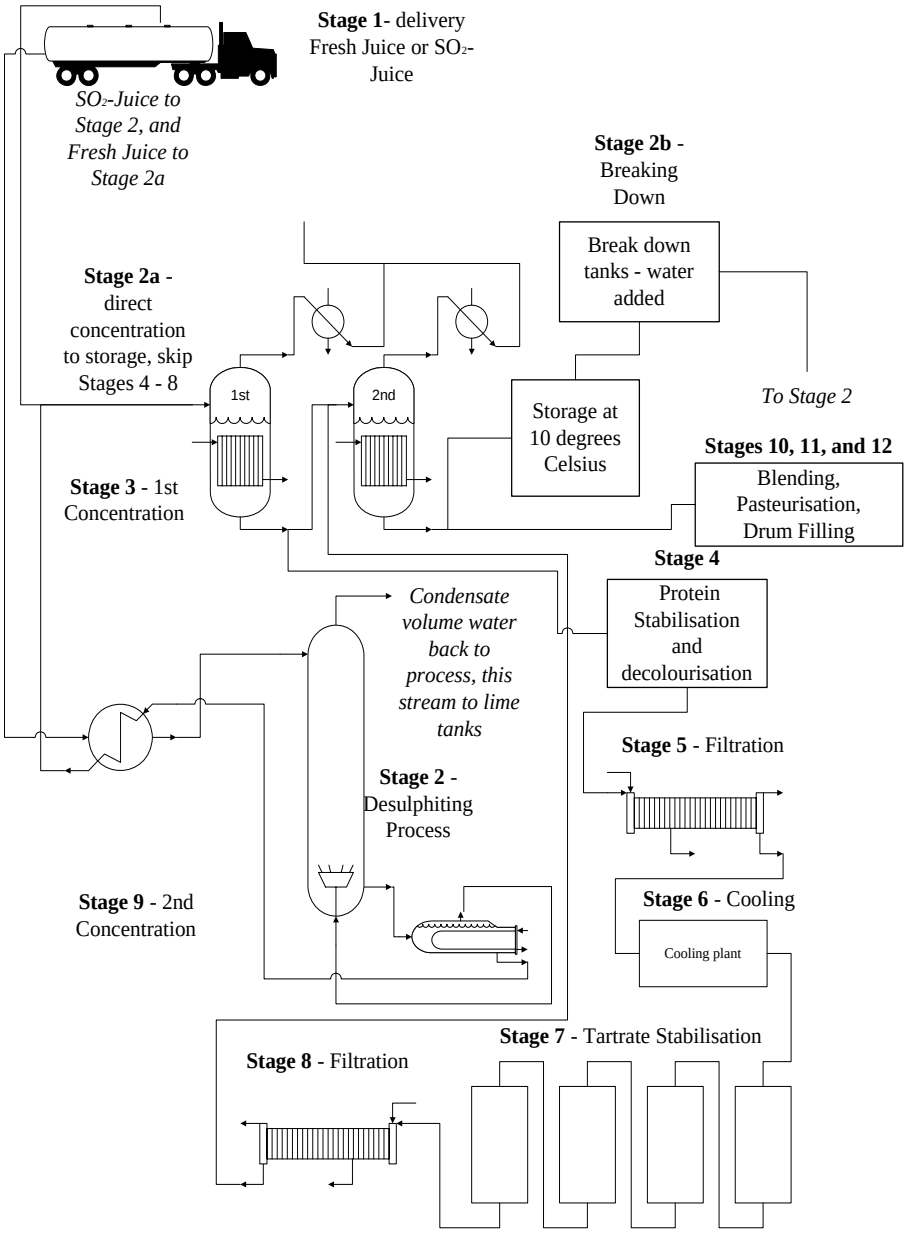


FIGURE 2-1 SIMPLIFIED PROCESS FLOW DIAGRAM OF THE CONVENTIONAL PROCESS

2.1.8 Stage 6 - Cooling

Cooling on this plant is performed by cooling water and plate exchangers. The cooling water is cooled in two stages in the cooling plant. The first stage is pre-cooling where the water is cooled to as low as 0^oC, and the second stage is crash-cooling where the water is taken to -4^oC. This is done at a water flow rate of 18000 L/hr.

2.1.9 Stage 7 - Tartrate Stabilisation

The juice is sent to the cold stabilisation tanks where it is cooled to a temperature of -4^{o}C. Natural tartrates are recovered from cooling the juice. The juice is then dosed with a 55% (solids) tartrate solution at 30 g/L juice. The process is continuous, flowing through six tanks, each with a volume of 12000 L, at a flow rate of 16000 L/hr. Filtration then follows.

2.1.10 Stage 8 - Filtration

The same filter is used as in stage 5. The pH barely changes during tartrate stabilisation from 3.4 to 3.5, and should in fact remain constant since tartrates are merely naturally occurring salts.

2.1.11 Stage 9 - 2nd Concentration

The second concentration is basically the same as the first concentration with the main differences that the steam temperature is now 85^{o}C, the exit flow rate of the juice is 9000 L/hr, and its exit temperature is 10^{o}C.

2.1.12 Stage 10 - Blending

The juice concentrate is blended to the requirements of the clients. Some clients prefer lower sugar content, so the concentrate will be diluted to the ^{o}Brix specified by the client. This is done in blending tanks (of which there are three), each with a volume of 15000 L.

2.1.13 Stage 11 - Pasteurisation

The juice concentrate is standardised at the required ^{o}Brix as mentioned above. After standardisation the concentrate gets pasteurised. The concentrate is heated to a temperature of 86^{o}C and kept there for a few seconds. It is then cooled down again to 5^{o}C. The pasteurisation plant is able to provide 85% of the necessary heat by means of counter-current (regenerative) heating. This then implies that only 15% of the heat is to be

supplied by the steam kettles. The pasteurisation plant consists of three units, namely:

- The generative heating section;
- The hot water heating section:
- The glycol cooling section.

The regenerative heating section is maintained by a counter current flow of concentrate from the hot water section with concentrate fed to the pasteurisation plant. The hot water section is heated by the hot water system of the plant, which makes use of steam to heat the water. The glycol cooling section cools the concentrate by counter current flow of the cooled concentrate from the regenerative heating section with cold glycol.

2.1.14 Stage 12 - Drum filling/storage

The product is then put into drums at a volumetric flow rate of 3750 L/hr. These drums are then stored at ambient temperature after filling until delivery or collection. Citric acid is usually added to increase the acid content to about 2 g/L to prevent sugar crystallisation (if required).

2.2 LITERATURE STUDY

2.2.1 The browning problem in juice and methods of dealing with it

Two major methods of dealing with the browning problem in juices, juice concentrates, and wines are found in literature. They are:

- The prevention of the browning reactions by treating the clear juice in some way as to prevent browning;
- Curing of the juice by removing the brown colour formed during storage.

2.2.1.1 Prevention

The following summarised references contain some information on preventing browning in wine and/or juice.

Cantarelli et al. (1971) investigated the addition of formaldehyde for the prevention of browning of white wines. The addition of formaldehyde or hexamethylenetetramine (HMTA) to grape musts was marked by reduced phenolics in wines. The formaldehyde selectively precipitated the non-tannin flavonoid fraction and effectively slowed oxidative browning ("maderization") in white wines. Similar detanninising effects were obtained with the insoluble PVP (polyvinylpirrolidine) also tested during this investigation. However, in their case the tannic flavonoid fraction is selectively eliminated, resulting in a decolourisation of the wines and a lower stability to maderization. The PVP was added after fermentation. Other detanninising agents that was also tested and compared with formaldehyde were: gelatin and casein, added to the must before fermentation. The concentration of formaldehyde residues appears to be inversely related to the flavonoid contents of wines. The excess of formaldehyde added to the must reacts with the sulphur dioxide, resulting in high residues of SO_2 and formaldehyde in the wines. Although this method of adding formaldehyde to the musts seemed effective, the residual formaldehyde is still a problem. Due to the interaction of the sulphur dioxide, there remain some difficulties in determining the amount of formaldehyde or HMTA, which has to be added to the must. Furthermore, to determine the exact dosage of formaldehyde or HMTA, one has to have a very good indication of the amount of non-tannin flavonoids present in each batch of must. A certain amount of formaldehyde reacts only with a certain amount of the non-tannin flavonoids. The rest is residual and has to be removed in some way. Formaldehyde is a carcinogen and residual formaldehyde remains a problem.

Kelly and Finkle (1969) investigated the action of a ring-cleaving enzyme in preventing oxidative darkening of juices. Catechol-type compounds such as chlorogenic acid are the natural substrates for polyphenol oxidase, a natural occurring enzyme in fruit juices. The dark pigments in juices are produced when catechol type compounds, i.e. dihydroxyaromatic compounds, present in fruit or juice, are oxidized in air through the action of catechol oxidase (or polyphenol oxidase) to polymeric quinones. The first step in this reaction involves the formation of an o-quinone. The quinone is then polymerised to dark pigments during further oxidation. Modifying the phenolic substrates in an irreversible manner so that they can no longer participate in the darkening reaction, can prevent the above reaction. The following three methods were used to alter the substrates: (1) O-methylation of catechol compounds; (2) an alkaline buffer treatment of cut fruit; and (3) oxidative ring opening of catechol compounds. Since this investigation was done on apples and apple juice, we will only discuss methods (1) and (3). Method (1) involves an enzymatic O-methylation of the catechols by treatment with the enzyme O-methyltransferase and a methyl donor such as S-adenosylmethionine. Since the O-methylation reaction is irreversible and the methylated products cannot be oxidized by catechol oxidase, oxidative darkening is then eliminated. Method (3) involves the oxidative ring opening of the catechol compounds. The enzyme used to accomplish this in apple juice was the ring-cleaving oxygenase, protocatechuate 3,4-dioxygenase (PC-ase). This enzyme converts protocatechuic acid to an open chain acid, beta-carboxymuconic acid. In a similar manner, it attacks other catechol acids, cleaving the aromatic ring and thus, preventing browning. These methods will not be given further attention in this study.

Peterson and Caputi (1967) studied the effects of Na+ exchange, H+ exchange, and H+ exchange followed by OH- exchange on browning of four dry white wines and compared them in the presence- and absence of

oxygen. Wines browned more when oxygenated than when stripped with N_2, and ion-exchanged wines browned less than the untreated wines. H^+ exchange was more effective than Na^+ exchange in inhibiting the browning of all wines tested. H^+ exchange followed by OH^- exchange practically eliminated browning, but wines treated in this manner were water-white and contained little wine character. Evidence is also presented in this report that two distinct types of browning may occur simultaneously in wine, oxidative and non-oxidative. Oxidative involves the polymerisation of phenols and the oxidation of these polyphenols, whereas, non-oxidative possibly involves nitrogenous compounds such as amino acids condensing with carbonyl compounds. Caputi et al. (1969) further investigated residual PVP in wine and possible hazardous effects thereof.

Investigations done by Flores et al. (1988) on three vintages indicated that White Riesling juice processed without SO_2 and clarified by ultra-filtration (UF) tended to develop sediments on storage. This study analysed the possible effect of oxidation (processing with and without SO_2) and pre-UF treatment of juices with enzymes and fining agents on juice flux, colour (browning), composition, and stability. White Riesling juice was ultra-filtered with a Romicon Lab-5 pilot-scale hollow-fibre unit, operated in a batch mode, with membrane of nominal molecular weight cut-off (MWCO) of 10000 Daltons. Grapes were processed with and without SO_2, and the effects of treatment of settled press juice with Rohapect VR Super (VRS, mainly pectinase and protease) and of fining with bentonite, gelatine, and silica sol before UF were investigated. Juice parameters evaluated included total protein, pectin, phenol, colour ($A_{420\ nm}$), and stability to heat/cold testing. Pre-UF treatment with enzymes and fining, increased flux. Sediments were found to contain large amounts of proteins and phenolics and trace amounts of pectin and neutral polysaccharides. Sediment formation and instability to heat testing of UF permeates processed without SO_2 were prevented with pre-fining. Up to 99% of

protein, 90% of pectin, 84% of colour, and low variable phenolics were retained by the 10000-dalton-MWCO membrane. However, pre-UF treatment can increase protein and pectin in permeates. During UF, there is a significant increase in the soluble protein and water-soluble pectin passing through the membrane with increasing volume concentration ratio (VCR, process time). It is concluded that it is not only the quantity, but also the nature/state of compounds such as proteins, phenolics, and pectins and their interactions that results in instability. At present research is done on refining ultra-filtration techniques to such an extent that it becomes commercially acceptable (Borneman et al., 2001).

In a study done by Panagiotakopoulou and Morris (1991) juices from two different white grape cultivars, Aurore and Cayuga, received 13 different wine treatments to determine the effects on browning in wine. The additives used separately and in combination, were: ascorbic acid, hypo-phosphorous acid, thiodipropionic acid, Trolox-C, stannous chloride, Sporix, and SO_2. All chemicals, accept SO_2, added to wine were made from nitrogen-sparged juice. One batch of the wine received no treatment. All treatments were stored at 20^oC and 37^oC for nine months and exposed to air after three and six months storage to accelerate browning. The use of SO_2 during bottling resulted in the least browning, and the use of SO_2 during crushing resulted in the most browning. Wine treated with the other chemicals had less browning than the wine produced from the nitrogen-sparged juice but had more browning than the untreated wine. The addition of ascorbic acid combinations reduced browning to a greater extent than any of the other chemicals or combinations.

Lee et al. (1990), Oszmianski & Lee (1990), Lee et al. (1985), McLellan et al. (1995), Lee & Whitaker (1994), and Kime & Lee (1987) investigated various aspects of the inhibitory effect of honey on polyphenol oxidase. They specifically gave attention to the possible reduction of SO_2 in juice

and wine by using honey. From their results it appears that honey proved to be an efficient inhibitor of oxidative browning.

2.2.1.2 Cure

This section contains the summary of a few references containing information on the treatment of brown juice and/or wine.

Brú et al. (1995), treated different samples of dry sherry wine with several adsorbent compounds at different dosages. The evolution of the wine over a period of two years was then recorded. The following adsorbents were used: four types of activated carbon, two types of casein (Potassium Caseinate), two types of PVPP (Polyclar VT and Divergan W), and three adsorbent resins (XAD-761 Duolite, S-861 Duolite, and Micron 96). In the same way two control samples were prepared; one treated with Sodium Bentonite and the other without. The adsorbents were added to the wine in three dosages; 100, 500, and 2500 mg/L. It was concluded that, of all the adsorbents tested, the soluble casein and caseinates gave the best results in terms of browning removal. This was the case for low, medium, and high dosages of the compounds.

John Daumé (2001) gives some information in his website on a few fining agents used before bottling. Casein: Potassium Caseinate can improve both flavour and colour in slightly oxidized wines. Polyclar/PVPP: Microscopic, insoluble nylon that binds with some phenolic compounds can remove colour precursors and prevent enzymatic browning/pinking in whites. It may also clean up an imperfect wine's odour/taste. Can remove anthocyanin colour, as in too-red blush wine. Eisenman (1991) elaborates in his paper on these fining materials.

Escolar et al. (1995) studied the decolourising effects of different quantities of activated carbon adsorbent on the colour of sherry samples (Fino white). Wine samples were treated with accurately weighed amounts of activated

carbon and mechanically shaken in a thermostatic bath at 25^0C for one hour to reach equilibrium, then centrifuged at 5000 rpm for five minutes and filtered with a 0.25-micrometer membrane filter. Data gathered showed that during the decolourisation process all the chromatic parameters varied, agreeing with the progressive disappearance of the colour yellow due to the chemical adsorption of the colouring compounds of the Fino wine, since the graphitised structure of the adsorbent shows affinity for polyphenolic compounds responsible for the colour. The colour change was measured through the use of chromatic parameters determined by the CIELAB 76 system, using transmittances taken at 1-nm intervals throughout the visible spectrum. The effect of the adsorbent on the colour of the wine has been expressed by equations that relate the values of the chromatic parameters with the quality of the added adsorbent. These equations show that the decolourisation process follows a Freundlich-type isotherm. He concluded, that, by using these equations, it is possible to predict the amount of carbon adsorbent necessary to attain a desired colour.

Pilone (1977) investigated methods determining the tartaric acid in wine and he does not specifically give attention to the decolourisation of wine. He does, however, use two methods of decolourisation before the tartaric acid concentration is determined. An activated carbon (Darco KB Activated Carbon, Atlas Chemical Industries Inc.) and a resin (20-50-mesh Bio-Beads-SM-2, Bio-Rad Laboratories) were used.

The POLYCLAR web page (www.iscorp.com, 2001) advertises two registered PVPP products to use in the wine industry, namely, Polyclar V, and Polyclar VT. These are used for stabilising wine to either prevent or remedy oxidation problems, caused either during the winemaking process, or due to high levels of grape rot and mould. In addition, Polyclar stabilisers are used to improve wine affected by over ageing or poor

storage conditions. Polyclar stabilisers are insoluble in wine and are completely removed in the lees by either filtration or rapid settling techniques. They are compatible with all fining agents and can also allow SO_2 levels to be reduced.

Mennet and Nakayama (1969) tried casein, hide powder, nylon-66 powder, and Polyclar-AT (an insoluble polyvinylpirrolidine) as adsorbents. Adsorption isotherms in model systems and wine are evaluated for their adherence to the Freundlich isotherm. The effect of acidity on adsorption is investigated. The adsorption of the hydroxybenzoic acids by Polyclar-AT does not appear to be selective as determined by similar equilibrium constants for all the acids tested. The non-selectivity is due to the requirement for adsorption, which is the presence of one hydrogen-bonding donor. The acidity of a solute exerts a negative effect on its adsorption, although the effect is not strong enough to cause selective adsorption.

2.2.2 The chemistry of browning reactions

In this study it is necessary to have some understanding of how browning reactions occur, what their preferred environments are, as well as how these reactions could be impeded.

Researchers Kramling & Singleton (1965), Dutson & Orcutt (1984), Mayen et al. (1997) and Garza et al. (1999) collectively mention four basic pathways by which grape juice concentrate (GJC) can possibly undergo browning. They are: enzymatic oxidative browning, non-enzymatic oxidative browning, non-enzymatic browning (the Maillard reaction), and caramelisation.

We will group enzymatic- and non-enzymatic oxidative browning, since they are both oxidative, and explain each pathway in the next few paragraphs.

2.2.2.1 Oxidative browning

Oxidative browning is divided into two pathways, namely, enzymatic, and non-enzymatic. The main difference between these two pathways is the catalyst. In enzymatic oxidative browning the catalyst would be an enzyme, whereas the catalyst for non-enzymatic oxidative browning would be an acidic medium.

Enzymes are large proteins that act as catalysts for biological reactions. An enzyme is very specific in its action and will often catalyse only one specific reaction. Enzymes do not affect the equilibrium constant of a reaction and cannot bring about chemical changes that are otherwise unfavourable. Enzymes act only to lower the activation energy of a reaction, thereby making the reaction take place more rapidly (McMurry, 1996).

Some of the phenolics involved with oxidative browning reactions can be seen in Figure 2-2.

2.2.2.1.1 Enzymatic oxidative browning

This pathway involves enzymatic catalysts such as Polyphenol Oxidase (Phenolase, PPO), or Peroxidase to assist in the formation of o-quinones from phenolics (or antioxidants) in the juice, which polymerises to form brown-coloured polymers. Figure 2-3 indicates the pathway to brown polymers with PPO as catalyst.

Enzymes, of which the activity is not limited, can cause a serious reduction in the quality of the juice. Phenolase is relatively unstable to heat and can be inactivated by a mild heat treatment, whereas Peroxidase is much more heat resistant and needs higher temperatures for its destruction (Mayen et al., 1997).

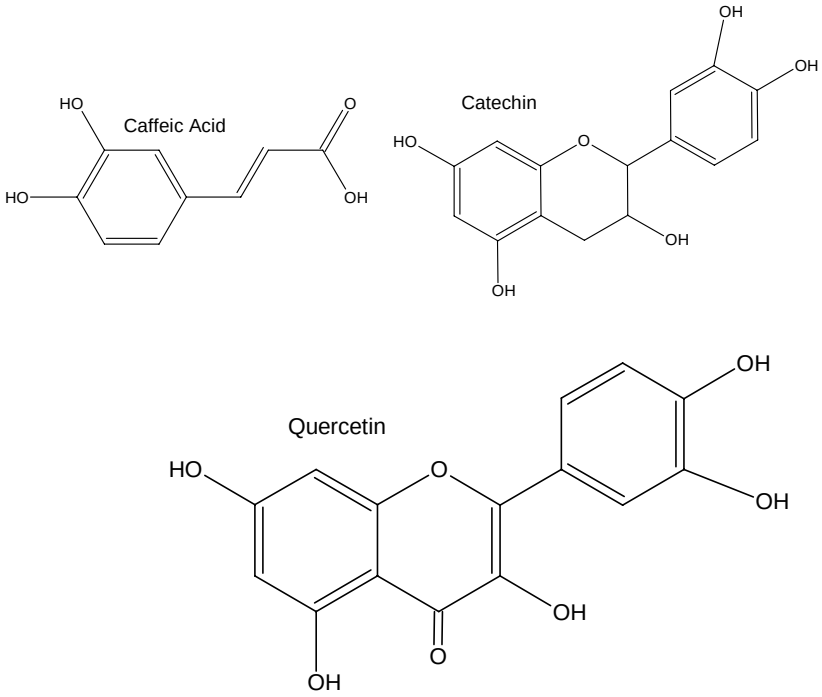


FIGURE 2-2 A FEW ANTIOXIDANTS/PHENOLICS PARTICIPATING IN OXIDATIVE BROWNING REACTIONS

During the manufacturing process of the concentrate, the juice is subjected to mild heat in the concentration stages. It is subjected to somewhat higher heat during pasteurisation just before shipping. Not all enzymes involved in the browning reactions are inactivated by these "heat treatments". Their activity however, is significantly inhibited.

2.2.2.1.2 Non-enzymatic oxidative browning

Non-enzymatic oxidative browning, otherwise known as auto-oxidative browning, is catalysed by an acidic medium (See Figure 2-4).

As can be seen when Figure 2-3 and Figure 2-4 are compared, the products are quite similar, and both of them can be seen as a brown colour in the juice.

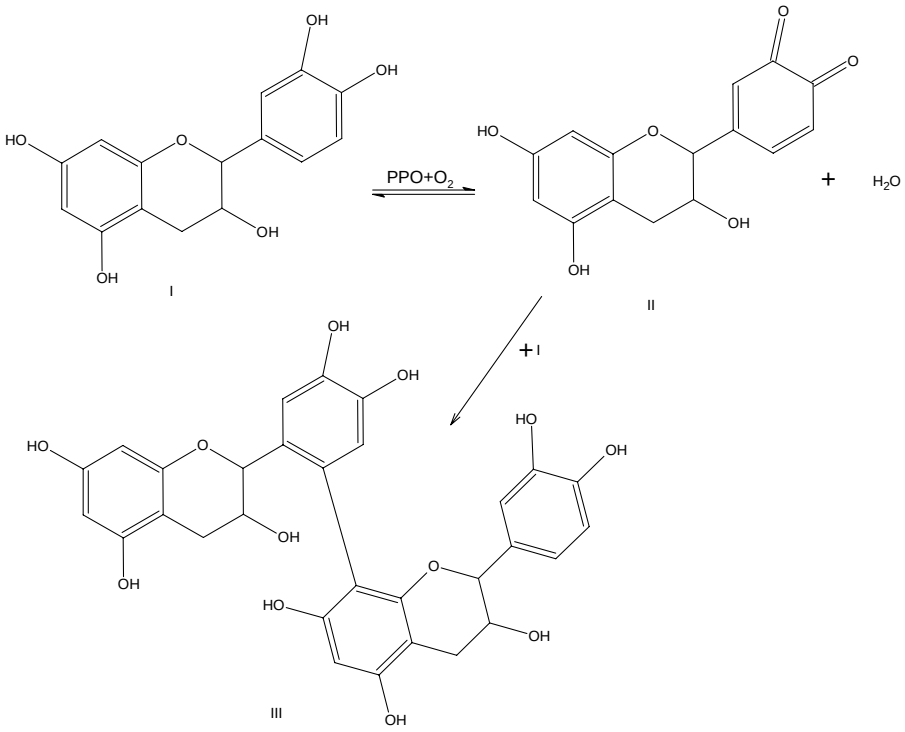


FIGURE 2-3 ENZYMATIC BROWNING OF CATECHIN, THE FORMATION OF A BROWN POLYMER

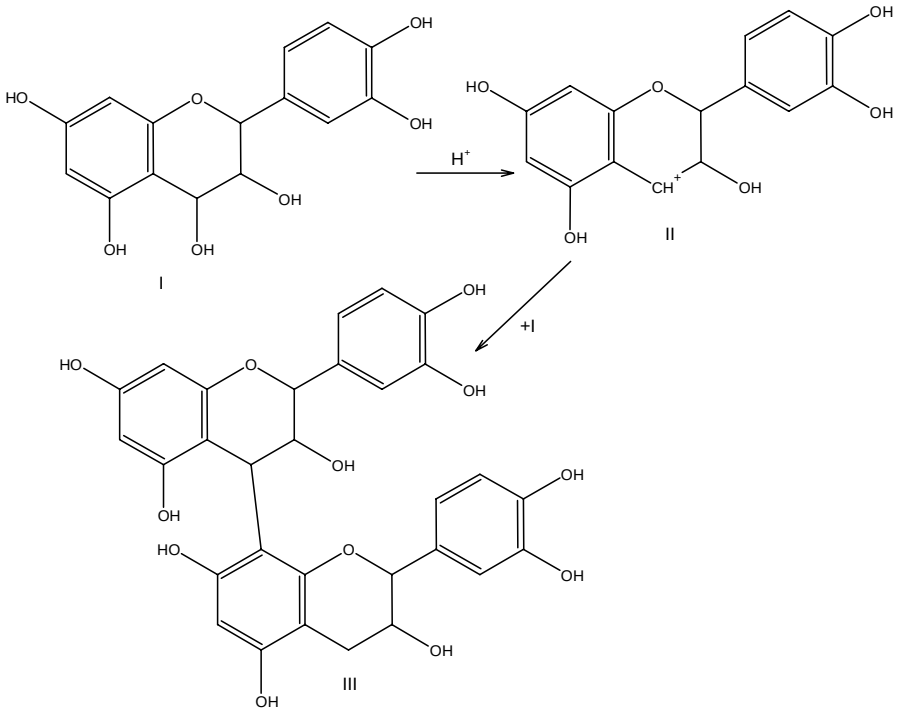


FIGURE 2-4 NON-ENZYMATIC OXIDATIVE BROWNING OF A LEUCOCYANIDIN

2.2.2.2 Non-enzymatic browning (The Maillard reaction)

The chemistry of the Maillard reaction is not fully understood in the context of this study. Although many studies have been conducted on the flavour formation of this sundry reaction, not much knowledge has been gathered concerning colour formation. The following paragraphs is a summary of the results from research work done by the following researchers: Resnik & Cherife (1979), Yeo & Shibamoto (1991), Arena et al. (2000), Petriella et al. (1985), Boston & Boyacioglu (1997), Beudo et al. (2001), Bozkurt et al. (1999), Litchfield et al. (1999) and Nagaraj & Monnier (1995).

The Maillard reaction consists of three stages (early stage, advanced stage and final stage) and depends upon factors such as pH, time, temperature, concentration of reactants and reaction type.

2.2.2.2.1 Early Stage

This stage involves the condensation of an amino acid with a reducing sugar to form Amadori or Heyns rearrangement products via an N-substituted glucosylamine, otherwise known as a Schiff base (Figure 2-5).

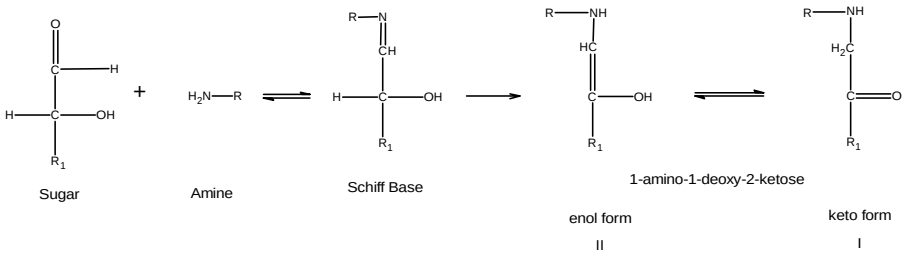


FIGURE 2-5 FORMATION OF SCHIFF BASE AND AMADORI COMPOUNDS

2.2.2.2.2 Advanced Stage

The degradation of the Amadori and Heyns rearrangement products can occur via four or five possible routes. We will only focus our attention on one of them, namely the pathway to the formation of 5-hydroxymethylfurfural (HMF), since HMF is a known precursor in

browning reactions. Figure 2-6 indicates the pathway to the formation of HMF.

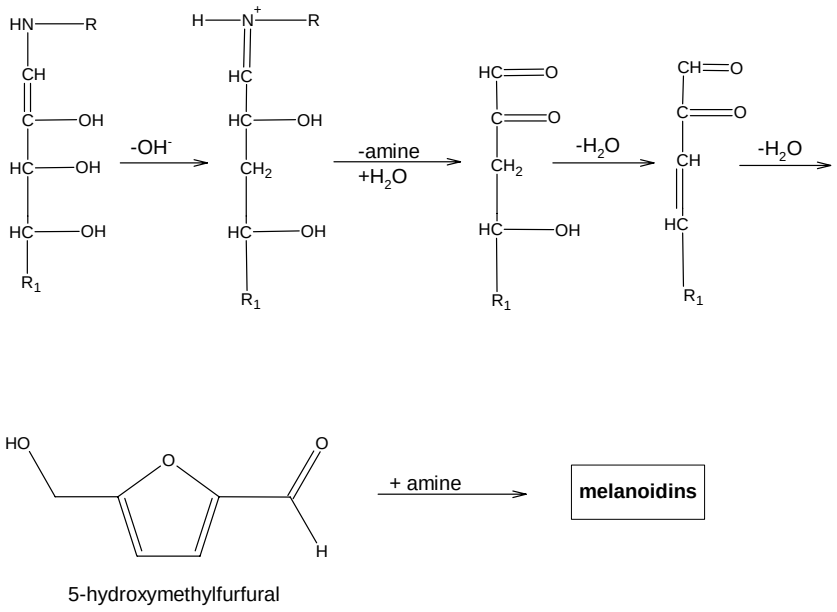


FIGURE 2-6 THE MAILLARD REACTION PATHWAY TO HMF AND MELANOIDINS

2.2.2.2.3 Final Stage

The final stage of the Maillard reaction is characterised by the formation of brown nitrogenous polymers and co-polymers. As mentioned earlier very little is known about the chemical nature of these products. What is known, however, is that these colour compounds can be grouped into two general classes - low molecular weight colour compounds, which comprises of two to four linked rings, and the melanoidins, which have much higher molecular weights. Colour development increases with increasing temperature, time of heating, and decreasing pH.

2.2.2.3 Caramelisation

Only a slight overview of caramelisation is given here since this pathway to browning has no significant contribution in the process in this study.

Caramelisation is also a non-enzymatic browning reaction, but one that involves the heat-induced decomposition of sugars, normally

monosacharides. They undergo initial enolisation, known as the Lobry de Bruyn-A. van Eckenstein rearrangement, and progress to subsequent complex reactions, such as dehydration, dicarboxylic cleaving and aldol condensation. The reaction generally releases H^+, thus the pH of the solution decreases with time (Lee & Lee, 1997)

2.2.2.4 Pathways to the formation of HMF

In the juice and juice concentrate studied, the concentration of 5-hydroxymethylfurfural (HMF) is of great importance. Gomis et al. (1991) has shown that HMF can be used as a determinative factor for the browning potential of juice concentrate. HMF is, of course, a precursor to browning as can be seen in Figure 2-6 and it is important to gain knowledge on how this compound is formed. From literature (Graza et al., 1999, and Feather, 1982) two pathways are observed.

The first is as shown in Figure 2-6, where the formation of HMF is amine assisted (the Maillard reaction). The second pathway is an acid catalysed reaction (non-enzymatic oxidative browning). According to Feather (1982) there is a great deal of similarity between the dehydration of a sugar in acidic solution and in the presence of amines. This similarity can clearly be seen when the reactions in Figure 2-6 and Figure 2-7 are compared. The formation of a 2-Furaldehyde (such as HMF) via acid catalysis is shown in Figure 2-7. Further reaction with an amine will yield a melanoidin, which is visible as a brown colour in high concentrations.

According to Feather (1982) the reducing sugars decompose at much milder conditions in the presence of amino groups (amines) than for the acid catalysed reaction.

2.2.2.5 Preferred environments of browning pathways

The preferred environment of the various pathways of browning reactions is summarised in Table 2-1.

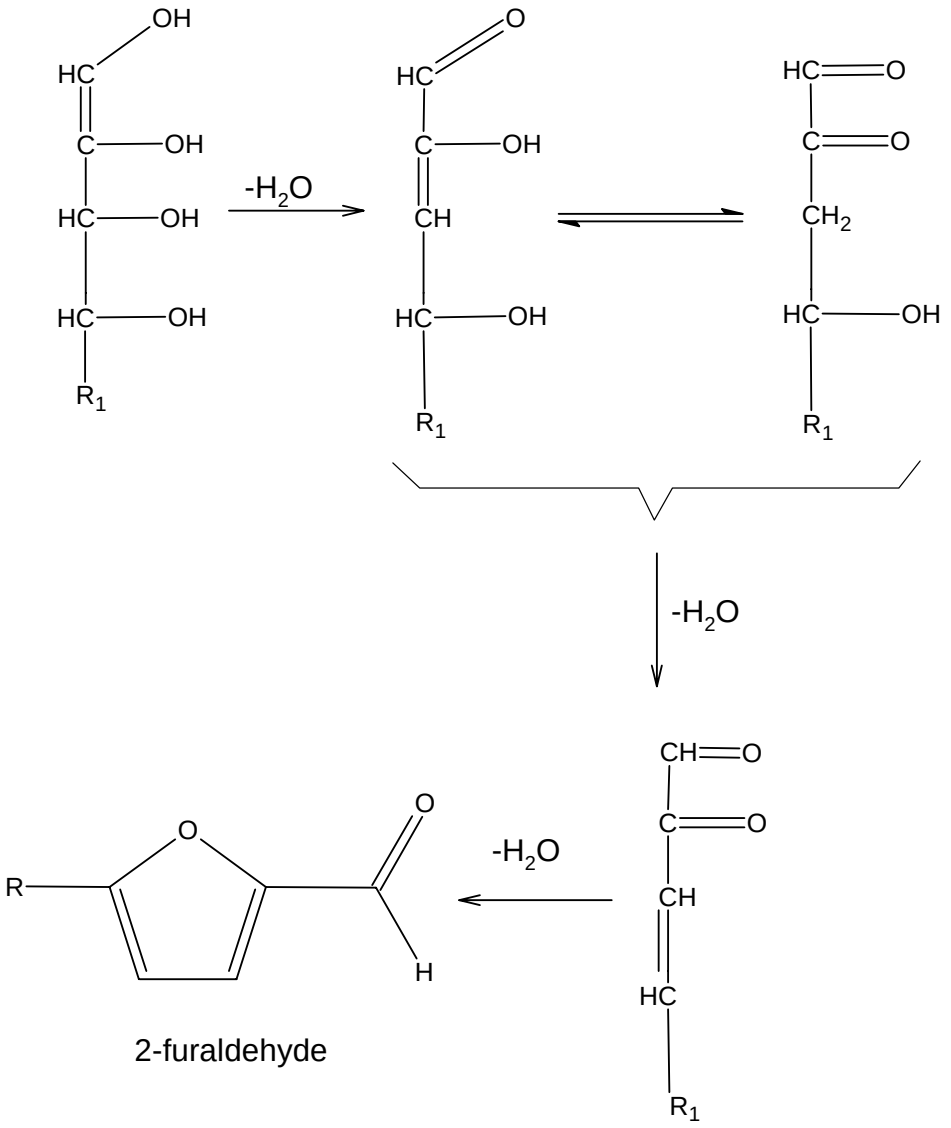


FIGURE 2-7 ACID CATALYSED SUGAR DEHYDRATION (THE FORMATION OF HMF)

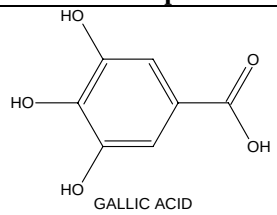
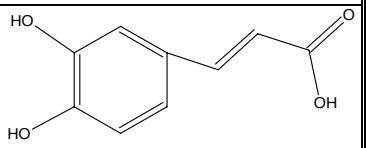
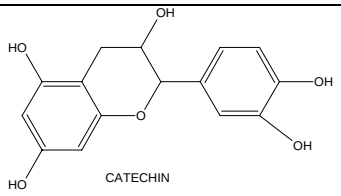
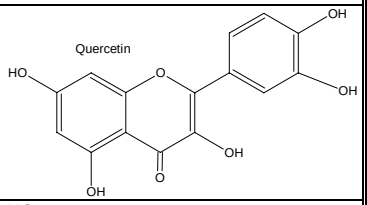
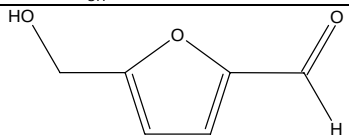
TABLE 2-1 A SUMMARY OF THE PREFERRED ENVIRONMENTS OF THE BROWNING REACTIONS

Browning Reaction	Preferred Environments
Enzymatic Oxidative browning	Mild temperatures, mild acidic environment
Non-Enzymatic Oxidative Browning	Acidic environment, higher temperatures
The Maillard Reaction	Acidic environment, high temperature
Caramelisation	Acidic environment, very high temperatures

2.2.2.6 Grouping of some of the phenolics

Table 2-2 contains some of the major groups of grape juice phenolics and examples of them are given.

TABLE 2-2 EXAMPLES OF GRAPE JUICE PHENOLICS

Major groups	Members	Example
Hydroxybenzoic Acids	Gallic Acid Protocatechuic Acid Vanillic Acid	 GALLIC ACID
Hydroxycinnamic Acids	Caffeic Acid Chlorogenic Acid Ferulic Acid *p*-Coumaric Acid	 Caffeic Acid
Flavan-3-ols	Catechin Epicatechin	 CATECHIN
Others	Quercetin Resveratrol	
Furfurals	HMF 2-Furaldehyde	 5-Hydroxymethylfurfural

The examples given in Table 2-2 were chosen as initial representatives of the phenolics and furfural involved in this study. Added to these was Resveratrol.

2.2.3 Applications and limitations of adsorption products

This section contains information on the applications and limitations of some adsorption products mentioned in the previous sections. Other topics

mentioned are: operating parameters, regeneration/reactivation/ disposal determination, and operating and maintenance costs. Also some technical guidelines will be given on how to decide when alternative adsorption media may be selected in lieu of activated carbon.

2.2.3.1 Carbon adsorption

2.2.3.1.1 Applications

Some typical rules of thumb for types of compounds that are amenable to carbon adsorption are as follows:

- Larger molecules adsorb better than smaller molecules;
- Non-polar molecules adsorb better than polar molecules;
- Non-soluble or slightly soluble molecules adsorb better than highly soluble molecules;
- Based on the polarity or solubility (or both) of the molecule being adsorbed, pH may have an influence on the extent of adsorption;
- Temperature increases the rate of diffusion through the liquid to the adsorption sites, but since the adsorption process is exothermic, increases in temperature may reduce the degree of adsorption. This temperature effect is negligible in water treatment applications and ambient vapour phase applications.

2.2.3.1.2 Chemicals Adsorbed

The following are examples of chemicals adsorbed:

- Alcohols are poorly adsorbed since they are very soluble and highly polar;
- Aldehydes are highly polar, and as molecular weight increases, the polarity decreases, and adsorbability increases;
- Amines are similar in structure to ammonia (NH_3), except the nitrogen is bonded to an organic group. Adsorption is limited by

polarity and solubility. Typical amines in grape juice would be proteins and amino acids. It has been shown that these compounds decrease the adsorption potential of activated carbon;

- Chlorinated aromatics, and chlorinated aliphatics are low-polarity and low-solubility compounds, which make them generally quite adsorbent;
- Glycols are water-soluble and not very adsorbent. Glucose and fructose can be included in this category. Both are naturally occurring sugars in grape juice;
- Higher molecular weight organic compounds will generally be more adsorbant owing to adsorptive attraction relative to size.

2.2.3.1.3 Operating Parameters

The following parameters should be taken into consideration when designing an adsorption process:

- Contact time - the time the activated carbon will have to be in contact with the liquid to remove the undesired compounds efficiently;
- Adsorbent volume - the volume of activated carbon needed to remove undesired compounds to a specified concentration in the contact time determined;
- Equipment needed - including pumps, mixing facilities, contact tanks, and filtration facilities;
- Regeneration, reactivation and disposal.

2.2.3.1.4 Regeneration, Reactivation, and Disposal

This section is specifically focused on granular activated carbon with some attention given to powder activated carbon (as used on the concentrate manufacturing plant).

As contaminants are adsorbed, the carbon's adsorptive capacity gradually decreases. When the carbon's adsorptive capacity is reached, it is considered "spent," and it must be regenerated, reactivated, or disposed of.

Regeneration usually involves removing the adsorbed contaminants from the carbon using temperatures or processes that drive the contaminants from the carbon but that do not destroy the contaminants or the activated carbon. A common regeneration process introduces steam into the spent carbon bed, volatilising the contaminants and restoring the carbon's capacity to what is called its "working capacity." Steam regeneration does not completely remove adsorbed contaminants. Another common process uses a hot inert gas, such as nitrogen, to remove the contaminants. The stripped volatiles are compressed, and recovered as liquid in a condenser. A third process is pressure swing adsorption. Pressure swing adsorption uses the fact that adsorption capacity is directly proportional to the partial pressure of the contaminants in the surrounding environment. The contaminants are adsorbed at a high pressure (providing higher partial pressure of the contaminant to be adsorbed), and then desorbed at a lower pressure where the capacity is reduced. These regeneration processes are usually run on-site and inside the adsorption vessel. All regeneration processes produce a waste stream that contains the desorbed contaminants. For example, steam regeneration produces a mixture of water and organics from the condensed desorbed vapour. This is not at all practical for use in powdered activated carbon regeneration. Powdered activated carbon is firstly filtered from the liquid media. This is done by means of a diatomaceous earth filtering aid. To separate these two is virtually impossible. Regeneration will not be an attainable task.

Other than thermal reactivation at elevated temperatures, regeneration techniques will result in some contaminants remaining adsorbed and unaltered within the carbon particle. These contaminants will be occupying

"high energy adsorption pores, or sites," and lower temperature regenerants (< 260°C) or capacity corrections will not be able to provide sufficient energy to reverse the adsorptive force. Carbon having these residual contaminants remaining in the high-energy adsorption sites will likely have much shorter runs before breakthrough. Protein and amino acids are known to contaminate activated carbon by occupying these high-energy adsorption pores.

Spent carbon reactivation off-site, involves removing the adsorbed contaminants from the spent activated carbon in a process that is a modification of the one that initially activated the carbon. The contaminants are desorbed and destroyed in the high temperature (typically in excess of 800°C), pyrolising atmosphere of the reactivation furnace. Several types of furnaces are available, such as rotary kilns and multiple hearths. The furnaces can be heated by a fuel such as natural gas or fuel oil or by electricity. Off-site carbon reactivation manufacturers reactivate spent carbon in large capacity (5 to 60 tons/day) furnaces. While furnaces of this capacity are not typically cost effective for a single hazardous waste site, smaller furnaces that may prove cost effective are available for on-site use from a few manufacturers. Reactivation furnaces only produce reactivated carbon, air emissions, and some carbon fines. No organic wastes are produced. Again, with the filtering aid and powdered activated carbon, this option is not extremely viable, unless another, inexpensive method of filtration exists.

2.2.3.1.5 Criteria for Determining When to Use On-site Regeneration, Reactivation or Off-site Reactivation, or Disposal

- On-site reactivation requires space and utility support for the equipment. It also usually requires an air pollution permit for the furnace afterburner. If the site cannot provide the land or utility support, or if obtaining the required permit is not practical, the

spent carbon must rather be regenerated on-site (if possible) or reactivated off-site;

- At some sites, the availability or turn-around times for off-site carbon re-supply may be impractical. In these situations, on-site regeneration or reactivation will be required or the site can provide sufficient storage for both fresh and spent carbon to eliminate the constraint of response time by outside suppliers;
- Studies indicate that on-site thermal reactivation is not economical if carbon usage is less than 500 to 2000 lb/day (227 to 909 kg/day). Other studies have found that carbon reactivation unit cost rises rapidly if carbon usage is less than 5000 to 6000 lb/day (2272 to 2727 kg/day);
- When carbon is regenerated on-site, some contaminants may not be desorbed. For example, GAC containing organic contaminants with high boiling points may need to be reactivated instead of regenerated;
- There are several cases where regeneration or reactivation of the spent carbon will not be feasible or will be prohibitively expensive. In these cases, the spent carbon must be disposed of.

2.2.3.2 Non-Carbon Adsorption

Modified clay, polymeric adsorbents, and zeolite molecular sieves are also currently used as adsorbents in a variety of applications. Some of these adsorption media are used primarily as pre-treatment for activated carbon. For example, these media may be used to remove compounds that may, through physical or chemical interactions, degrade the effectiveness of the activated carbon. As mentioned earlier amines such as proteins and amino acids can significantly degrade the effectiveness of activated carbon during the decolourisation of grape juice, due to fouling.

Some polymeric adsorbents is much more selective than activated carbon, removing only the grape juice phenolics. By adsorbing the single compounds of concern, the working capacity of these polymeric adsorbents will exceed the capacity of activated carbon, which loses its working capacity to competitive adsorption of other compounds.

Primary selection criteria for using these alternative adsorbents and systems include the effectiveness of adsorbing the contaminants of concern and the overall lifecycle cost compared to using activated carbon. For most vapour or liquid applications, both the proper alternative adsorbent and activated carbon will adequately adsorb the contaminants of concern. The alternative adsorbents usually have higher capital costs and lower operations and maintenance (O&M) costs. So, for short-term (2 years or less) projects, such as a one time spill remediation, the alternative adsorbent will typically not be as cost effective as activated carbon systems. For long-term projects, the lower O&M costs of the alternative adsorbent can make activated carbon less cost effective.

2.2.3.2.1 Technical Guidelines for Choosing Adsorption Media

A summary of the technical information needed to evaluate when alternative adsorption media may be selected in lieu of activated carbon is as follows:

- Media description;
- Adsorption system description;
- Availability;
- Estimated purchase and operating cost;
- Advantages and disadvantages for the application;
- Organic chemicals and contaminant ranges that can be adsorbed;
- Adsorption isotherms;

- Regeneration methods;
- Safety data and considerations, including loading, unloading, and handling methods;
- Applications;
- Pressure drop through the media - not applicable to this study;
- Effects of temperature and relative humidity;
- Any proprietary ownership and use limitations.

2.2.3.2.2 Modified clays

It was decided to add this paragraph due to the use of bentonite clay during protein stabilisation of grape juice. This material can be a mixture of anthracite and bentonite clay or bulk clay. The clay in both media has been treated with quaternary amine, which makes the surface of the clay much more active. Reports have shown that these clays remove BOD and COD, amongst other things, from liquid streams. The material can remove up to 60% of its weight in oil and other organic compounds; however, it cannot be regenerated. Disposal options include it being used as fuel if the oil adsorbed has sufficient heating value. Otherwise the type of organic contaminants the clay has adsorbed governs disposal. Typically, the spent material has been incinerated, blended into cement kiln fuel, or treated biologically (e.g., landfarming), or placed in a landfill. Modified clay is usually applied as a pre-treatment unit upstream of activated carbon in order to prevent the activated carbon to be fouled by contaminants.

2.2.3.2.3 Polymeric Adsorbents

When mentioning polymeric adsorbents, attention will be given to specifically polyvinylpolypyrrolidone (PVPP).

The following characteristics relates to what has been discussed in the above sections on design criteria, contact time, regenerative capabilities, etc.:

- Polyvinylpolypyrrolidone (PVPP) is a hygroscopic, amorphous polymer that is insoluble in water, wine, acids, alkali and all organic solvents;
- PVPP is compound specific and has a special affinity for polyphenolic compounds in the juice and wine decolourisation application;
- PVPP has very fast adsorption kinetics, which will allow for a quicker contact time;
- PVPP is less reactive than activated carbon, allowing for fewer engineered safety controls on the system (not really applicable to juice concentrate manufacturing);
- PVPP can be regenerated at low temperatures, allowing nearly all systems to use on-site regeneration. This is achieved by washing it with a water-ethanol solution. Due to the fact that PVPP is also a fine powder, this might again not be a viable attribute, since it has to be filtered from the juice as well.

2.2.4 Background on products chosen for this study

Three adsorption products were chosen for comparison in terms of adsorption efficiency. These products were:

- NORIT CA 1 (a chemically activated carbon);
- NORIT SA 4 (a steam activated carbon);
- POLYCLAR V (PVPP, polyvinylpolypyrrolidone, a synthetic polymer).

The reasons for the choice of these products are as follows:

- NORIT CA1 - The product currently used in the conventional process. This product was chosen to generate a standard to which the other products can be compared to;

- NORIT SA4 – This product has a much greater desorption capability and can more easily be regenerated than CA1;
- PVPP - Due to the physical differences from activated carbon, the specificity to adsorb browning precursors and brown pigments, and to compare the running costs of utilising a different adsorption product to activated carbon.

2.2.4.1 NORIT CA 1

2.2.4.1.1 Description

NORIT CA 1 is a chemically activated carbon manufactured by the phosphoric acid process. It is proven that this carbon combines a high adsorptive capacity with excellent filtration characteristics and can be used in a large range of applications in the food and chemical industry. It is said to be especially effective in adsorbing high molecular organics such as proteins and large colour bodies. It is a powdered activated carbon and CHEMIMPO SA (PTY) LTD donated samples.

2.2.4.1.2 Working methodology

In order to explain the working methodology of activated carbon, one should first give some attention to the manufacturing process. Activated carbon can be manufactured from carbonaceous material, including coal (bituminous, subbituminous, and lignite), peat, wood, or nutshells (i.e., coconut) (US Army Corps of Engineers, 2001 and Hassler, 1974).

The manufacturing process consists of two phases, carbonisation and activation. The carbonisation process includes drying and then heating to separate by-products, including tars and other hydrocarbons, from the raw material, as well as to drive off any gases generated. Heating the material to 400–600°C in an oxygen-deficient atmosphere, that cannot support combustion, completes the carbonisation process. The carbonised particles are then “activated” by exposing them to an activating agent, such as steam at high temperature (yielding a steam activated carbon) or

phosphoric acid (yielding a chemically activated carbon). The steam burns off the decomposition products from the carbonisation phase to develop a porous, three-dimensional graphite lattice structure (similarly the acid dissolves the impurities). The size of the pores developed during activation is dependant on the time they are exposed to the steam. Longer exposure times result in larger pores. A simplified three-dimensional graphite lattice pore structure of a typical activated carbon particle is shown in Figure 2-8.

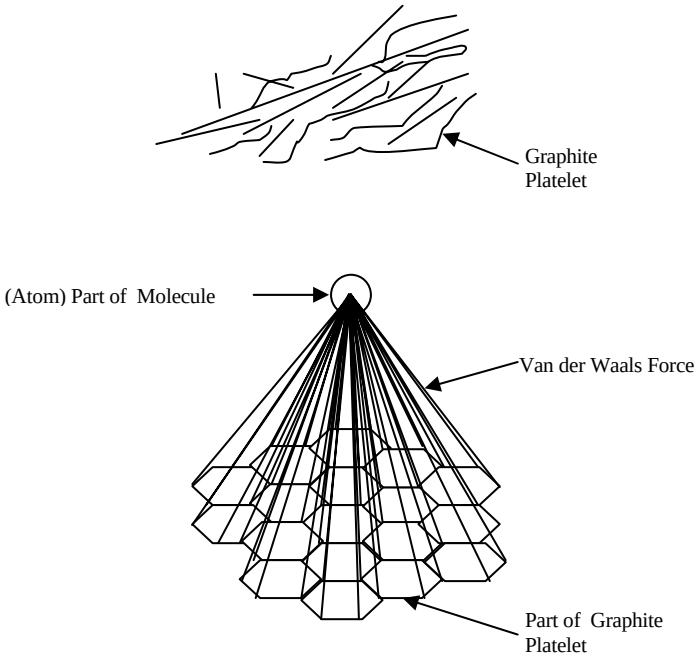


FIGURE 2-8 THREE-DIMENSIONAL GRAPHITE LATTICE PORE STRUCTURE OF A TYPICAL ACTIVATED CARBON PARTICLE

At the bottom of Figure 2-8, the simplified working methodology of activated carbon is explained. Less polar compounds are drawn to each other by forces known as Van der Waals Forces (or Londen Dispersion Forces in general). An atom (as part of a larger molecule) is drawn to graphite surface by these forces. It adsorbs onto the surface due to its preference to a less polar environment. In this way, less polar compounds can be removed from water (which is polar). This is termed adsorption.

To explain adsorption in another way would be to compare it with an extraction procedure where a less polar substance can be removed from, e.g. water (polar), by e.g. ethyl acetate (little polarity and insoluble in water). The less polar substance prefers the ethyl acetate, since it will move to where it experiences the least resistance from its environment. This is one of the basic laws of physical science.

2.2.4.1.3 Specifications

Table 2-3 contains a few of the general specifications of NORIT CA1 as obtained from the product sheet. An in-house analysis report of the adsorption capabilities and the original product sheet can be found in Appendix A.

TABLE 2-3 GENERAL SPECIFICATIONS OF NORIT CA1

Particle size D_{50}	41 μm
pH	2.0 - 3.5
Moisture (as packed)	10 w/w%
Filtration time	8 min.
Apparent density	300 kg/m^3
Internal surface area (B.E.T.)	1400 m^2/g

2.2.4.2 NORIT SA 4

2.2.4.2.1 Description

NORIT SA 4 is a steam activated carbon suitable for a large range of applications like decolourisation and purification of various food products and chemicals and also for potable water treatment. This carbon is said to be especially suitable for removal of polycyclic aromatic hydrocarbons from vegetable oils and fats. It is also a powdered activated carbon and is supplied by the same supplier as NORIT CA1.

2.2.4.2.2 Working methodology

The working methodology of this activated carbon is essentially exactly the same as for the previous one. Thus, refer to the working methodology of NORIT CA1.

2.2.4.2.3 Specifications

Table 2-4 contains a few of the general specifications of NORIT SA4 as obtained from the product sheet. An in-house analysis report of the adsorption capabilities and the original product sheet can be found in Appendix A.

TABLE 2-4 GENERAL SPECIFICATIONS OF NORIT SA4

Particle size D_{50}	+/- 76 μm
pH	alkaline
Moisture (as packed)	10 w/w%
Filtration time	21 min.
Apparent density	490 kg/m^3
Internal surface area (B.E.T.)	650 m^2/g

2.2.4.3 Polyclar V

2.2.4.3.1 Description

PVPP (polyvinylpolypyrrolidone) is sold under the brand name "Polyclar V." It is manufactured in the form of very small, round plastic beads. PVPP is a light weight plastic similar to nylon, and this material is completely insoluble in water or wine. PVPP is mainly used to remove "browning" or "pinking" pigments from white or blush wines. It is used to remove oxidized odours and for removing small amounts of bitter phenolic compounds. PVPP is often used to clarify juice pressed from mouldy grapes.

2.2.4.3.2 Working methodology

The exact mechanism in which PVPP adsorbs the phenolic compounds is still under investigation. A few suggestions of how these compounds adsorb to PVPP and detach from haze active protein in beer have been made. These are mentioned in Siebert and Lynn (1998).

Mechanism 1:

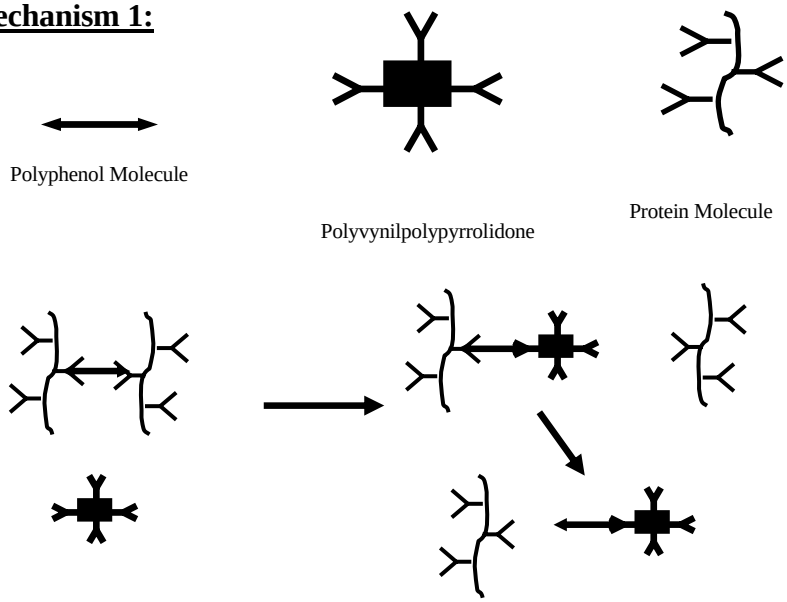


FIGURE 2-9 MECHANISM 1 - PVPP WITH SAME BINDING SITES AS PROTEINS

Mechanism 2:

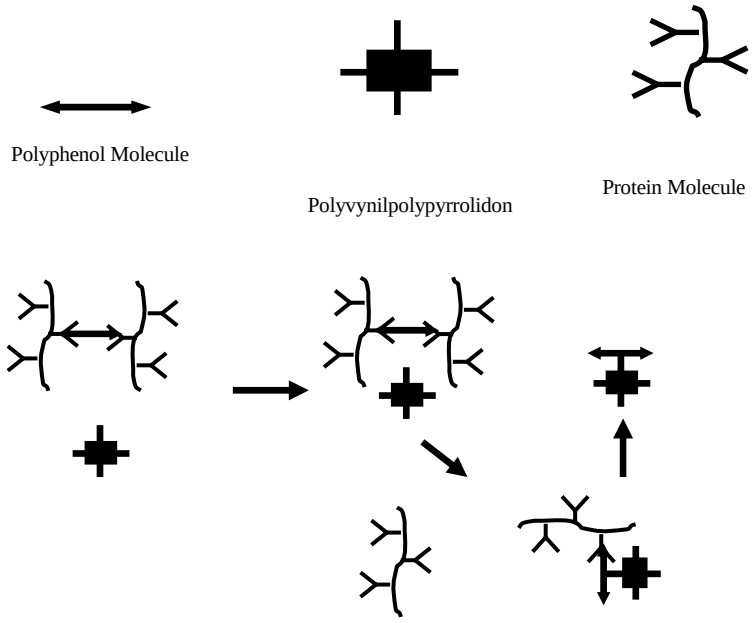


FIGURE 2-10 MECHANISM 2 - PVPP WITH DIFFERENT BINDING SITES THAN PROTEINS

In Figure 2-9 and Figure 2-10, the suggested possible adsorption mechanisms of PVPP are compared. Which one of the two, if any, is of

importance, is uncertain. It is, however, a certainty that PVPP makes use of an adsorption mechanism.

2.2.4.3.3 Specifications

Table 2-5 contains a few of the general specifications of Polyclar V as obtained from the product sheet (Appendix A).

TABLE 2-5 GENERAL SPECIFICATIONS OF POLYCLAR V

Particle size D_{50}	13 μm
pH	5.0 - 8.0
Moisture (Karl Fischer)	5% max.
Filtration time	Rapid
Apparent density	Not available
Adsorptive capacity (catechin removal)	55%

2.3 SUMMARY

Several research projects were undertaken by researchers in the past to investigate the browning reactions in wines and juices. This was done mainly to get a clearer understanding of how the reaction mechanisms work and to either inhibit these reactions, or to effectively deal with their side effects, e.g. browning, etc. It was found that at least four pathways to browning exist, namely:

- Enzymatic oxidative browning;
- Non-enzymatic oxidative browning;
- Non-enzymatic browning (the Maillard reaction);
- Caramelisation.

Each of these chemical reaction pathways has preferred environments in which they occur.

From the description of the conventional process it can be gathered that:

- The direct concentrate of the fresh juice still contains all the natural occurring amines (proteins, amino acids and enzymes);

- The media (concentrate) is acidic;
- The media is exposed to relatively high temperatures during the concentration process for relatively long periods of time.

All these are in favour of at least some of the browning reactions.

The most common precursors to browning gathered from the literature survey are: gallic acid, vanillic acid, caffeic acid, catechin, epicatechin, quercetin, and HMF. HMF was found to be indicative of the browning potential of GJC. Two pathways to HMF formation were observed from literature. These are:

- The Maillard reaction;
- Non-enzymatic oxidative browning.

Selection guidelines for selecting specific adsorption media were discussed.

With a better understanding of how browning in GJC can occur, it is necessary to determine which browning pathway is the most predominant and investigate how to inhibit this reaction.

3 ANALYSIS: METHOD DEVELOPMENT

The objective of the analysis method selection and method development was to qualify and quantify a number of the browning precursors in the grape juice and GJC (As summarised in Section 2.2.2.6). No simultaneous method of analysis exists for the qualification and quantification of some grape juice phenolics (catechin, resveratrol, catechuic acid, caffeic acid, etc.) and furfurals (2-methylfurfural and HMF). The following paragraphs depict the difficulties experienced during method development for simultaneous analysis of the abovementioned compounds.

3.1 METHOD DEVELOPMENT

A wide range of analysis methods was tested, and most had some shortcomings. This section trails the path followed to come to the decision made regarding the method of analysis, and which compound to be analysed for.

The methods used and developed in this section are based on the work done by the following researchers: Kermesha et al. (1995), Nagel & Wulf (1979), Vanhoenacker et al. (2001), Palmer & Brandes (1974), Bickler (2001), Saitta et al. (2001), Yaun & Chen (1999) and Zoecklein et al. (1997). The final method of analysis was developed at the Department of Biochemistry (University of Stellenbosch).

3.1.1 Qualitative and quantitative classification of the phenolics in GJC

During the classification procedure of the various phenolics, quite a few methods of analysis were put to trail. Most of these methods delivered inadequate results and better ways to analyse the juice in terms of phenolics concentration were sought.

It was initially estimated that the size of the polymerised phenolics would be the order and excess of 1000 grams per mol (corresponding to oxidative browning reactions). A method of analysis that would give information on the mobility and size of the molecules were initially tried (MALDI-TOF).

The following few paragraphs give some background on the various methods tried/used, the runs performed and preparations preceding it, the problems experienced, and the method decided on.

3.1.2 Background on Methods Used

3.1.2.1 MALDI-TOF (www.srsmaldi.com, 2002)

MALDI-TOF (Matrix-Assisted Laser Desorption Ionisation Time-Of-Flight) has proven to be one of the most successful ionisation methods for the mass spectrometric analysis and investigation of large molecules. MALDI is a method that allows for vaporisation and ionisation of non-volatile biological samples from a solid-state phase directly into the gas phase.

The sample (analyte) is suspended or dissolved in a matrix (» 1000x molar excess). Matrices are small organic compounds that are co-crystallized with the analyte. It seems that the presence of the matrix, spares the analyte from degradation, resulting in the detection of intact molecules as large as 1 million Daltons. The matrix is a small organic compound (e.g., in our case, Trans-3-Indoleacrylic Acid (IAA)) with certain properties such as high absorption at the laser beam that prevents the decomposition of fragile samples. The phenomenon is not well understood but it is believed that the matrix molecules absorb the energy of the laser and gently transfer this energy to the sample, preventing decomposition. It also promotes the ionisation process. Most matrices are acids.

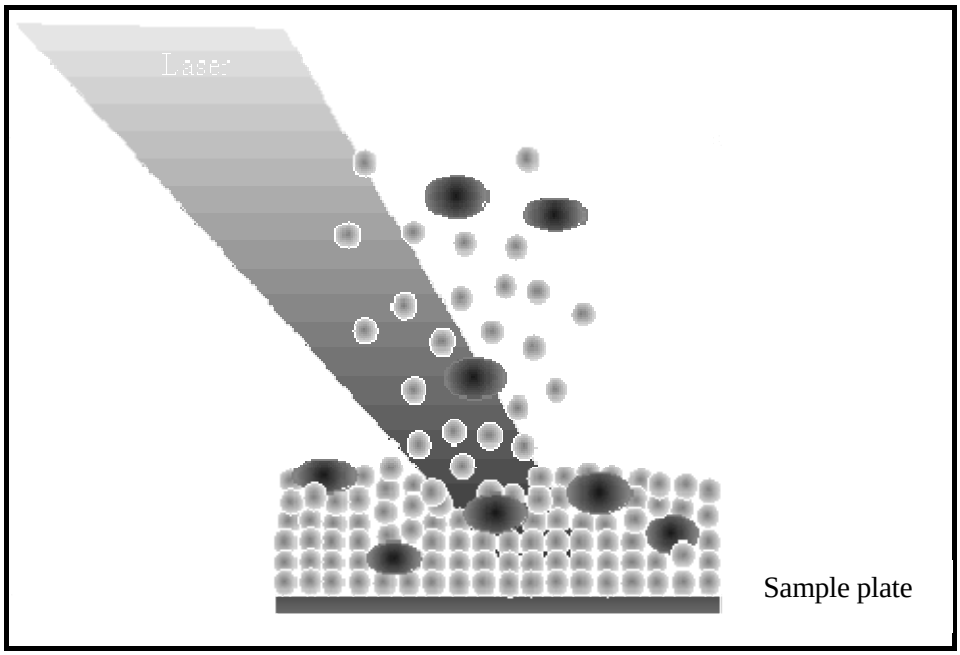


FIGURE 3-1 MALDI-TOF

During sample preparation, the matrix and analyte are mixed and the mixture is dried on a probe, or, as it is more commonly known, on a sample plate.

A laser beam, serves as desorption and ionisation source in MALDI. Upon laser irradiation, the matrix and analyte molecules are desorbed and ionised. The matrix plays a key role in this technique by absorbing the laser light energy and causing part of the illuminated substrate to vaporize. (The matrix molecules absorb most of the incident laser energy minimizing sample damage and ion fragmentation). A rapidly expanding matrix plume carries some of the analyte into the vacuum with it and aids the sample ionisation process. They are then accelerated by high voltage and separated based on the time it takes them to travel a field-free drift region. Since all ions gain the same kinetic energy, $KE=1/2mv^2$, the ones with a larger mass travel at a lower velocity and therefore arrive at the detector later than the smaller ions. A spectrum of ion intensity as a function of the travel time is recorded.

Once the sample molecules are vaporized and ionised they are transferred electrostatically into a time-of-flight mass spectrometer (TOF-MS) where they are separated from the matrix ions, and individually detected, based on their mass-to-charge (*m/z*) ratios and analysed. High transmission and sensitivity, along with theoretically unlimited mass range are among the inherent advantages of TOF instruments. Detection of the ions at the end of the tube is based on their flight time, which is proportional to the square root of their *m/z*.

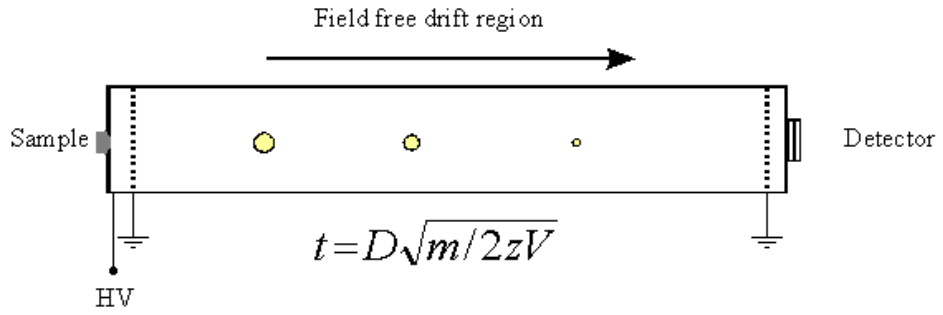


FIGURE 3-2 TIME OF FLIGHT (T) IN THE FIELD-FREE REGION (D = DISTANCE TRAVELLED BY ION, M/Z = MASS TO CHARGE RATIO, V = GUIDE VOLTAGE)

3.1.2.2 HPLC

High performance (sometimes pressure) liquid chromatography or HPLC as more commonly known is versatile method of analysis and finds application in a very wide field. HPLC is basically a normal liquid chromatography process, but faster, operating under higher pressure, and in most cases with a higher resolution than normal LC. A simplified diagram that explains the working of liquid chromatography will be sufficient to explain HPLC in general. A sample gets injected into a buffer solution (carrier liquid), which flows through a column that usually has a specified usage. In this column the various compounds are separated based e.g. on their polarity. For example a C18-column allows polar compounds to move through it freely, whereas less polar compounds tend to remain in column for longer periods of time. From the column it usually goes to a detector, either an ultraviolet detector, or mass spectrometer. The results

then get registered and a liquid chromatogram is produced. See Figure 3-3. (www.ionsource.com, 2002)

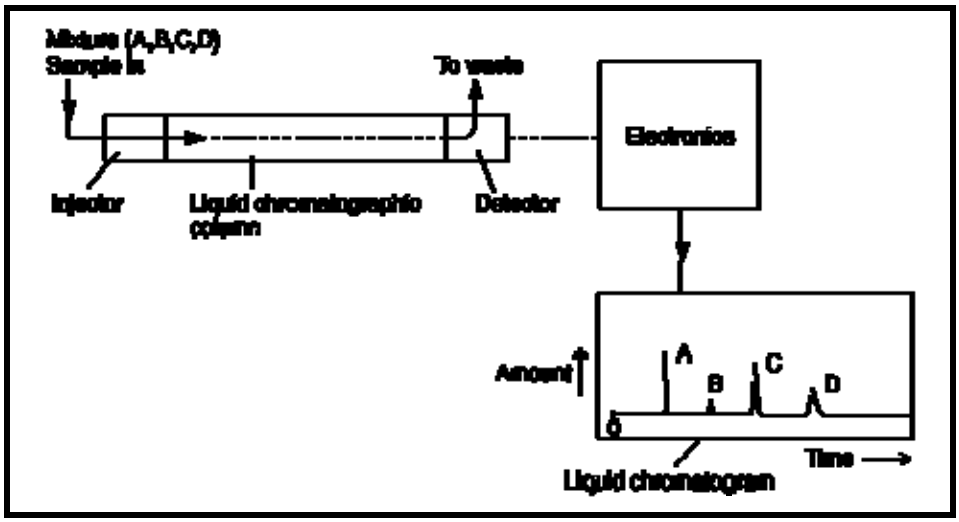


FIGURE 3-3 LIQUID CHROMATOGRAPHY, A SIMPLIFIED ILLUSTRATION

3.1.2.3 Atmospheric Pressure Mass Spectrometry, ESI, APcI

Ionisation of polar compounds can be achieved by using atmospheric pressure ionisation (API). One of the most successful techniques of API is electro spray ionisation (ESI). In the ESMS laboratory this method is mostly used to ionise molecules derived from plants. If ESI is not effective in the ionisation process, another API method, namely, atmospheric pressure chemical ionisation (APcI) can be used. This is a more robust technique where less polar compounds can be ionised.

Quantifying of ions can be achieved by setting the instrument to express the intensity of an ion in terms of concentration. Calibration curves are established by plotting intensities of known concentrations of pure standards against the concentrations. Unknown concentrations of an ion can thus be read from the calibration curve if the intensity of the specific ion can be determined.

The identity of a specific ion is presented by the mass of this ion. During negative ionisation, where e.g. phenolics are analysed by ESI mass

spectrometry, the mechanism is ionisation deprotonation. The ion that is observed is thus the $[M-H]^-$-ion, which has one mass unit less than the molecular ion. If the mass spectrometer is set to detect this one specific ion, the area underneath the chromatographic peak obtained will be an expression of the concentration of this ion. It is, however, possible for other ions in an extract to have the same mass. To increase the specificity of the detection the molecular ion gets fragmented inside the instrument, and the most dominant fragment is then taken as representative of the substance. To explain this detection technique (known as MS/MS) it is necessary to shortly mention the configuration of the instrument:

The mass spectrometer that is used in the ESMS-laboratory (University of Stellenbosch) is known as a triple quadropole instrument. It describes an instrument existing of two quadropole analysers that is positioned in line with each other, with a fragmentation cell in between. Ions that leave the ionisation source (ESI or APcI), is separated by means of their mass in the first quadropole that yields a mass spectrum. If it is necessary to fragment a specific ion, the first quadropole can be set to only let this ion through to the fragmentation cell. In this cell, Argon collides with the ion yielding fragmentation of the ion. These fragment ions can then be separated in the second quadropole in terms of their masses, yielding a fragmentation spectrum.

For quantification the second analyser is set to only let the most dominant fragment ion through, which falls on the ion-detector en gets registered. The intensity of this peak is then taken as an indication of the original molecular ion. This step increases the specificity of the detection to such an extent that the result can be taken as representative of the original ion of importance, even if there was other molecular ions with the same masses present.

The presence of a mixture of ions that is ionised simultaneously can adversely affect the ionisation of a specific ion. It is thus better to separate the ions to some extent in order to ensure that different ions reach the ionisation source at different times. It is for this reason that a separation technique is used before the ionisation process. In the ESMS laboratory (University of Stellenbosch), HPLC is mostly used to achieve this separation. The whole process is then known as LC/MS/MS and a simplified illustration of this entire process can be seen in Figure 3-4.

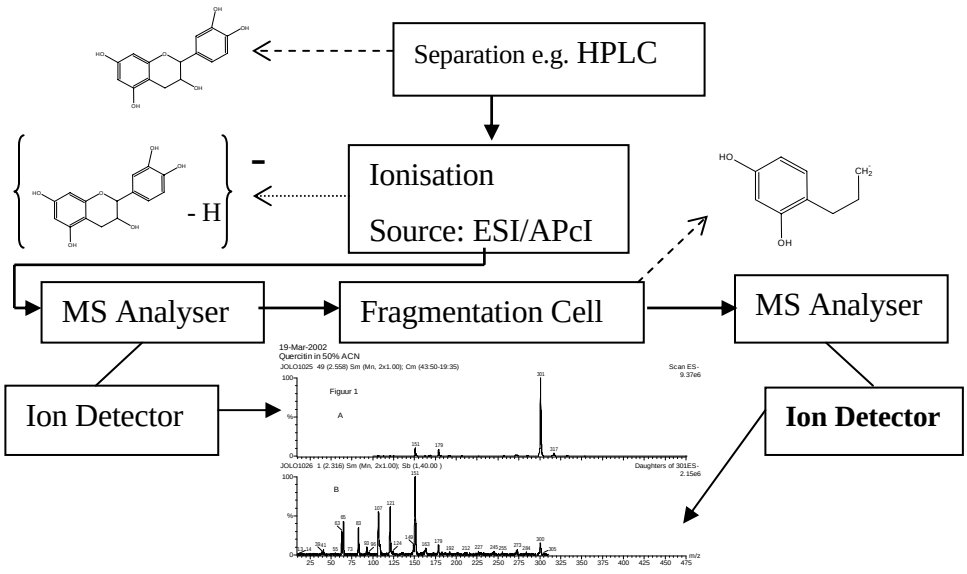


FIGURE 3-4 SIMPLIFIED ILLUSTRATION OF LC/MS/MS

Although LC/MS/MS is a powerful detection technique that can be used for successful quantification, it is often necessary to rid the sample of impurities that can interfere with the detection. A Grape juice sample contains sugars and salts that are highly water-soluble and can be removed by liquid/liquid extraction. Liquid/liquid extraction can also be used to concentrate certain compounds and to purify the sample. Such samples are thus usually extracted with e.g. ethyl acetate. The aqueous phase will then contain all the sugar etc., while phenolics will be contained in the organic

phase. Evaporation of the organic phase then yields a concentrated, enriched extract of the sample.

3.1.3 Trials

3.1.3.1 MALDI-TOF MS

3.1.3.1.1 Sample Preparation:

Six grape juice samples were prepared, each in a different way, to try and develop the best possible method of analysis. The preparations of the samples are described below, from A1, being sample one, to E, being sample six. The analyses were conducted at the University of Cape Town.

A1. 2 ml of diluted GJC was put through a C18-cartridge to capture the phenolics. The eluent was discarded. The cartridge was washed with 1ml of 100% methanol (MeOH) and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix, trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

A2. The same cartridge as was used in A1, was washed with 1ml of 100% methanol (MeOH) again and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix, trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

B. 2 ml of diluted GJC were put through a C18-cartridge to capture the phenolics. The eluent was discarded. The cartridge was washed with 1ml of 1% Acetic Acid Solution (CH_3COOH) and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix,

trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

C. 2 ml of diluted GJC was put through a C18-cartridge to capture the phenolics. The eluent was discarded. The cartridge was washed with 1ml of 25% MeOH, 75% H_2O Solution and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix, trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

D. 2 ml of diluted GJC was put through a C18-cartridge to capture the phenolics. The eluent was discarded. The cartridge was washed with 1 ml of 50% MeOH, 50% H_2O Solution and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix, trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

E. 2 ml of diluted GJC was put through a C18-cartridge to capture the phenolics. The eluent was discarded. The cartridge was washed with 1 ml of 75% MeOH, 25% H_2O Solution and the eluent gathered in a vial. The vial was put in a Speedy Vac until the volume evaporated to about 50 μL. 1 μL was taken from the vial and mixed with 1 μL of the matrix, trans-3-indoleacrylic acid (IAA), put onto the sample plate and inserted into the MALDI.

From all these, a mass spectrum was collected and studied to determine the validity of each spectrum. The validity of the spectra was determined by the ease of which a known molecular weight of a phenolic substance present in grape juice could be spotted.

3.1.3.2 HPLC runs conducted at the University of Cape Town

3.1.3.2.1 For the sake of interest

For the sake of interest and to see whether sensible results could be obtained, an HPLC run was conducted with a pooled sample of the fractions used in the MALDI analysis. This result proofed to be quite remarkable, since it correlated in some way with a reversed phase HPLC profile gathered during a study on the purification procedure for polyphenols in crude grape seed extract.

3.1.3.2.2 Sample Preparation

10 µl fractions were collected from each of the samples A1, A2, C, D, and E and pooled to make a representative sample. 10 µl from the representative sample was taken and injected into the HPLC system.

3.1.3.2.3 The Procedure and Equipment

The column used was a Jones Chromatography RP C18 Column, with outer diameter of 2 mm, and pore size of 3 µm. The buffers used were: A, 0.1% Trifluoro-acetic acid (TFA) in water; and B, 0.1% TFA in Acetonitrile (absolute). The flow rate was 0.7 ml/min with the following gradient: 0 - 5 minutes 100% A, 5 - 45 minutes gradually increase B to 100%, 45 - 50 minutes gradually decrease B to 0%, 61 minutes stop operation.

3.1.3.2.4 Determination of changes in phenolics content during storage

Two more HPLC runs were conducted at the University of Cape Town, one on a GJC that has been in storage for a few months, and the other a GJC that has been in storage for a few days. This was done to try and determine the changes in phenolics content that occurs during long terms of storage. No real sample preparation was performed except that the juice

was filtered through a 2-micron filter. The procedure followed was exactly the same as mentioned above.

Twenty-one peaks were gathered from each run, each in a different vial. These were then brought to Stellenbosch for qualification of the components on the ES-MS.

3.1.3.3 ESI and APcI

Before we could proceed with these methods of analysis, it was necessary to press grapes in order to retrieve juice or samples. The following paragraph simply states the procedures followed in doing this.

3.1.3.3.1 Retrieving Samples

According to a cliental manager of one of the leading GJC manufacturers in South Africa, the predominant grape cultivar received for 2002 was Colombar. It was thus decided to use Colombar for the fresh juice sample.

Four crates of Colombar grapes, approximately 87 kg in weight, were put through the following procedure:

- De-stemming - All four crates were put through a crusher/de-stemmer to remove the stems from the grapes. The pulp was gathered in four, clean 20 liter buckets;
- SO_2-solution was added to each bucket to the amount of 30 mg/L;
- The pulp was then put through a small-scale bag press to separate the must/juice from the skins and pips. A yield of approximately 40 liters of juice was achieved;
- 3 ml of a 10 ml Papidase®/l distilled water solution was added per litre of grape juice. (A total of 120ml of the solution.) Papidase®, a pectolytic enzyme from Anchor Yeast®, is added to the juice to allow all the suspended solids to settle;

- 16 ml of a 5 ml Bentonite/l distilled water suspension was added per litre of grape juice (A total of 640 ml of the suspension). Bentonite is used in the wine industry for protein stabilisation. The Bentonite causes the proteins to coagulate and settle out;
- The juice was left overnight at 15^{o}C to allow the solids and proteins to settle;
- The supernatant fluid (juice) was drawn off and a spatula point of Actistab® from Gist-brocades® was added. Actistab® stops any fermentateous activity. The remaining juice was approximately 35 liters;
- The remaining juice was stored at -4^{o}C for 48 hours, allowing for tartaric acid crystallisation, and thus, tartrate stability;
- Bentostab® solution was used to test for protein stability, and it was found to be protein stable;
- The juice was then stored at 10^{o}C for the rest of the analysis period.

3.1.3.3.2 Sample Preparation

The sample preparation procedure is as follows:

- Ten 3ml grape juice samples were put into separate vials;
- To each vial 3 ml of Ethyl Acetate (EA) were added;
- A cap was placed on each tube and then vortexed for 30 seconds;
- The layers were allowed to separate and were centrifuged for two minutes at 2000 rpm;
- 2.4 ml of the supernatant fluid (EA with phenolics/polyphenolics) of each tube was drawn off and placed in clean tubes;
- A spatula tip of Sodium Carbonate (Na_2SO_4) was added to each of these to remove the remaining water;

- These vials were then centrifuged for another two minutes at 2000 rpm;
- 2 ml were drawn from the "dried" vials and were put into 4 ml vials, and evaporated at 35^{o}C under Nitrogen;
- The concentrates were then frozen for preservation.

3.1.3.3.3 Procedure

For an explanation of the procedure, refer to Figure 3-4 and the paragraph on Atmospheric Pressure Mass Spectrometry, ESI, APcI.

3.1.4 Inadequate results

3.1.4.1 MALDI-TOF MS

As mentioned earlier the MALDI-TOF analysis proof to be great at the classification of larger molecular weights (1000+). Initially it was thought that the phenolics in the juice were at a stage of polymerisation where the molecular weights are about 1000 g/mol or more. This thought proved to be in error as could be seen in Figure 3-5. Although only a mass to charge ratio range of 0 to 1000 are shown it can be seen that the peaks die away at an m/z of approximately 800. Further attempts to determine whether there are any phenolics or derivatives distinguishable proved to be a strenuous exercise since there are literally hundreds of peaks.

The matrix used in a MALDI-TOF MS analysis also has a spectrum. The matrix in this case analysis was trans-3-indoleacrylic acid (IAA), and its spectrum can be seen at the bottom of Figure 3-5.

Since the matrix also has a spectrum, difficulty distinguishing between the peaks generated by the matrix and the peaks generated by the analyte were experienced. The spectrum of the matrix also duplicates itself in the spectrum of the analyte (since it is mixed with the analyte), but not necessarily in the same intensity of the spectrum of the matrix alone. Comparing the spectra of the matrix with that of the analyte with a m/z-

range of 0 to 500 (see Figure 3-6), it can be seen why it is difficult to distinguish between the two.

However, one positive result was that the MALDI-TOF analysis confirmed that the browning taking place in the juice concentrate is not of an oxidative nature. This conclusion can be made based on the fact that oxidative reactions yield molecules in excess of 1000 g/mol.

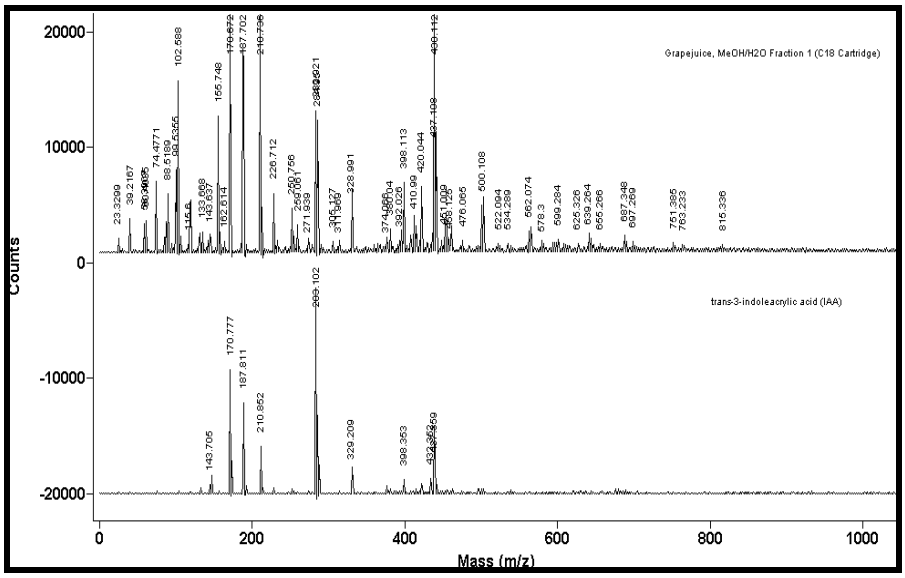


FIGURE 3-5 MASS SPECTRA OF ANALYTE AND MATRIX (MALDI-TOF)

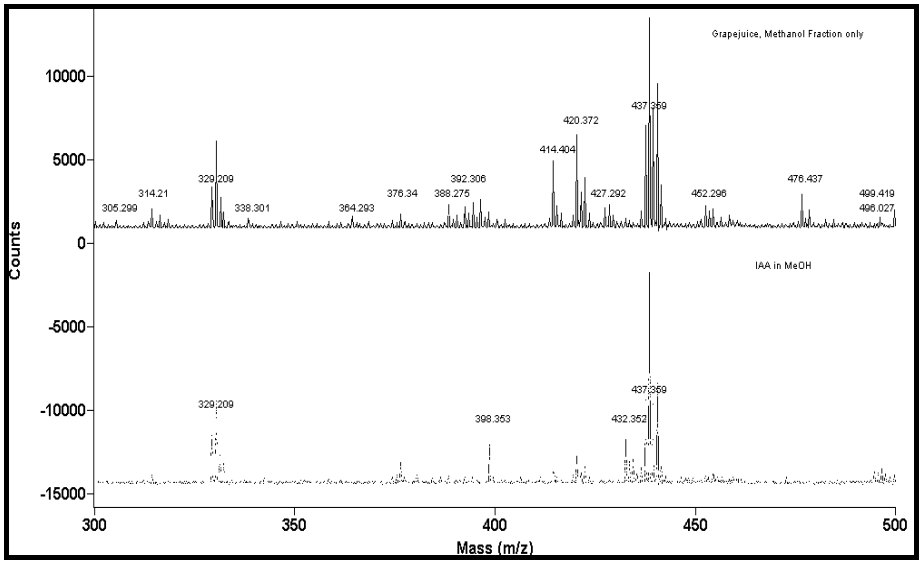


FIGURE 3-6 MASS SPECTRA WITH SMALLER M/Z-RANGE

3.1.4.2 HPLC conducted at UCT

The results obtained from the runs conducted at the University of Cape Town are as follows:

For the run conducted for the sake of interest of the pooled sample, the chromatogram as shown in Figure 3-7 was yielded. The chromatogram shown in Figure 3-8 is the typical profile of grape seed extract. Although it is not technically correct to compare the two, since the methods of analyses differ, there are some similarities between the two chromatograms. Figure 3-9 depicts the technically incorrect presentation of the comparison between the two profiles.

Qualification of the different compounds should first be performed to deem this comparison conclusive.

In the runs conducted to determine the change in phenolic content during storage some other problems were experienced. No sample pre-treatment (i.e. extraction, etc.) were performed except to put the juice through a 2-micron filter. A legion of compounds thus passed through the HPLC, producing profiles difficult to analyse. Figure 3-10 and Figure 3-11 shows the profiles gathered from the HPLC runs for the old (spent a few months in storage) and new (spent a few days in storage) GJC samples, respectively.

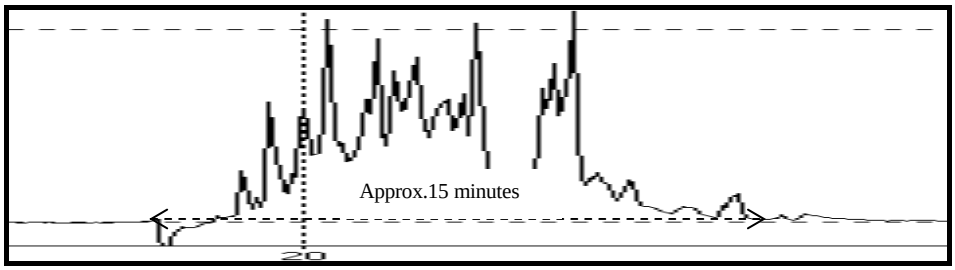


FIGURE 3-7 CHROMATOGRAM OBTAINED FROM POOLED SAMPLE

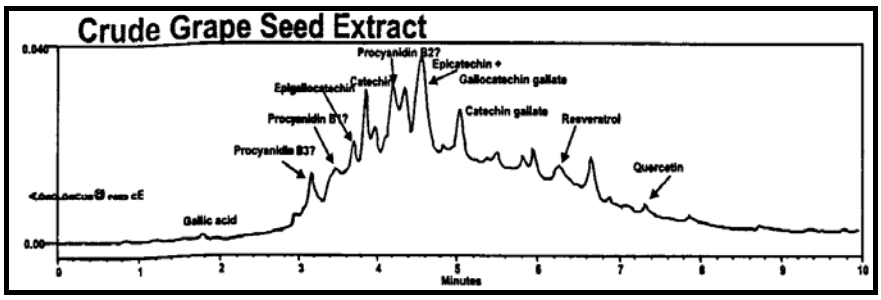


FIGURE 3-8 CHROMATOGRAM OBTAINED FROM A GRAPE SEED EXTRACT

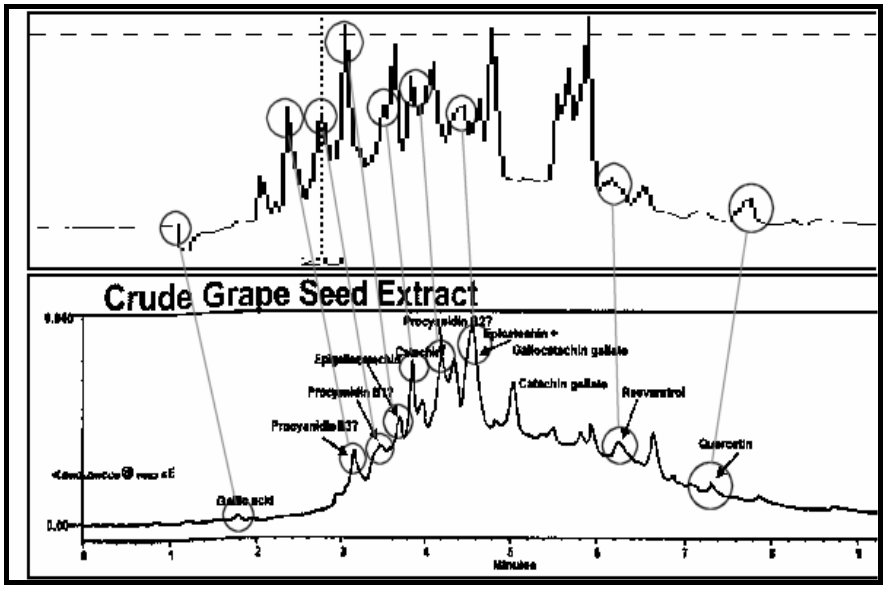


FIGURE 3-9 TECHNICALLY INCORRECT COMPARISON OF THE TWO PROFILES

In comparing Figure 3-10 and Figure 3-11 two peaks that differ significantly are observed. An interest developed to see what exactly these peaks were, since some conclusions concerning the changes during storage of the concentrate might be possible. Figure 3-12 shows these changes more clearly.

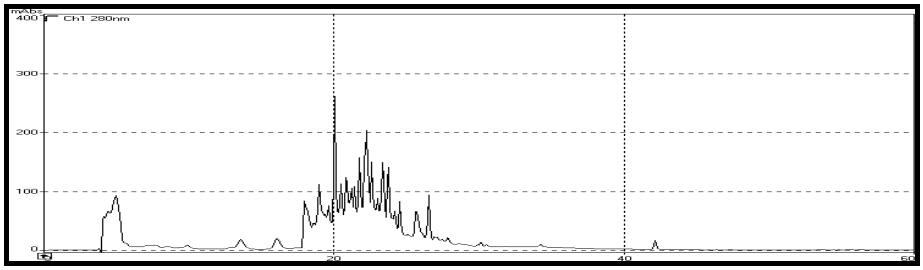


FIGURE 3-10 CHROMATOGRAPH OF OLD GJC SAMPLE

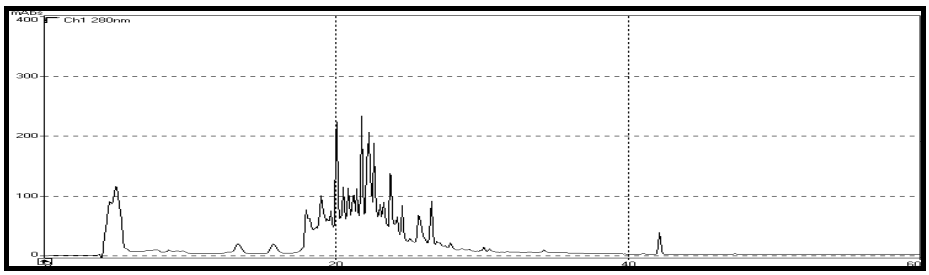


FIGURE 3-11 CHROMATOGRAPH OF NEW GJC SAMPLE

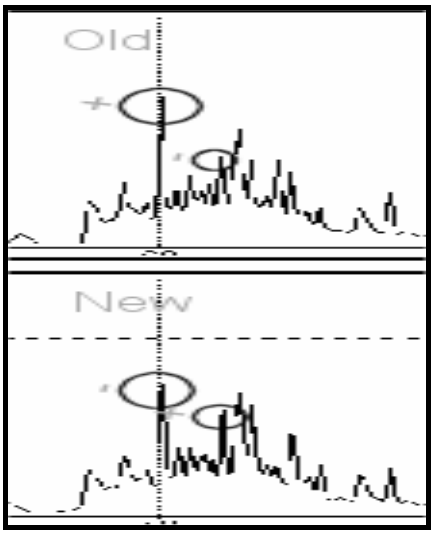


FIGURE 3-12 COMPARISON OF PROFILES

The encircled peak on the left of Figure 3-12 increases during storage while the peak on the right decreases during storage. The assumption was made that some chemical shift is taking place during storage. However, this could only be confirmed by qualification and quantification of the peaks. A total of 21 peaks were gathered in separate vials for further analysis.

3.1.4.3 Electrospray Ionisation-LC/MS/MS

Pure standards were dissolved in acetonitrile to a concentration of 1 mg/ml. These solutions were then diluted to 50 μg/ml. The injection volume was 5 μl injected into buffer solution A: acetonitrile/water: 50/50 (v/v). The flow rate to the ESI source was 20 μl/minute. The first analyser was then used

to obtain a spectrum of each standard. The molecular anion: $[M-H]^-$ of each standard is then fragmented. The fragmentation spectrum is obtained from the second analyser. The following figures denote the spectra of the individual (and pooled) compounds.

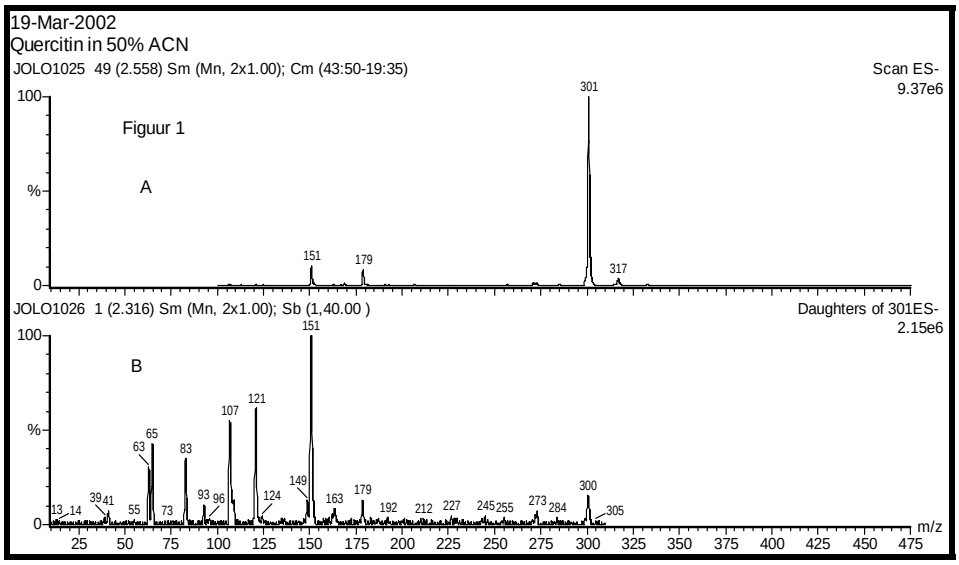


FIGURE 3-13 MASS SPECTRA OF QUERCETIN MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

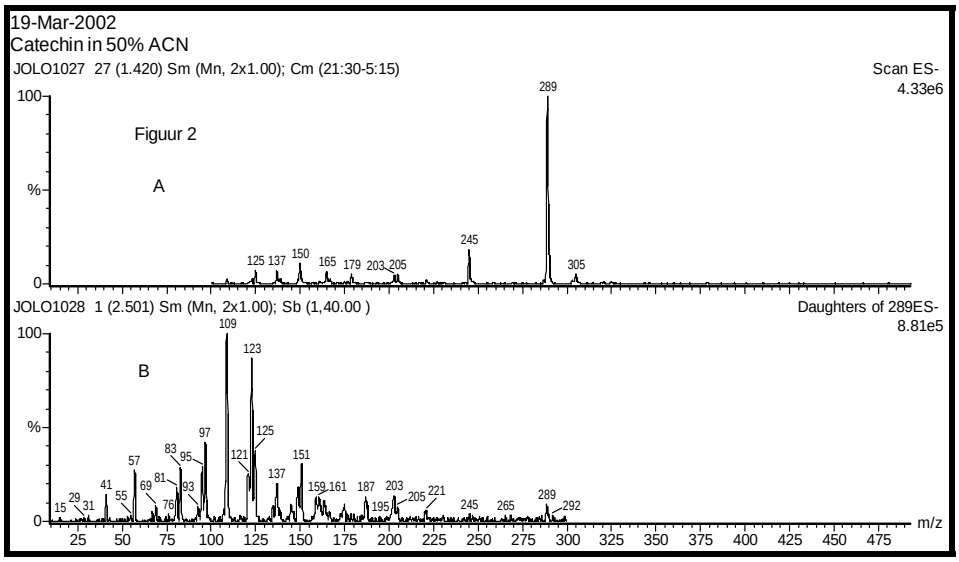


FIGURE 3-14 MASS SPECTRA OF CATECHIN MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

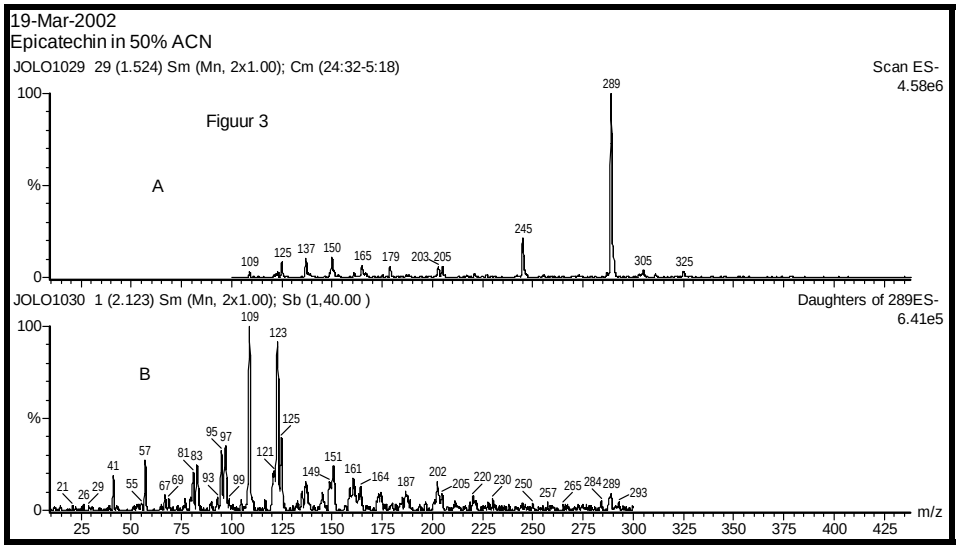


FIGURE 3-15 MASS SPECTRA OF EPICATECHIN MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

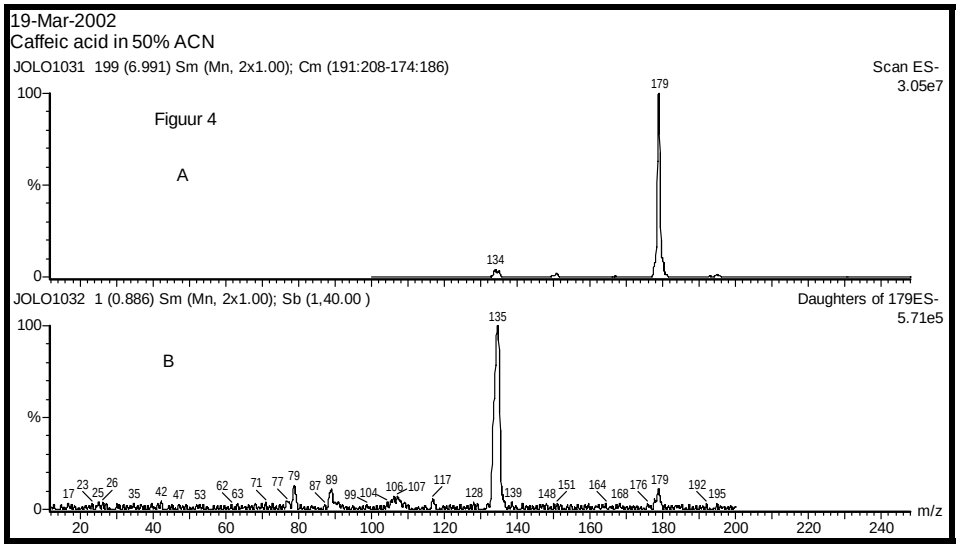


FIGURE 3-16 MASS SPECTRA OF CAFFEIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

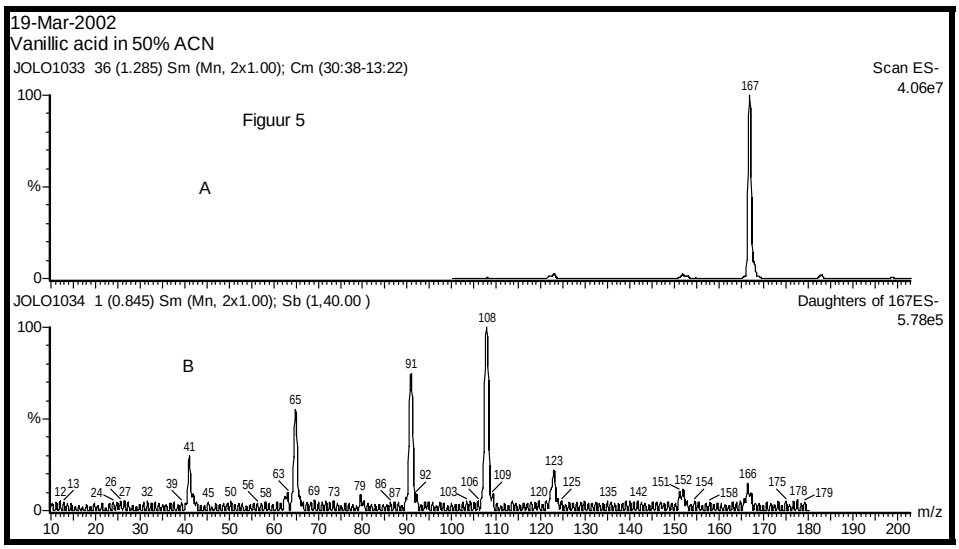


FIGURE 3-17 MASS SPECTRA OF VANILLIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

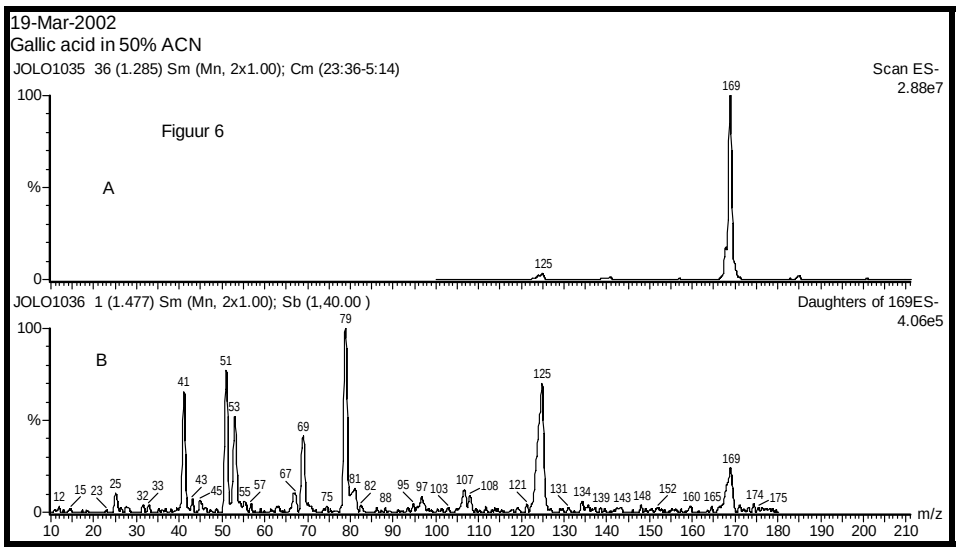


FIGURE 3-18 MASS SPECTRA OF GALLIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

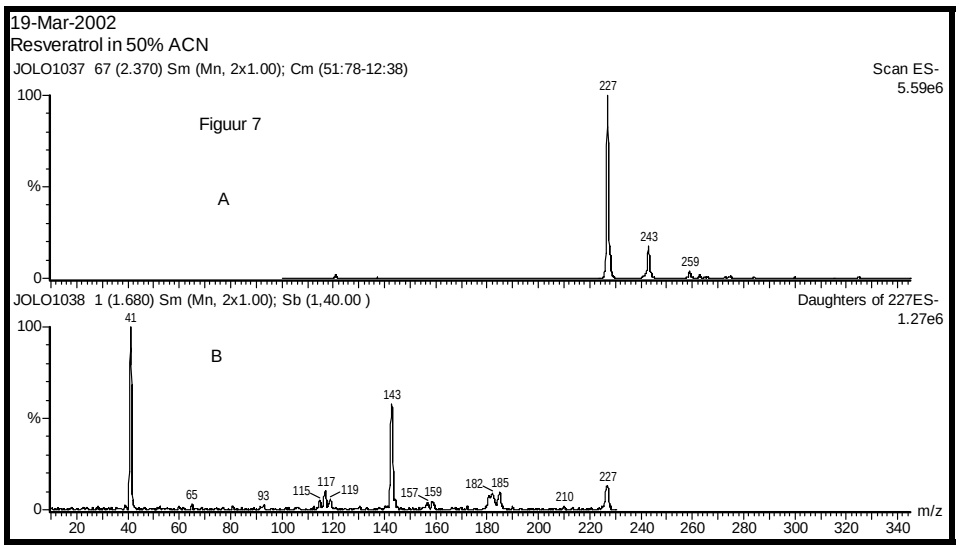


FIGURE 3-19 MASS SPECTRA OF RESVERATROL MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), ESI

All the spectra (Figure 3-13 to Figure 3-19) showed the $[M-H]^-$ molecular ion as the main component (base peak). Clear fragmentation patterns of these ions were obtained.

Figure 3-13 to Figure 3-19 indicate that electrospray ionisation could work for analysis of the phenolic compounds. With the furfural, 5-Hydroxymethylfurfural (HMF), another case was observed. Figure 3-20 indicates that electrospray ionisation did not sufficiently ionise HMF. The expected molecular ion at m/z = 125 show a low intensity ion. Another way of ionisation should thus be tried.

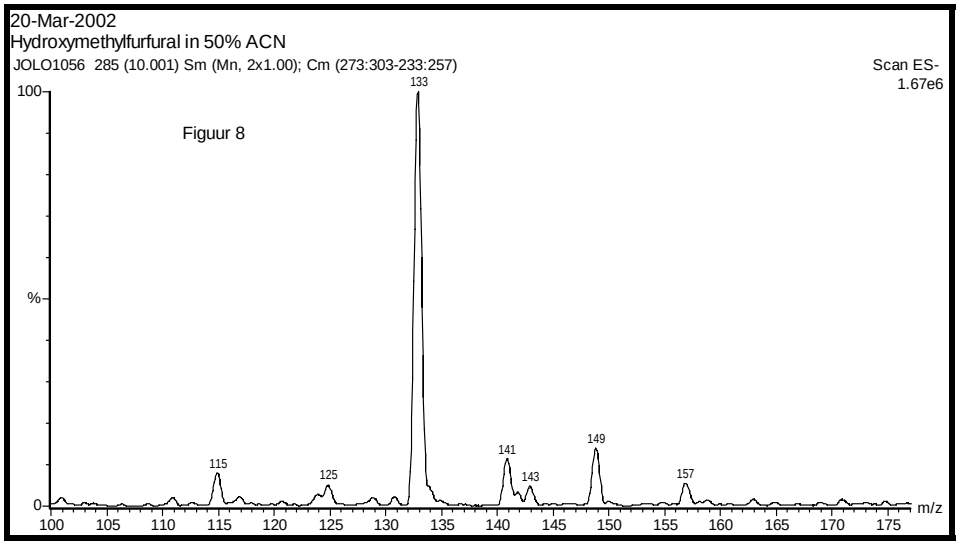


FIGURE 3-20 MASS SPECTRUM OF 5-HYDROXYMETHYLFURFURAL, ESI

Table 3-1 summarise the m/z's of the molecular- and fragment ion combinations of the various phenolics that ionised sufficiently.

TABLE 3-1 MOLECULAR- AND FRAGMENT ION COMBINATIONS, ESI

Substance	Mr g/mol	m/z of molecular-ion	m/z of fragment-ion
Quercetin	302	301	151
Catechin	290	289	109
Epicatechin	290	289	109
Caffeic Acid	180	179	135
Vanillic Acid	168	167	91
Gallic Acid	170	169	125
Resveratrol	228	227	143

With these combinations it will be possible to analyse the individual compounds in a mixture except for catechin end epicatechin, which has the same m/z values for both the molecular and fragmentation ions (refer to Figure 3-14 and Figure 3-15). To distinguish between catechin and epicatechin, a Phenomenex Luna C18(2)-column (with dimensions 2.00 x 150 mm and 3 µ packing) were installed between the injector and ionisation source. Buffer solution A (acetonitrile/water) was used at a flow rate of 100 µl/minute. Following this, the mass spectrometer was set to monitor the different combinations of molecular- and fragment ions, and the standards were injected.

Figure 3-21 shows the mass chromatogram during the analysis of quercetin. Each panel represents the chromatogram of a specific substance. Each substance is represented by their molecular- and fragment ion combination (refer to top right corner of each panel.) E.g., quercetin is represented by the combination 301>151. When quercetin is injected, there only appears a peak in the top panel. The retention time (R/t) of quercetin on the C18-column with buffer solution A, at a flow rate of 100 µl/minute, is 5.98 minutes (as shown above the peak).

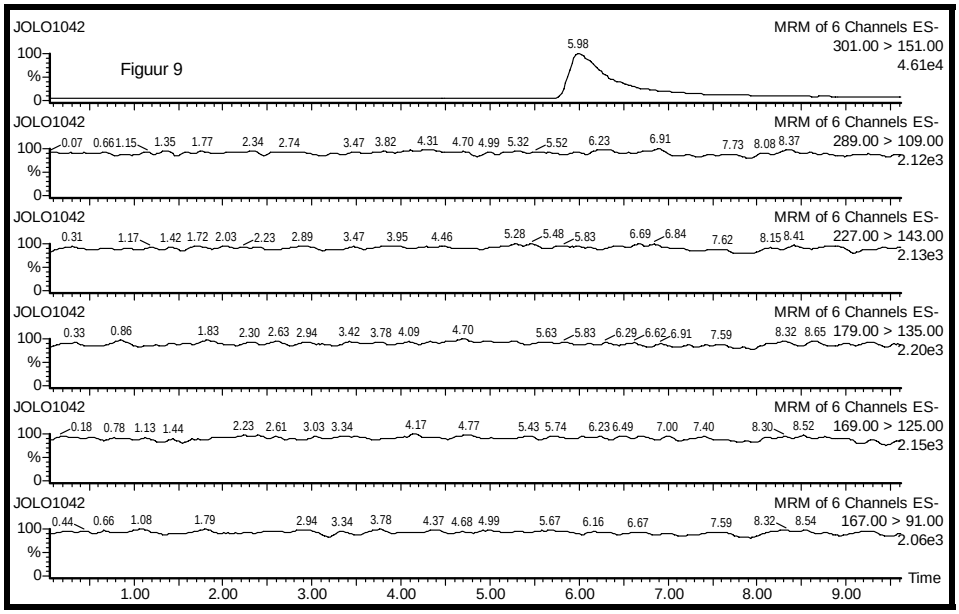


FIGURE 3-21 MASS CHROMATOGRAM OF QUERCETIN, ESI

The second panel from the top indicates catechin and epicatechin, which has the same combination ions, as stated previously. Figure 3-22 and Figure 3-23 show the chromatograms of catechin and epicatechin, respectively. These two compounds do not separate sufficiently to quantify them separately. This is demonstrated in Figure 3-24, a chromatogram of a mixture of the two.

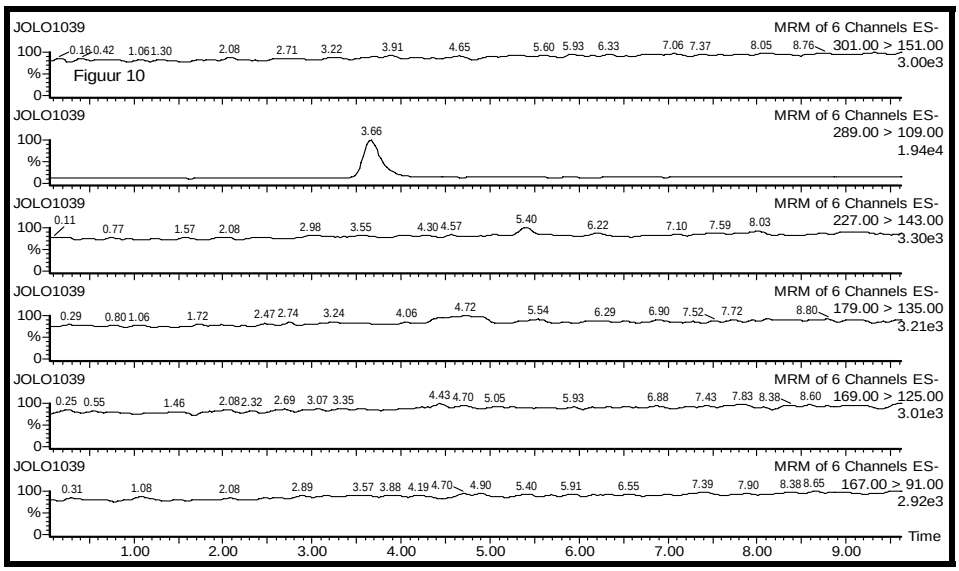


FIGURE 3-22 MASS CHROMATOGRAM OF CATECHIN, ESI

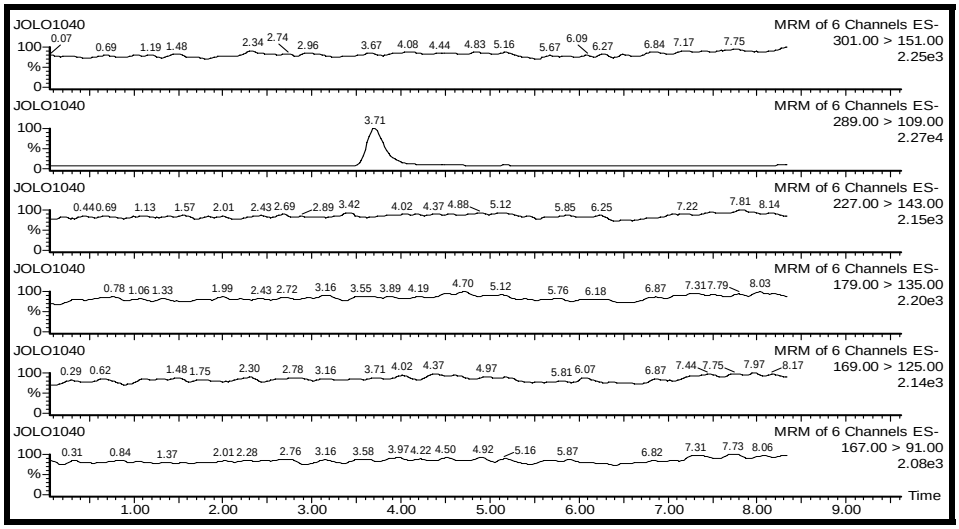


FIGURE 3-23 MASS CHROMATOGRAM OF EPICATECHIN, ESI

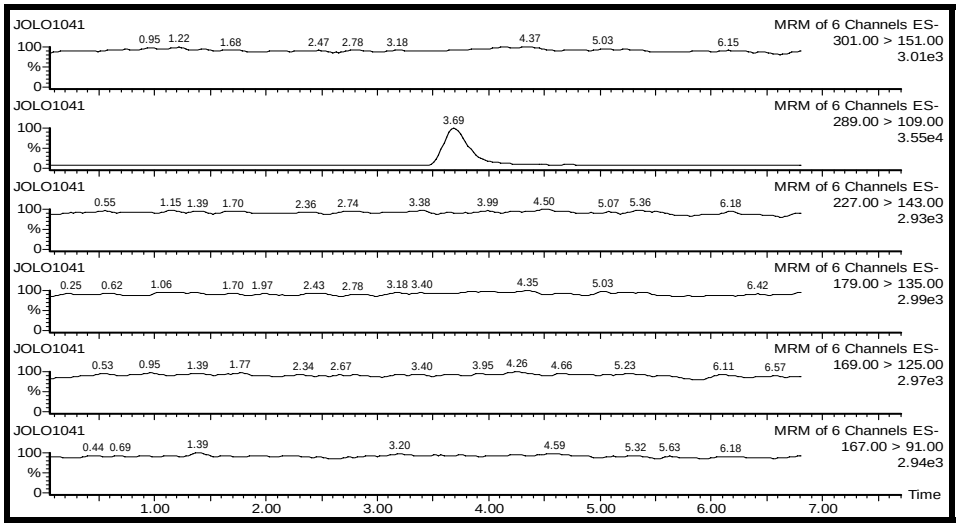


FIGURE 3-24 MASS CHROMATOGRAM OF CATECHIN/EPICATECHIN MIXTURE, ESI

Figure 3-25, Figure 3-26, Figure 3-27, and Figure 3-28 show the chromatograms of caffeic acid, gallic acid, vanillic acid, and resveratrol respectively. These figures indicate that the specificity of this detection, with the exception of catechin and epicatechin, is good enough for individual substance analysis.

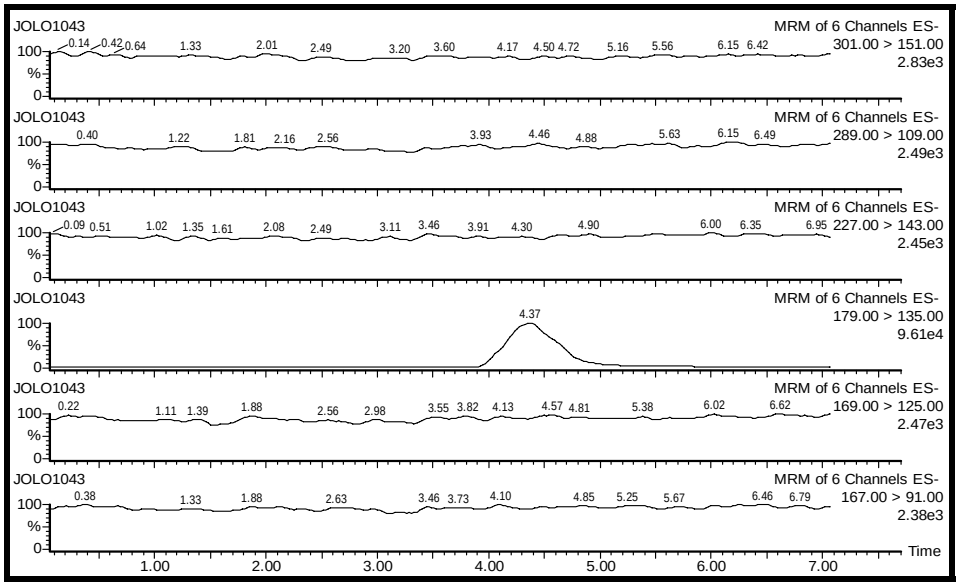


FIGURE 3-25 MASS CHROMATOGRAM OF CAFFEIC ACID, ESI

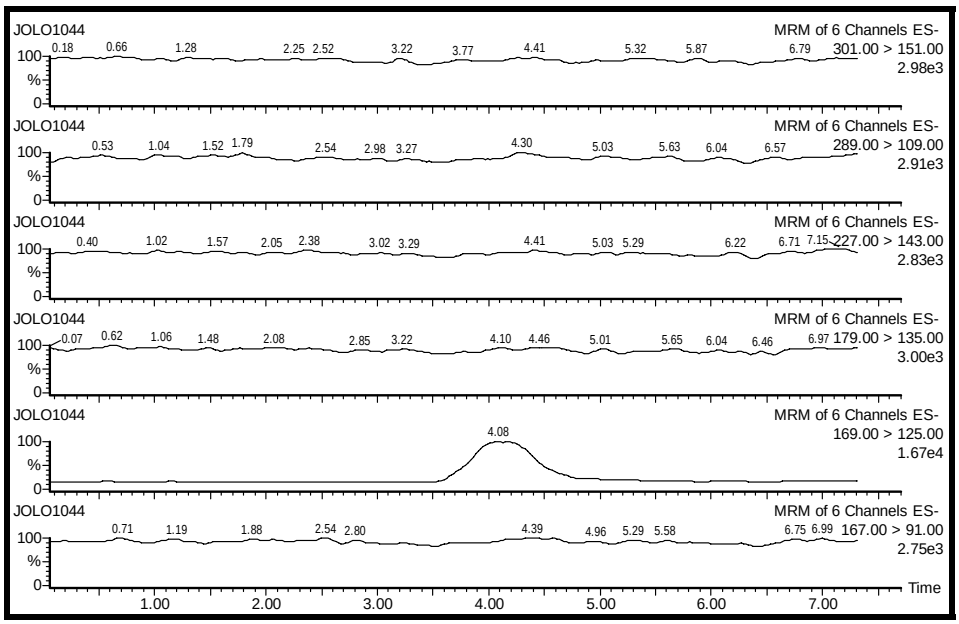


FIGURE 3-26 MASS CHROMATOGRAM OF GALLIC ACID, ESI

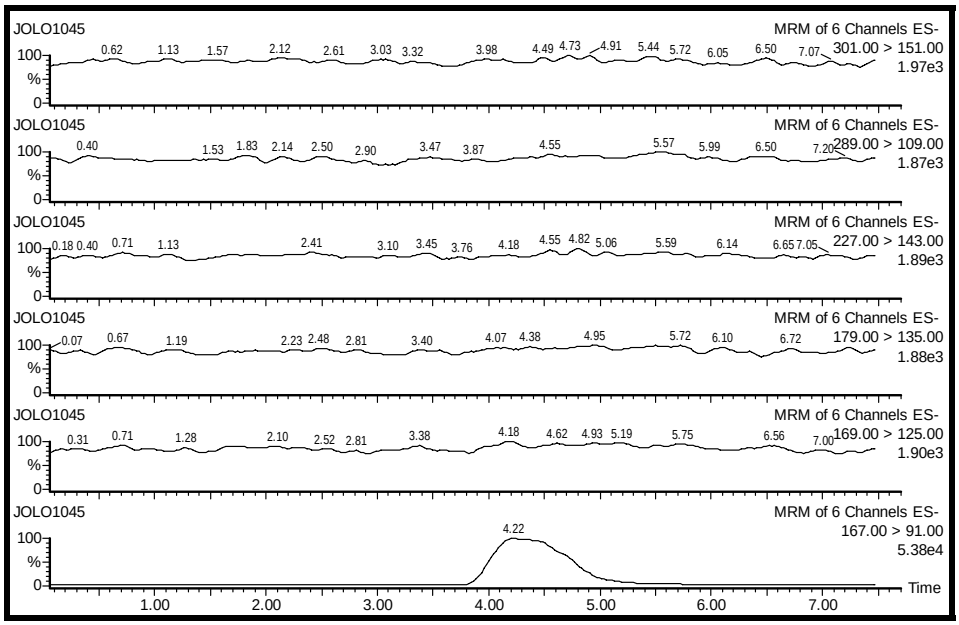


FIGURE 3-27 MASS CHROMATOGRAM OF VANILLIC ACID, ESI

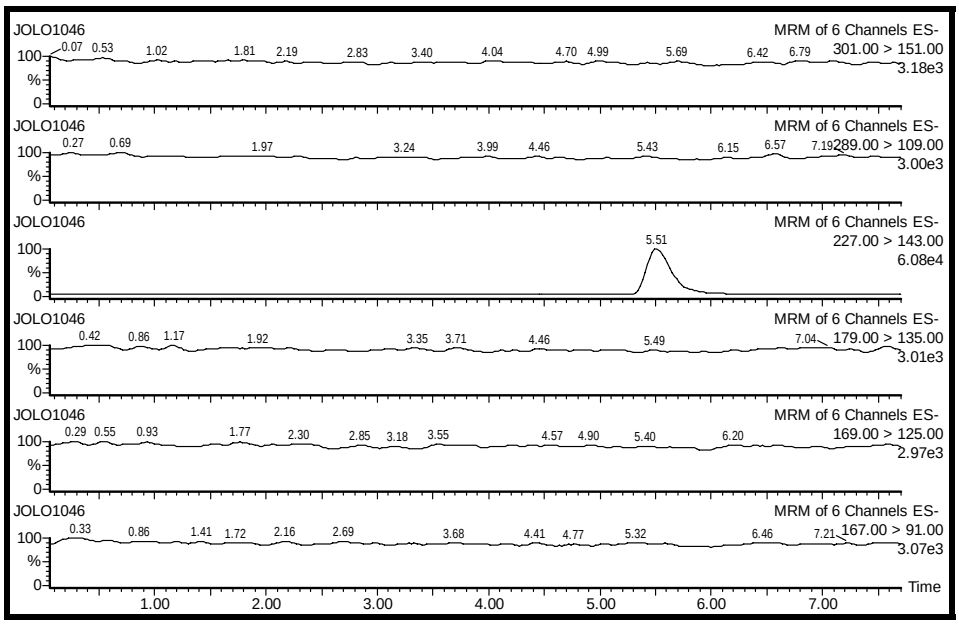


FIGURE 3-28 MASS CHROMATOGRAM OF RESVERATROL, ESI

The success of ESI in terms of the detection of the phenolics is quite apparent. However, the most important compound to be analysed for is HMF. A method of analysis for both the phenolics and HMF were still to be developed.

3.1.5 Near successful results

3.1.5.1 Atmospheric Pressure chemical Ionisation-LC/MS/MS

It was necessary to develop an analytical method that could also detect HMF. Another ionisation technique, namely atmospheric pressure chemical ionisation (APcI), was used to see if HMF could be effectively ionised. If so, it would be advantageous if the other compounds to be measured could also be ionised with the same technique. Double analysis of each sample will thus be avoided. For this reason all the abovementioned compounds were subjected to APcI analysis. During APcI a molecular cation of the type $[M+H]^+$ is generated, and it was again necessary to determine the molecular- and fragment ion combinations for each of the compounds. This was done in the same way as the ESI analysis above. Each standard was diluted in buffer solution A, to 50

µg/ml. 10 µl was injected into a stream of solution A with a flow rate of 50 µl/minute. The spectra obtained can be seen in Figure 3-29 to Figure 3-35.

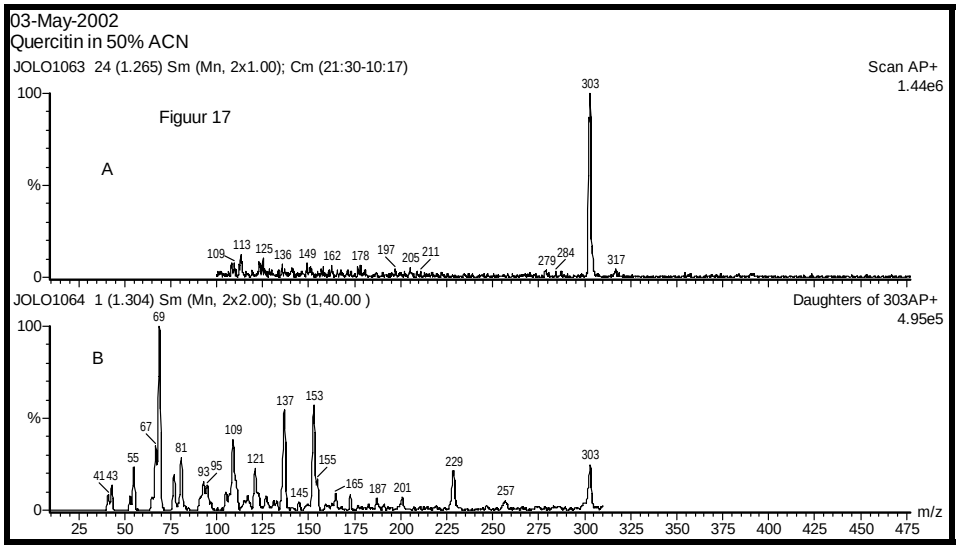


FIGURE 3-29 MASS SPECTRA OF QUERCETIN MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

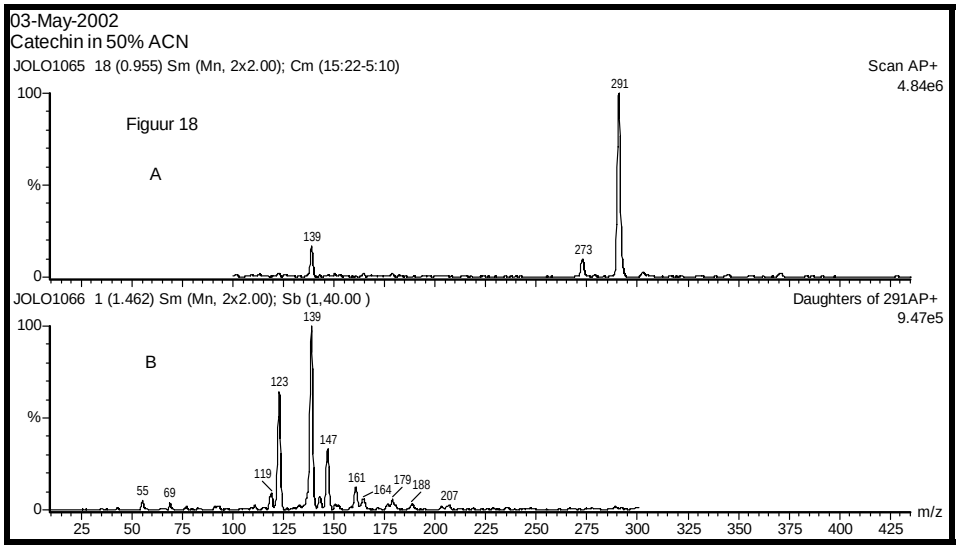


FIGURE 3-30 MASS SPECTRA OF CATECHIN MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

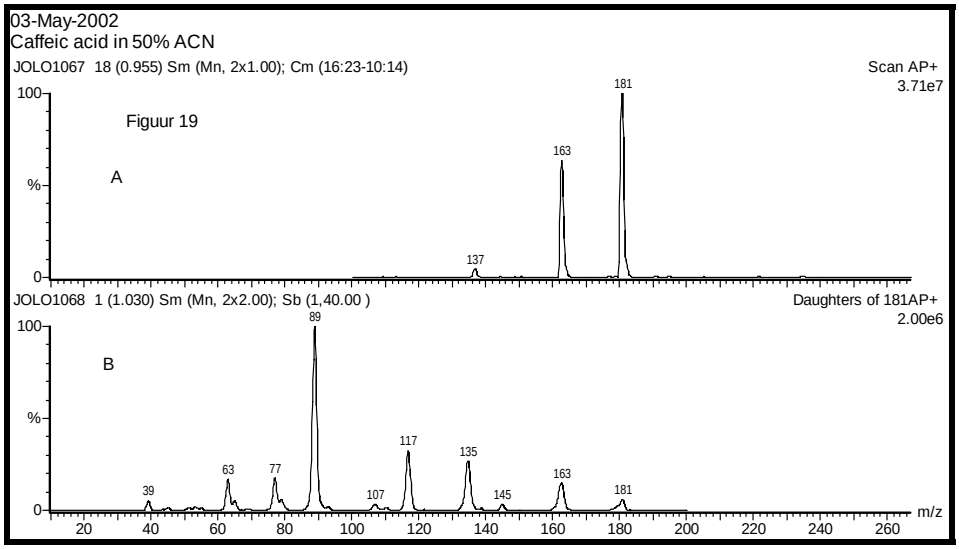


FIGURE 3-31 MASS SPECTRA OF CAFFEIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

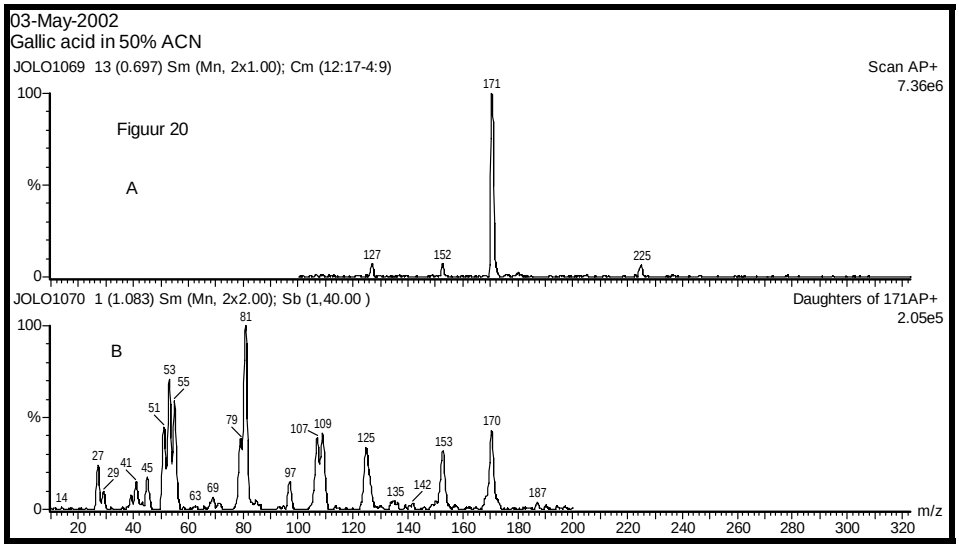


FIGURE 3-32 MASS SPECTRA OF GALLIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

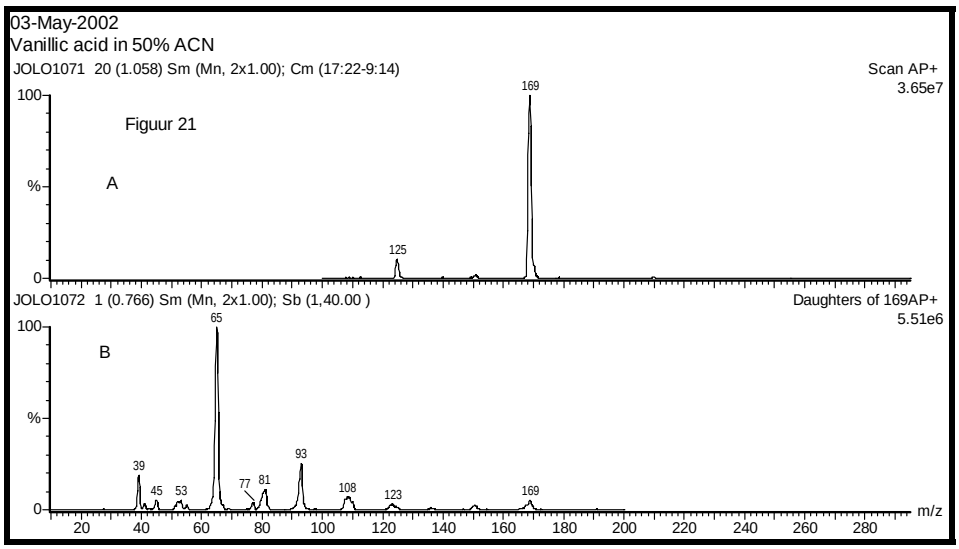


FIGURE 3-33 MASS SPECTRA OF VANILLIC ACID MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

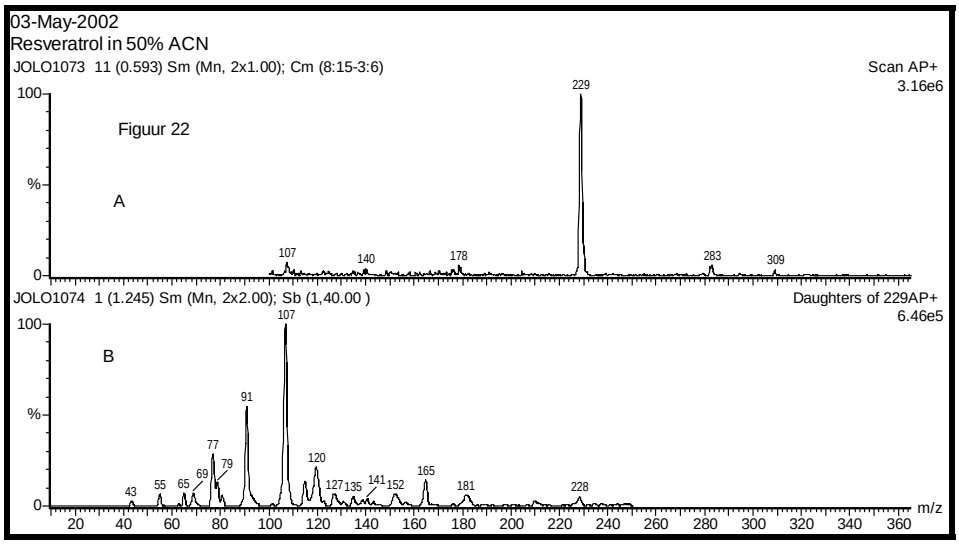


FIGURE 3-34 MASS SPECTRA OF RESVERATROL MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

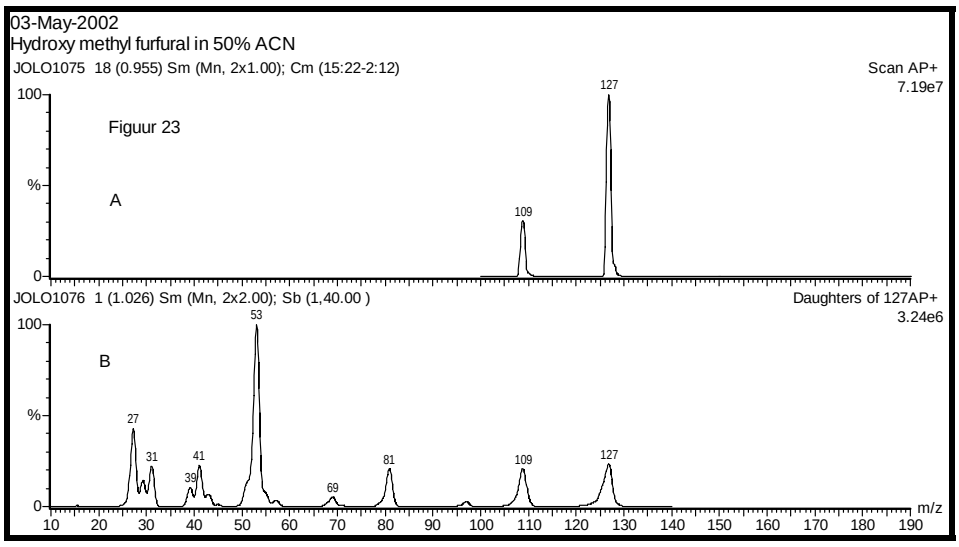


FIGURE 3-35 MASS SPECTRA OF HMF MOLECULAR ION (A), AND FRAGMENTATION SPECTRA (B), APCI

As in the case of ESI analysis the molecular- and fragment ion combinations represent the various compounds. Table 3-2 summarises the combinations obtained from APcI analysis.

TABLE 3-2 MOLECULAR- AND FRAGMENT ION COMBINATIONS, APCI

Substance	Mr g/mol	m/z of molecular-ion	m/z of fragment ion
Quercetin	302	303	69
Catechins	290	291	139
Caffeic Acid	180	181	89
Vanillic Acid	168	169	65
Gallic Acid	170	171	81
Resveratrol	228	229	107
HMF	126	127	53

Figure 3-36 and Figure 3-37 show the chromatograms of the different compounds injected separately. There was no separation performed in this case and it was merely done to show that sufficient detection is obtained for all the compounds based on their molecular- and fragment ion combinations.

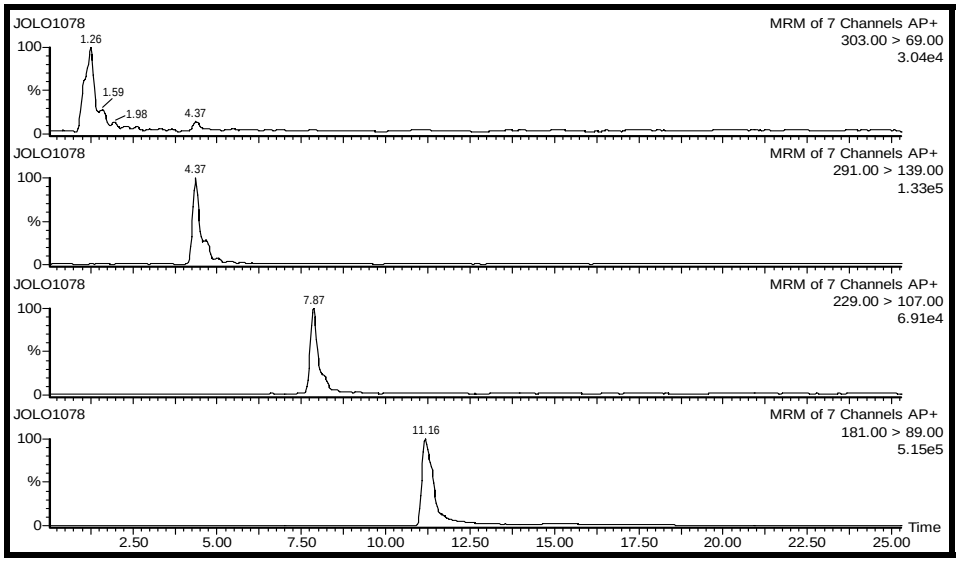


FIGURE 3-36 COMPOUNDS INJECTED SEPARATELY

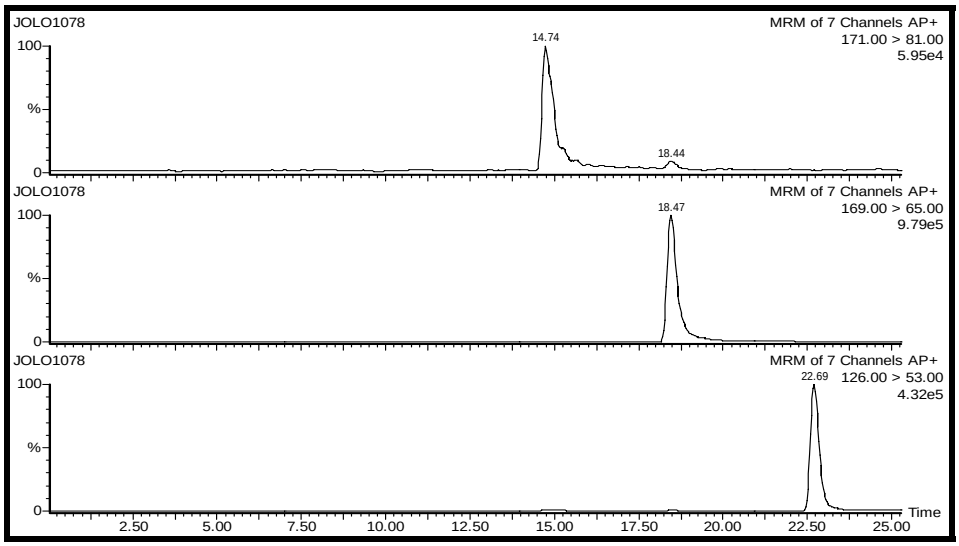


FIGURE 3-37 COMPOUNDS INJECTED SEPARATELY

It was assumed that this detection technique has the potential to analyse all the compounds of interest with one injection. To test this statement an extract of 10 ml grape juice with ethyl acetate was dissolved in 200 µl buffer solution A. The resulting chromatogram is shown in Figure 3-38 and Figure 3-39, indicating detection of all compounds.

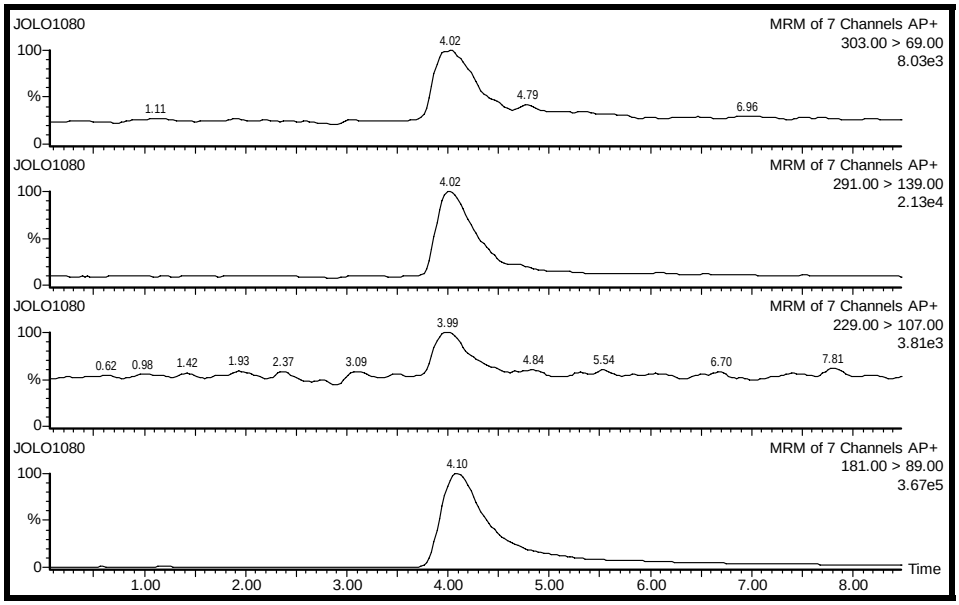


FIGURE 3-38 JUICE SAMPLE INJECTED FOR DETECTION, APCI

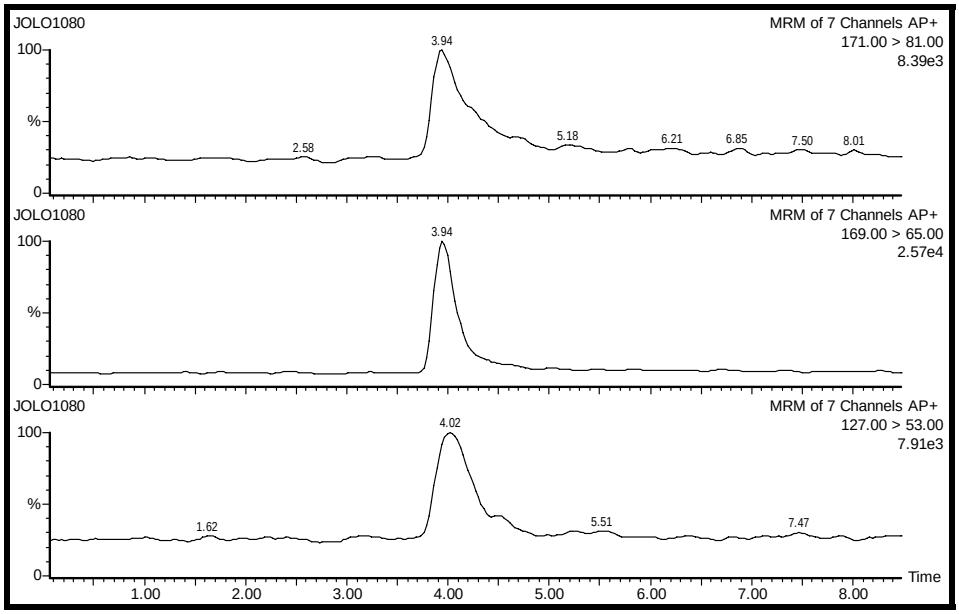


FIGURE 3-39 JUICE SAMPLE INJECTED FOR DETECTION, APCI

Due to technical problems with the analysis facilities the extracted samples had to stored for extensive periods. Although the extracted samples were stored at $-5^{o}C$, the APcI method did not deliver repeatable results after long storage periods, except for HMF. It was then decided to analyse for the most important indicator of browning potential only, namely HMF. A

final method of analysis was developed, namely, positive electron spray ionisation followed by MS-MS (+ESI-MS-MS).

3.1.6 Selected method of analysis: +ESI-MS-MS

Ionisation of polar compounds can be achieved by using atmospheric pressure ionisation (API). One successful technique of API is electro-spray ionisation (ESI). An alternative to ESI is atmospheric pressure chemical ionisation (APcI). APcI is a more robust technique used to ionise less polar compounds.

Quantification of ions can be achieved by setting the instrument to express the intensity of an ion in terms of concentration. Calibration curves are established by plotting intensities of known concentrations of pure standards against the measured concentrations. Unknown concentrations of an ion can thus be read from the calibration curve if the intensity of a specific ion can be determined.

The identity of a specific ion is presented by the mass of this ion. During positive ionisation (e.g., where phenolics are analysed by ESI-MS-MS) the mechanism is ionisation de-hydroxylation. The ion that is observed is thus the $[M\text{-}OH]^+$-ion, which has seventeen (sixteen for oxygen plus one for hydrogen) mass units less than the molecular ion. If the mass spectrometer is set to detect this specific ion, the area underneath the chromatographic peak obtained will be an expression of the concentration of this ion. It is, however, possible for other ions in an extract to have the same mass. To increase the specificity of the detection the molecular ion is fragmented inside the instrument, and the most dominant fragment is then taken as representative of the substance. To explain this detection technique (known as MS/MS) it is necessary to briefly describe the configuration of the instrument.

The mass spectrometer that is used in the ESMS-laboratory at the University of Stellenbosch (Department of Biochemistry) is known as a triple quadropole instrument. It describes an instrument consisting of two quadropole analysers that is positioned in series, with a fragmentation cell in between. Ions leaving the ionisation source (ESI or APcI) are classified by mass in the first quadropole *via* a mass spectrum. To qualify the specific ion (i.e. to fragment it), the first quadropole can be set to let this ion through to the fragmentation cell. In the fragmentation cell, Argon gas molecules collide with the ion, fragmenting it. The fragment ions are classified in the second quadropole via a second mass spectrum, otherwise known as a fragmentation spectrum.

For quantification the second analyser is set to let the most dominant fragment ion through to be registered by the ion-detector as a peak in a chromatograph. The intensity of this peak gives an indication of the original molecular ion concentration.

A mixture of molecules ionised simultaneously can adversely affect the ionisation of a specific ion. This adverse effect is minimised by separating the different molecules before ionisation. HPLC is commonly used to achieve this separation. The entire process is then known as LC-ESI-MS-MS as shown in Figure 3-40.

The chromatographic column used in the HPLC is sensitive to impurities in the feed sample. Impurities result in a decrease in separation efficiency. Sample pre-treatment (e.g. liquid-liquid extraction) usually eliminates this problem.

Grape juice contains sugars and salts that adversely affect the mobility of molecules in a chromatographic column. These sugars and salts are water-soluble. An organic liquid (less polar than and insoluble in water) is used to extract low polarity phenolics from grape juice since these phenolics

will preferentially dissolve in the less polar liquid. This extraction is called liquid-liquid extraction. Ethyl acetate is typically used to extract phenolics from grape juice.

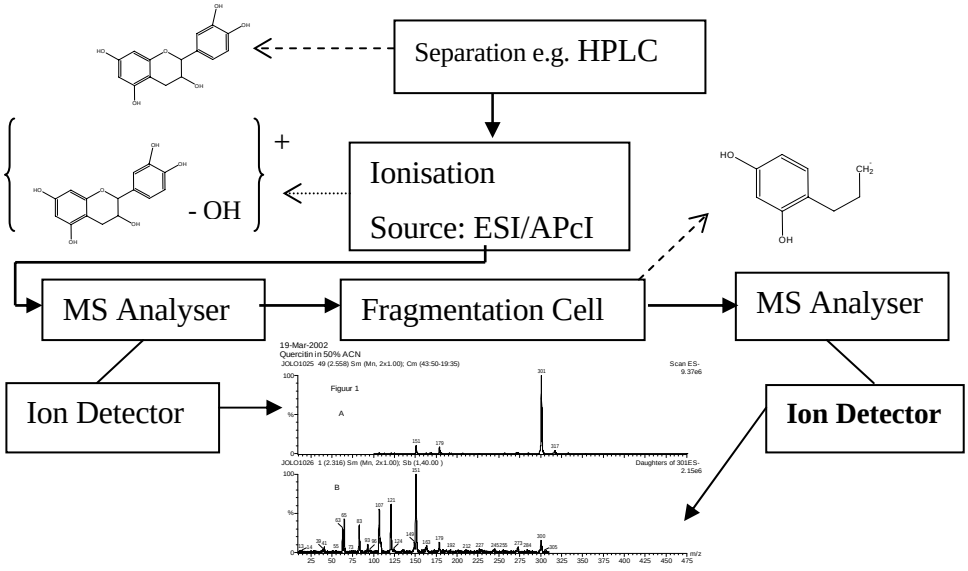


FIGURE 3-40 SIMPLIFIED ILLUSTRATION OF LC-ESI-MS-MS

3.1.6.1 Preparation of calibration curves

9.32 mg HMF was added to 4.36032 g ethanol. This is equivalent to 2.136 mg HMF/g ethanol or 13.391 µmol HMF/ml ethanol (stock solution). Dilution D1 was prepared by adding 1.0198 g of the stock solution to 17.53802 g of solvent (methanol:water:formic acid, 50:50:0.1). The equivalent of 1.029 µmol HMF/ml was obtained.

The calibration standards were prepared by adding additional solvent to D1. The standards (S1, S2, S3, S4 and S5) were prepared as follows:

S1: 5 µl of D1 was added to 995 µl solvent to obtain dilution D2. To 400 µl of D2 was added 600 µl solvent. The equivalent of 2.06 nmol HMF/ml was obtained.

S2: 5 µl of D1 was added to 995 µl solvent. The equivalent of 5.15 nmol HMF/ml was obtained.

S3: 10 µl of D1 was added to 990 µl solvent. The equivalent of 10.315 nmol HMF/ml was obtained.

S4: 25 µl of D1 was added to 975 µl solvent. The equivalent of 25.75 nmol HMF/ml was obtained.

S5: 50 µl of D1 was added to 950 µl solvent. The equivalent of 51.5 nmol HMF/ml was obtained.

S6: 100 µl of D1 was added to 950 µ solvent. The equivalent of 103 nmol HMF/ml was obtained.

The above calibration standards were analysed and the respective measured concentrations noted. Figure 3-41 to Figure 3-44 show the four independent calibration curves plotted at different stages during sample analysis. Linear trend lines were plotted onto the data sets to give an indication of the linearity of the plots.

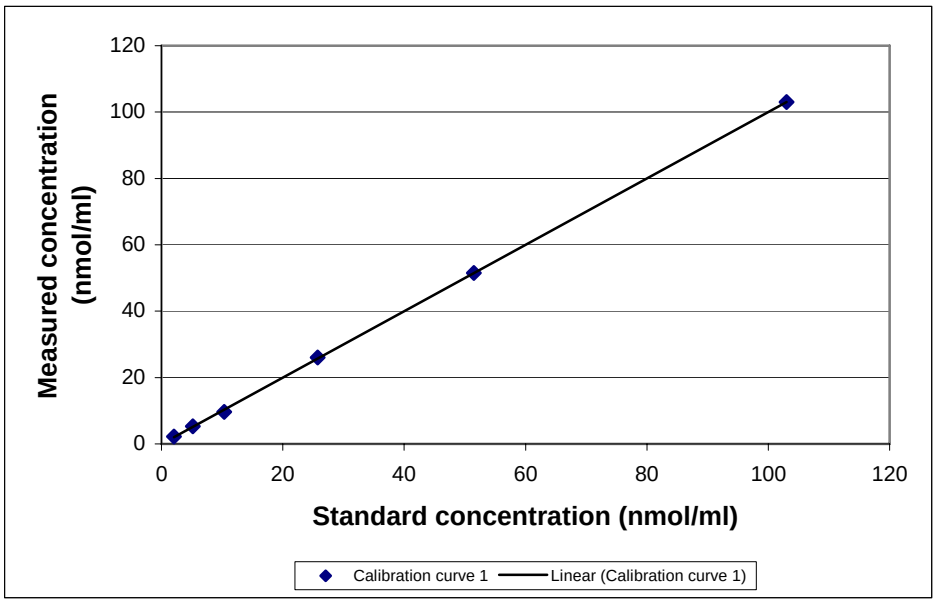


FIGURE 3-41 CALIBRATION CURVE 1

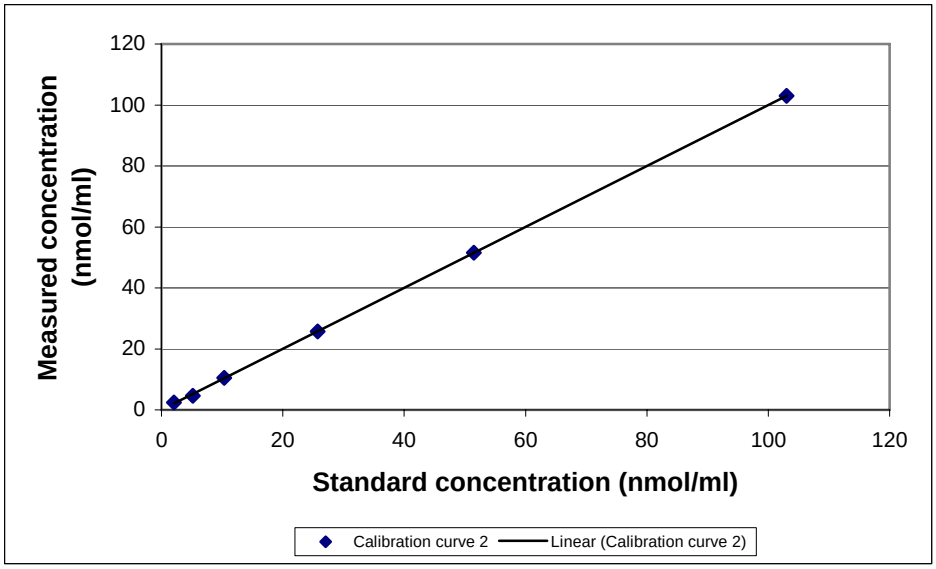


FIGURE 3-42 CALIBRATION CURVE 2

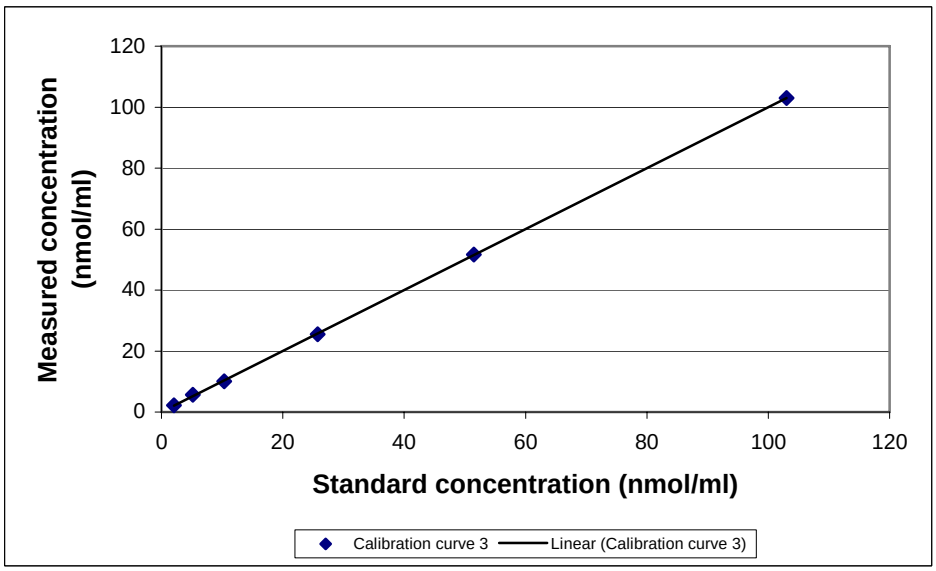


FIGURE 3-43 CALIBRATION CURVE 3

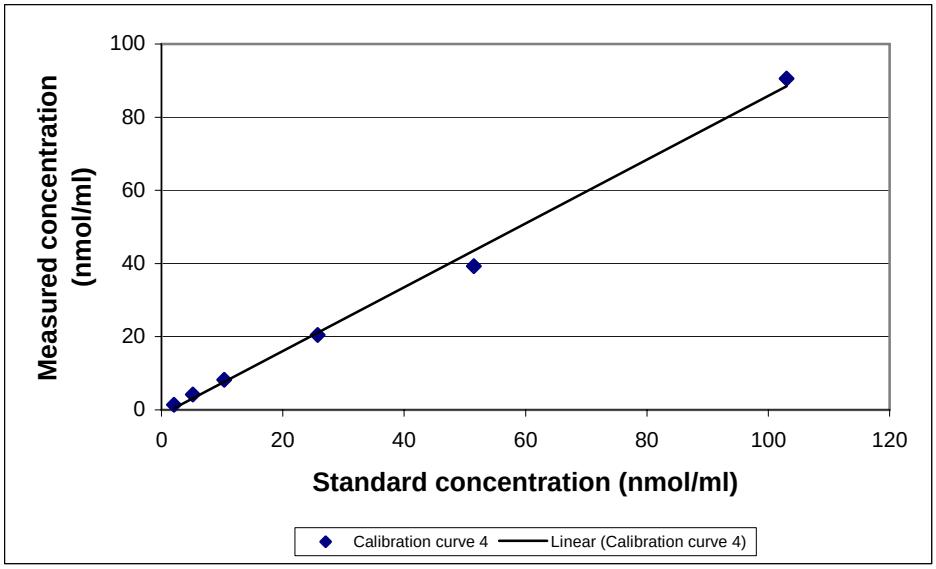


FIGURE 3-44 CALIBRATION CURVE 4

The R-squared values for the above data sets are 0.99992, 0.99993, 0.99994 and 0.99561, respectively. This indicates sufficient linearity and thus good repeatability of the method of analysis.

3.1.6.2 Sample pre-treatment: Liquid-liquid extraction

The following extraction method was used throughout this study, except where stated otherwise:

- With a Gilson Pipetman P1000 pipette, measure 2 ml of juice or GJC from each sample;
- Empty pipette into a 10 ml vial;
- Add 2 ml of ethyl acetate to the sample;
- Seal the vial and shake thoroughly to for approximately 10 seconds to bring the two liquid phases into proper contact;
- Allow the two phases to separate;
- Extract 1.5 ml of the supernatant (ethyl acetate extract) off with a clean pipette and empty pipette into a clean 10 ml vial;

- Add 1.5 ml ethyl acetate to the remaining juice and ethyl acetate extract, seal the vial and shake thoroughly for approximately 10 seconds;
- Extract 1.7 ml of the supernatant off with a clean pipette and add to the previous 1.5 ml;
- Pipette 1 ml of the pooled sample;
- Dry the 1 ml of the pooled sample under nitrogen gas and at a temperature of 37^{o}C;
- When dry, seal vial and store at -5^{o}C.

The dried samples are dissolved with 1000 µl of solvent added to each vial, mixed for 20 seconds and left overnight at 4°C before analysis.

3.1.6.3 LC-ESI-MS-MS analytical equipment and procedure

The chromatographic column used was a C18 Phenomenex Luna 2.0 mm x 150 mm, 3 µm aperture. The mobile phase was solvent (methanol:water:formic acid, 50:50:0.1) delivered at a flow rate of 100 µl/minute by an Agilent 1100 series HPLC system. The analyte injection volume was 10 µl.

The effluent from the column was directed into the electro-spray ionisation source of a Micromass Quattro triple quadropole mass spectrometer. Ionisation was in the positive mode with the capillary voltage at 3.0 kV and the cone voltage set at 35 V. The source temperature was 100°C. All other lenses were set for maximum detection of the required ions.

The analysis was based on multiple reaction monitoring. The molecular ion of HMF was selectively directed into the fragmentation cell, where dissociation of the molecular ion was accomplished by collisionally induced dissociation at an argon pressure of 1.5X10^{-3} mbar and collision energy of 25 eV. The second analyser monitored the most prominent

fragment ion, resulting in a chromatographic peak. The integral of this peak reflected the relative concentration of HMF in the sample.

A calibration curve was produced by analysis of standards of HMF, which was employed to calculate the concentration of HMF in the analyte samples. This was accomplished by the Quantify software program, which is an integral part of MassLynx, the operating software of the mass spectrometer.

3.2 SUMMARY

During the search for an effective method of analysis, a few discoveries were made concerning the compounds that would be analysed. They are:

- The MALDI-TOF analysis showed compounds with molecular weights below 1000 g/mol;
- The HPLC run conducted at the University of Cape Town (UCT) (for the sake of interest) showed a significant similarity to a crude grape seed extract profile. A technical incorrect (and perhaps not so incorrect) assumption that could be made, is that the same phenolic compounds are present in both the GJC, and grape seed extract;
- The other HPLC run conducted at UCT (determination of changes in phenolics content during storage) yielded very similar results for an old and new juice concentrate. The only major differences were a reduction in one peak and an increase in another peak during storage. The rest of the peaks stayed the same. Difficulty in collecting these peaks for further analysis eliminated further attention to these results. However, these results confirmed changes during storage;

- The success of negative electron spray ionisation (ESI) in terms of the detection of the phenolics is quite apparent. However, the most important compound to be analysed for is HMF. This method of analysis proved to be insufficient to analyse for HMF and phenolics simultaneously.
- Due to technical problems with the analysis facilities the extracted samples had to stored for extensive periods. Although the extracted samples were stored at $-5^{o}C$, the APcI method did not deliver repeatable results after long storage periods, except for HMF;

Method selection and method development involved extensive work. Due to difficulty in reproduction of analysis results it was decided to analyse for one compound only, namely HMF. The selected method of analysis, focusing on the qualification and quantification of HMF only, is positive electron- spray ionisation with mass spectrometry (+ESI-MS-MS) preceded by a HPLC. Electron spray ionisation is an atmospheric pressure ionisation (API).

4 EXPERIMENTAL

With the completion of the method of analysis the samples generated during the experimental work could be analysed. The rest of this chapter will present the procedures used during the experimental work. Each of this study's objectives relating to experiments is discussed.

4.1 DETERMINING THE EFFECT OF THE CONVENTIONAL PROCESS ON THE CONCENTRATION OF HMF

This objective can be divided into two subsections, namely:

- The effect of the conventional process by analysis of samples taken after different stages of production;
- The effect of heat induced over a period of time on two grape juice samples, one protein and tartrate stable, and one not protein and tartrate stable.

4.1.1 The effect of the conventional process by analysis of samples taken after different stages of production

4.1.1.1 Sampling

The following samples were taken on the GJC manufacturing plant (Please refer to Figure 2-1):

- Mother tank (i.e. the tank in which the direct concentrate is stored or the tank in which the sulphur juice is stored.);
- After breakdown of the juice to the specified ^{o}Balling, depending on the client's requirements;
- After desulphurisation and before the first concentration;
- After the first effect concentration to approximately 35 ^{o}Balling;

- After the addition of Bentonite and Activated Carbon, just before filtration;
- Directly after filtration;
- After cooling to -4^{o}C but before filtration;
- After filtration and removal of tartrates;
- After second concentration to approximately 76 ^{o}Balling;
- After blending and before pasteurisation;
- After pasteurisation and before drum filling.

These samples were taken three times and each time for a different batch with different client requirements. We labelled the sample sets RA1 to RA11, RB1 to RB11, and RC1 to RC11, respectively.

RA1 to RA11 were of a direct concentrate diluted to 20 ^{o}Balling. This juice contained a sulphur content of approximately 400 mg/L. Before desulphurisation, four 25 L containers were filled with the diluted juice to be used in the experimental procedures that follow. This was done due to sulphur content (and thus resistance to fermentation), and to obtain a juice that would remain constant in constitution during a few weeks of storage at 10^{o}C. Two of the four containers were protein and tartrate stabilised.

4.1.1.2 Protein and tartrate stabilisation

The two 25 L containers were emptied into a larger container and to them was added an amount of a 10% bentonite:water (mass:volume) solution equivalent to 140 g dry bentonite/hL juice. The container was left overnight in a refrigeration room (4^{o}C) and the clear juice was drawn off the following morning and returned to the two 25 L containers (washed).

RB1 to RB11 were also of a direct concentrate with initial sulphur content of 120 mg/L, but this time diluted to 35^{o}Balling and skipping the desulphurisation step.

RC1 to RC11 were of a stored SO_2-juice (with a sulphur content of 1200 mg/L) that has not yet undergone any concentration or heat treatment.

RA, RB, and RC samples were collected in 1 litre plastic bottles with screw-on caps and kept cold to prevent fermentation where possible. Sugar concentration was measured with a CDS Balling/Temperature meter 0-35% sugar in a 100 ml-measuring cylinder. A viscometer could also be used, but due to the murkiness of some of the samples, it was decided that a conventional meter would be used. Since some of the samples contained much more sugar than the range of the Balling-meter would allow, the juice was diluted to within the range of the Balling-meter, measured, noted and reworked to a relative concentration (relative to 70 ^{o}Balling) by making use of an Excel-spreadsheet. The spreadsheet is given in Appendix B - Typical Calculations.

4.1.2 The effect of heat induced over a period of time on two grape juice samples, one protein and tatrate stable, and one not protein and tartrate stable

4.1.2.1 Experiment and sample treatment

Half a litre of the protein stable juice was put into a 1 litre round glass flask with three inline openings (one for sampling, one for a thermometer, and one for refluxing). The flask was put into a heating mantle and the heat setting was set to "one". The contents of the flask were brought to a boil under total reflux. The boiling temperature was registered at 100^{o}C and every twenty minutes a 10 ml sample was taken over a period of three hours. Each sample was allowed to cool down and after cooling a 2 ml representative sample was taken and put into a 10 ml vial. Each sample was then extracted with Ethyl Acetate, dried under nitrogen, and frozen away for analysis in exactly the same manner as described previously.

Half a litre of the protein unstable juice was subjected to exactly the same procedure as above.

This experiment was performed twice with both the protein stable and protein unstable juice to determine the repeatability of the experiment.

4.2 COMPARISON OF THE EFFECT OF THE ADSORPTION PRODUCTS ON THE CONCENTRATION OF HMF

The adsorption products that is used in the experiments of this section is:

- Polyclar V (PVPP);
- NORIT CA1 (A chemically activated carbon);
- NORIT SA4 (A steam activated carbon).

Protea Chemicals, Cape Town, sponsored the Polyclar V and Chemimpo (agents of NORIT), Cape Town, sponsored both the activated carbons. The objectives of these experiments were:

- To determine the effect of protein stability on all these products under various environments;
- To determine the efficiency of HMF adsorption of each of these products;
- To eventually determine the most cost effective and environmentally friendly product (will be discussed in Chapter 6).

This section is divided into two subsections:

- Determining optimum conditions for the products;
- The product profiles.

4.2.1 Determining optimum conditions for the products

The objectives of these experiments were to determine the effect of contact time, protein stabilisation, and temperature on the efficiency of CA1, SA4, and PVPP. The dosage of each product was slightly more than the average

industrial dosage. The dosages used during these experiments is summarised in Table 4-1.

TABLE 4-1 PRODUCT DOSAGES

Product	Dosage (g/Litre)
CA1	4
SA4	4
PVPP	0.5

4.2.1.1 Experiment and sample treatment

Table 4-2 summarises the experimental conditions. For every entry in the table and then again for every product in that entry, a volume of 0.5 litre of juice was put into a 1 litre glass beaker and the dosage mentioned above added. The beaker was then placed in a warm water bath, the temperature set at, e.g. 40^{o}C, and then left at that temperature for, e.g. half an hour after the addition of a product, e.g. CA1. This was done for each product, at every temperature, for every contact period, and for both a protein stable and protein unstable juice. After the completion of the contact time the 1 litre beaker was taken from the warm water bath and the contacted juice was filtered under vacuum. A Buchner filter with Advantec GC50 150 mm, 50-micron glass fibre filter paper (obtained from Kimix laboratory supplies, Cape Town) was used. 2 ml of the filtered juice was then put into a 10 ml vial extracted with Ethyl Acetate, dried under nitrogen, and frozen.

TABLE 4-2 CONDITIONS FOR DETERMINING OPTIMUM PRODUCT CONDITIONS

Time/ Temp	**Room(20^{o}C)**	**40^{o}C**	**60^{o}C**	**80^{o}C**
½ Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
1 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
3 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP
6 Hour	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP	CA1/SA4/PVPP

4.2.2 The product profiles

The objective of these experiments was to determine the effect of dosage volume and protein stability on the products' efficiencies.

Three different dosage volumes of each product were tested separately at a temperature of 55^{o}C, a contact time of 6 hours, and a juice volume of 0.5 litre.

4.2.2.1 Experiment and sample treatment

Half a litre of juice (protein stable or unstable) is again added to a 1 litre glass beaker, placed in a warm water bath (set at 55 ^{o}C), and allowed to reach equilibrium temperature. A desired dosage mass of a specific product is then added to the juice, stirred until homogenous, and left for a period of six hours. Table 4-3 contains the dosage masses of the products used in this experiment. This experiment was done on both a protein stable and unstable juice.

TABLE 4-3 DOSAGE MASSES FOR CA1, SA4, AND PVPP

Sample/Tech.	CA1	SA4	PVPP
1	0.5g	0.5g	0.05g
2	1.0g	1.0g	0.35g
3	2.5g	2.5g	0.6g

4.3 GENERAL OBSERVATIONS DURING EXPERIMENTAL WORK

The following observations were made during the experimental work:

- During addition of SA4 to heated samples, a foam formed releasing a smell resembling hydrogen sulphide (H_2S);
- Foaming was also observed with CA1 addition but in the absence of the smell resembling hydrogen sulphide (H_2S);
- A significant difference in colour between PVPP, CA1 and SA4 could be observed. SA4 treated juice was observed to be of yellow to slightly green colour, while CA1 caused the juice to loose most of its colour. PVPP treatment enhanced the colour of the juice to a bright yellow;
- Better settling of PVPP was observed with unstable juice;

5 RESULTS AND DISCUSSION

This chapter is dedicated to giving the results and observations gathered during this study (organized in order of the objectives of this study) and discussing the results where applicable.

5.1 BACKGROUND AND LITERATURE STUDY

As with most projects, the background and literature study contributed greatly to the directions chosen during the course of this study. A few important aspects covered include:

- The conventional process;
- Decolourisation products;
- Chemistry of discolouring (browning) reactions;
- Choosing adsorption products.

5.1.1 The conventional process

From the description of the conventional process it can be gathered that:

- The direct concentrate of the fresh juice still contains all the natural occurring amines (proteins, amino acids and enzymes);
- The media (concentrate) is acidic – typically with a pH of 3.6;
- The media is exposed to relatively high temperatures during the concentration process for relatively long periods of time (i.e. the desulphurisation process and concentration stages);

Juice is contacted with activated carbon (CA1) in the presence of bentonite during protein stabilisation.

5.1.2 Decolourisation products

Two major methods of dealing with the browning problem in juices, juice concentrates, and wines are found in literature. They are:

- The prevention of the browning reactions by treating the clear juice in some way as to prevent browning;
- Curing of the juice by removing the brown colour formed during storage.

Products tested and used in the prevention and curing of browning reactions included the following:

- Addition of formaldehyde or derivative (HMTA);
- PVP (polyvinylpirrolidine);
- Gelatine and casein;
- Ion exchange resins;
- Ultra-filtration;
- Adding preservatives such as ascorbic acid, thiodipropionic acid, stannous chloride, SO_2, etc.;
- Addition of honey;
- Other adsorption products.

Activated carbon is a non-specific adsorbent. Amines (proteins, amino acids and enzymes) seem to adversely affect the adsorption capacity of activated carbon due to this fact. Bentonite can rather be used as a pre-treatment to activated carbon. This could reduce the dosage volume of activated carbon significantly, and thus reduce the production of solid waste.

5.1.3 Chemistry of discolouring (browning) reactions

It was found that at least four pathways to browning exist, namely:

- Enzymatic oxidative browning;
- Non-enzymatic oxidative browning;
- Non-enzymatic browning (the Maillard reaction);
- Caramelisation.

Each of these chemical reaction pathways has preferred environments in which they occur. From the literature survey Table 5-1 was developed.

TABLE 5-1 A SUMMARY OF THE PREFERRED BROWNING REACTION ENVIRONMENTS

Browning Reaction	Preferred Environments
Enzymatic Oxidative browning	Mild temperatures, mild acidic environment
Non-Enzymatic Oxidative Browning	Acidic environment, higher temperatures
The Maillard Reaction	Acidic environment, high temperature
Caramelisation	Acidic environment, very high temperatures

The information gathered on the conventional process indicates that nearly all these reactions can take place on this plant.

The most common precursors to browning gathered from the literature survey are: gallic acid, vanillic acid, caffeic acid, catechin, epicatechin, quercetin, and HMF. HMF was found to be indicative of the browning potential of GJC. Two pathways to HMF formation were observed from literature. These are:

- The Maillard reaction;
- Non-enzymatic oxidative browning.

Protein stabilization performed during the conventional process removes most of the amines and mild heat treatment will inactivate the enzymes typically present in grape juice concentrate. The possibility of the pathway to browning being enzymatic oxidative browning is thus reduced.

5.2 DEVELOPMENT OF METHOD OF ANALYSIS TO EFFECTIVELY QUALIFY AND QUANTIFY HMF

Method selection and method development involved extensive work. Refer to Chapter 3 for the analysis method selection process. The selected method of analysis, focusing on the qualification and quantification of HMF, is positive electron-spray ionisation with mass spectrometry (+ESI-MS-MS) preceded by HPLC. Electron spray ionisation is an atmospheric pressure ionisation (API).

The method showed good repeatability (linearity/R^2-values of above 0.995). However, the chromatographic column seemed to foul slightly when a large number of samples were analysed consecutively. This can be observed in the retention time shift of the first sample analysed to the last sample analysed (Refer to Appendix C). The chromatographic column was cleaned after each set of samples (of which there was three) thereby resetting the retention time to the original. The column could be washed after each sample analysed to improve the consistency of retention time. However, 250 samples were analysed and cleaning of the column takes 30 minutes. This would amount to days of continuous analyses, which was simply not an option.

More attention should also be given to the method of extraction. Although the results obtained are sufficient to draw some conclusions, the effect of each of the steps of the extraction process on e.g., the HMF concentration, should be traced and optimised where applicable.

5.3 ADSORPTION PRODUCTS

The following general observations regarding the adsorption products were made:

- From the in-house analysis reports (Refer to Table 5-2), it can be seen that NORIT CA1 has a much higher adsorption capacity than NORIT SA4, but that NORIT CA1, does not desorb as well as NORIT SA4;

TABLE 5-2 CUMMULATIVE SORPTION AREA OF CA1 AND SA4

Product	Cummulative adsorption area (m^2/g)	Cummulative desorption area (m^2/g)
CA1	1184.9	667.6
SA4	239.2	234.4

- PVPP is a compound-specific adsorbent, has fast adsorption kinetics, can be quite easily regenerated (where required) and is less reactive than activated carbon.

5.4 INVESTIGATING THE EFFECT OF THE CONVENTIONAL PROCESS ON THE RELATIVE CONCENTRATION OF HMF

Juice samples were taken from three different batches during most stages of the GJC manufacturing process. The three batches included the following three mother tank juices:

- Direct concentrate diluted to 20 ^{o}Balling (Initial juice SO_2-content of 400 mg/L);
- Direct concentrate diluted to 35 ^{o}Balling and skipping desulphurisation (Initial juice SO_2-content of 120 mg/L);
- A stored SO_2-juice that has not yet undergone any concentration or heat treatment (Initial juice SO_2-content of 1200 mg/L).

The stages in the GJC manufacturing process were defined as follows (Please refer to Figure 2-1):

- Mother tank (i.e. the tank in which the direct concentrate is stored or the tank in which the sulphur juice is stored);
- After breakdown of the juice to the specified ^{o}Balling;

- After desulphurisation and before the first concentration;
- After the first effect concentration to approximately 35 ^{o}Balling;
- After the addition of Bentonite and Activated Carbon, before primary filtration;
- Directly after primary filtration;
- After cooling to -4^{o}C before secondary filtration;
- After secondary filtration and removal of tartrates;
- After second effect concentration to approximately 76 ^{o}Balling;
- After blending and before pasteurisation;
- After pasteurisation and before drum filling.

The relative HMF concentrations for each stage in the manufacturing process are plotted for each mother tank juice in Figure 5-1.

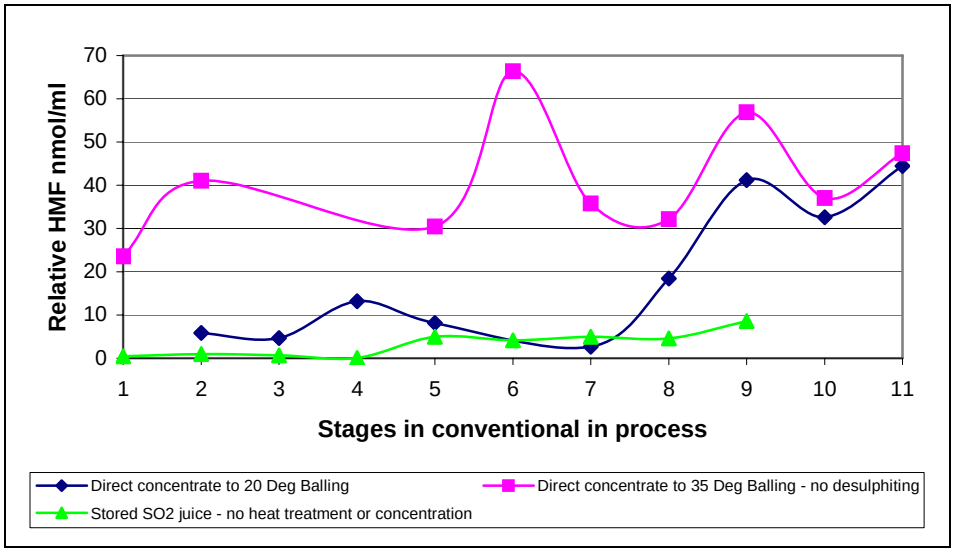


FIGURE 5-1 EFFECT OF CONVENTIONAL PROCESS AND STORAGE AT DIFFERENT CONDITIONS ON HMF CONCENTRATION DURING THE GJC MANUFACTURING PROCESS

The following observations can be made:

- The lower the initial sulphur content of the juice, the higher the HMF forming potential during manufacturing, and thus the higher the browning potential of the GJC;
- Sulphur juice has a much lower HMF forming potential than diluted direct concentrate juice. This can imply that storage of the direct concentrate increases the HMF forming (and thus browning) potential of the final concentrate;
- It is difficult to make comments regarding the effect of heat on the HMF concentration during manufacturing since concentration takes place during manufacturing. Further investigation regarding this aspect was thus required.

5.5 INVESTIGATING THE EFFECT OF HEAT ON HMF CONCENTRATION FOR BOTH A PROTEIN (AND TARTRATE) STABLE AND UNSTABLE JUICE

The initial relative concentration of HMF for all the following experiments is 4.55 nmol/ml. Figure 5-2 contains plotted data of a heat experiment to determine the effect of heat over time on the relative concentration of HMF. The experiment was run at 100^{o}C and samples were taken every twenty minutes for three hours. Duplicate experiments were done to determine the repeatability of the experiments as well as the consistency of the extraction procedure.

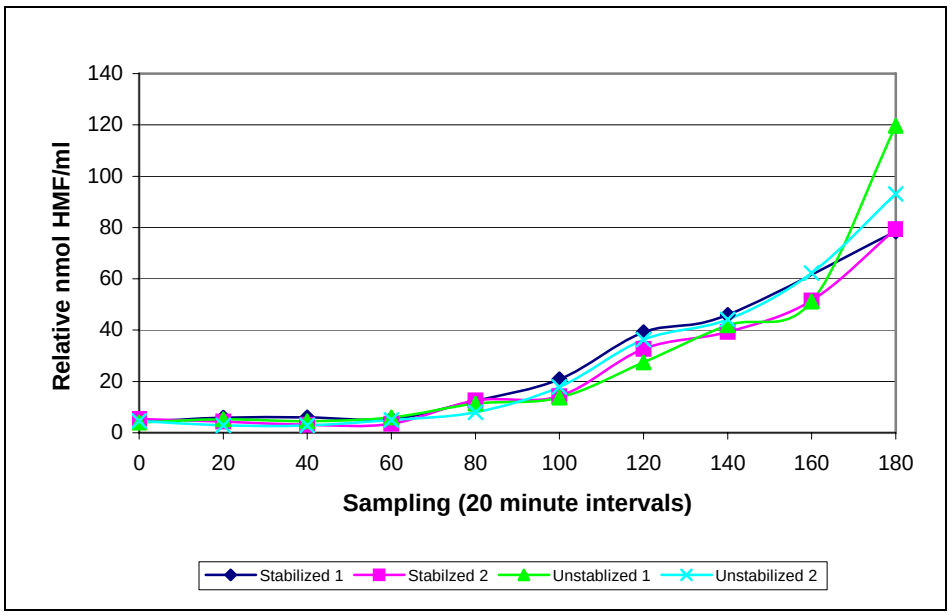


FIGURE 5-2 COMPARISON OF PROTEIN (AND TARTRATE) STABLE/UNSTABLE JUICE AT 100°C AND 20 MINUTE INTERVALS BETWEEN SAMPLING

The observation that HMF increases when the juice is exposed to high temperatures is confirmed in this experiment. Other observations include:

- The four experiments were run independently on four different days. The results show relatively good linearity. Regression of the protein stable and protein unstable results yield R^2-values of 0.984 and 0.931, respectively;
- The formation of HMF is in a high temperature environment is definitely time dependant. A lag phase during the first hour of heat addition indicates no change in HMF concentration. However, an increase in HMF is observed after an hour's heat addition;
- No conclusions regarding protein and tartrate stability can be made with regards to HMF concentration. The HMF in the unstable juice only starts to exceed the HMF in the stable juice after approximately three hours.

5.5.1 Rate of HMF formation based on analysis results from heat experiment

The average HMF concentrations of the four datasets above were used to evaluate the rate of formation of HMF at 100^{o}C and at constant volume. The HMF concentrations from 60 minutes onward were considered since little to no change in the HMF concentration was observed below 60 minutes (Refer to Figure 5-2).

A mole balance across the system (round glass flask under reflux) in terms of HMF yields Equation 5-1 (Fogler, 1999):

$$\frac{dC_{HMF}}{dt} = r_{HMF} \qquad \textbf{EQUATION 5-1}$$

Where C_{HMF} is the concentration of HMF (nmol/ml), t is time in seconds, and r_{HMF} is the rate of formation of HMF. Assuming a rate law with first order kinetics will include Equation 5-2:

$$r_{HMF} = kC_{HMF} \qquad \textbf{EQUATION 5-2}$$

Otherwise expressed as the rate of formation of HMF. k is the rate constant. Alternatively, if assumed that second order reaction kinetics applies, Equation 5-3 will be used instead of Equation 5-2:

$$r_{HMF} = kC_{HMF}^{2} \qquad \textbf{EQUATION 5-3}$$

Combining Equation 5-1 and Equation 5-2 (for evaluation of first order kinetics), rearranging and integrating, yields Equation 5-4:

$$\frac{1}{k}\left(\ln C_{HMF} - \ln C_{HMF,0}\right) = t \qquad \textbf{EQUATION 5-4}$$

If a plot of t *versus* ($\ln C_{HMF} - \ln C_{HMF,0}$) is linear, the rate of HMF formation is of the first order. Alternatively, in combining Equation 5-1 and Equation 5-3, rearranging and integrating, yields Equation 5-5:

$$\frac{1}{k}\left(\frac{1}{C_{HMF,0}}-\frac{1}{C_{HMF}}\right)=t$$ **EQUATION 5-5**

If a plot of t *versus* the factor in brackets yields a linear graph, the rate of HMF formation is of the second order.

The reaction order was estimated by evaluating the data in terms of first and second order reaction kinetics (Detailed calculations in Appendix B – Typical calculations). The corresponding data was plotted against time. Table 5-3 contains the reworked data used to plot the two graphs, Figure 5-3 for first order kinetics and Figure 5-4 for second order kinetics.

TABLE 5-3 REACTION RATE DETERMINATION

Min	Seconds	C_{HMF}	1st order	2nd order
			$\ln(C_{HMF}/C_{HMF0})$	$[1/C_{HMF0}-1/C_{HMF}]$
0	0	5	0.00	0.00
20	1200	11	0.80	0.11
40	2400	17	1.21	0.14
60	3600	34	1.92	0.17
80	4800	43	2.15	0.18
100	6000	57	2.43	0.18
120	7200	106	3.06	0.19

Plotting $\ln\left(\frac{C_{HMF}}{C_{HMF,0}}\right)$ *versus* time in seconds yields Figure 5-3. Correspondingly, plotting $\left[\frac{1}{C_{HMF,0}}-\frac{1}{C_{HMF}}\right]$ *versus* time in seconds yields Figure 5-4.

By observing the linearity of the plots in Figure 5-3 and Figure 5-4 the conclusion can be made that the rate of formation of HMF follows first order reaction kinetics rather than second order. Although the plot of first order reaction kinetics is not entirely linear, it is still a better estimation than second order kinetics. The rate constant, k, for first order reaction kinetics was calculated to be 4.452E-2 s^{-1}.

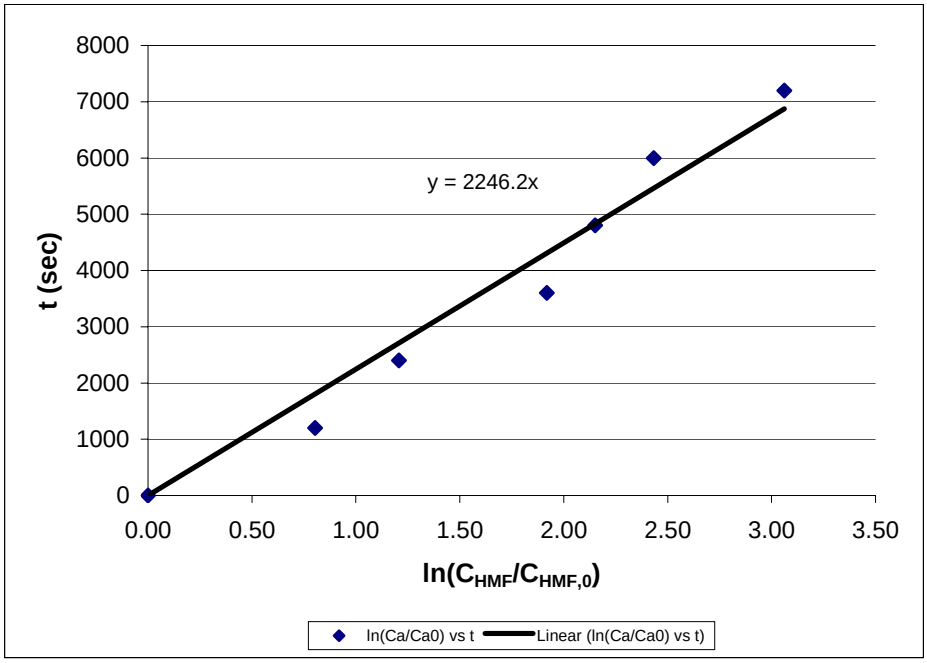


FIGURE 5-3 FIRST ORDER REACTION RATE OF HMF FORMATION AT 100^{O}C

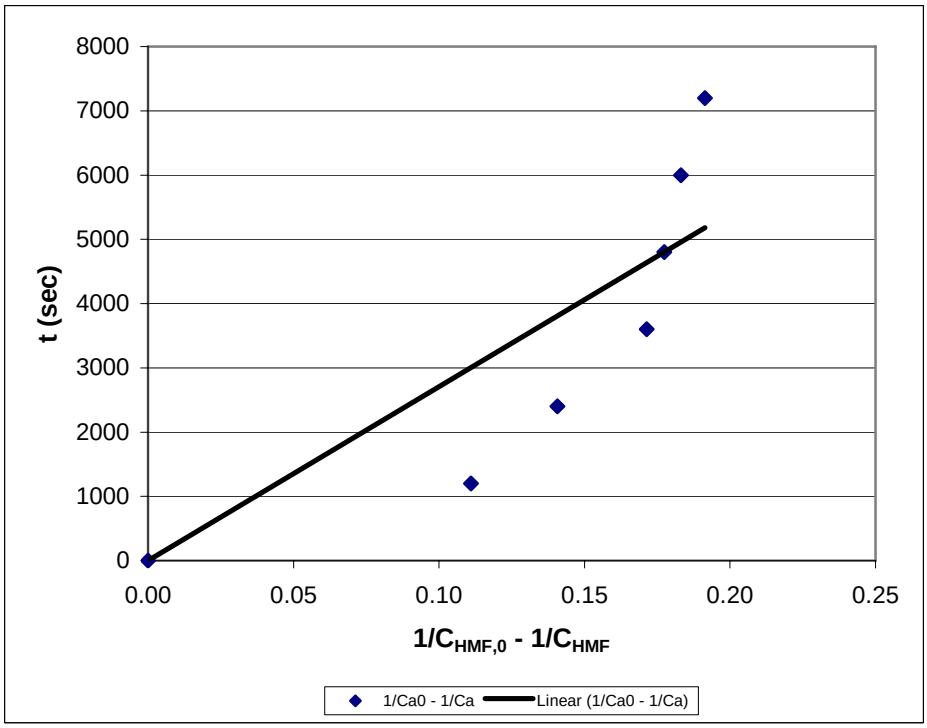


FIGURE 5-4 SECOND ORDER REACTION RATE OF HMF FORMATION AT 100^{O}C

5.6 PRODUCT PROFILES

The objective of the product profile experiments was to determine the effect of dosage volume and protein stability on the product adsorption efficiencies. Figure 5-5 to Figure 5-7 contain the plotted data of different dosages for CA1, SA4 and PVPP, respectively.

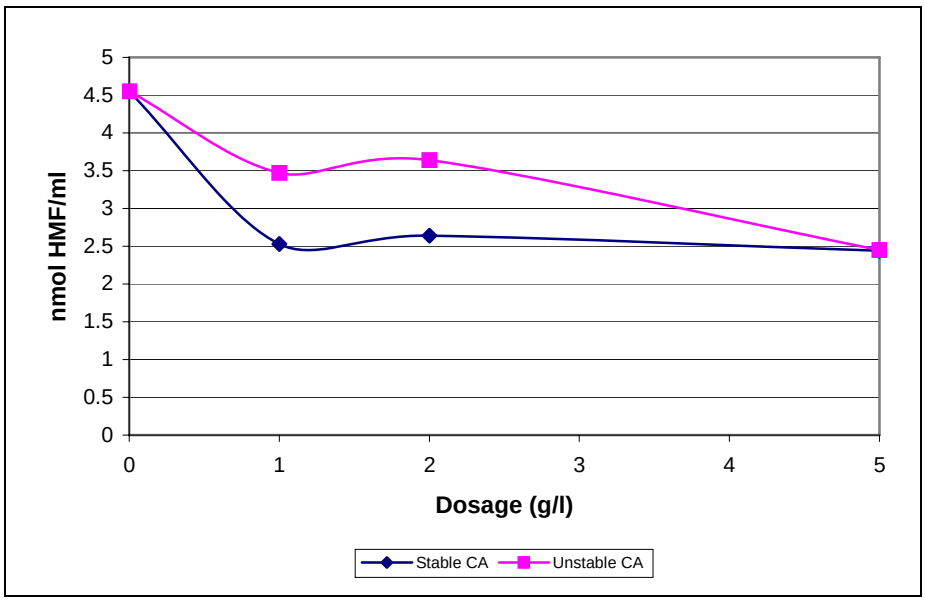


FIGURE 5-5 EFFECT OF CA CARBON DOSAGE DOSAGE ON BOTH STABLE AND UNSTABLE JUICE AT 20°C AND SIX HOURS CONTACAT TIME

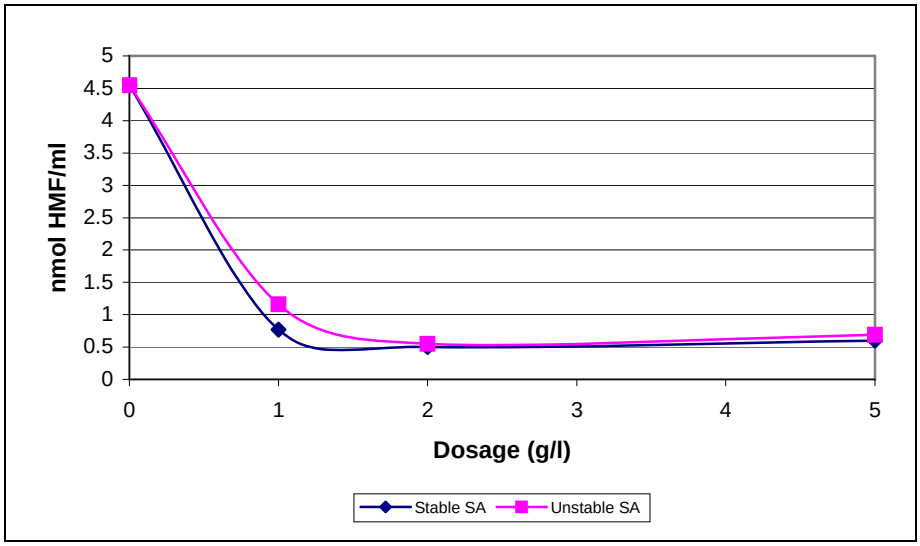


FIGURE 5-6 EFFECT OF SA CARBON DOSAGE ON BOTH STABLE AND UNSTABLE JUICE AT 20°C AND SIX HOURS CONTACT TIME

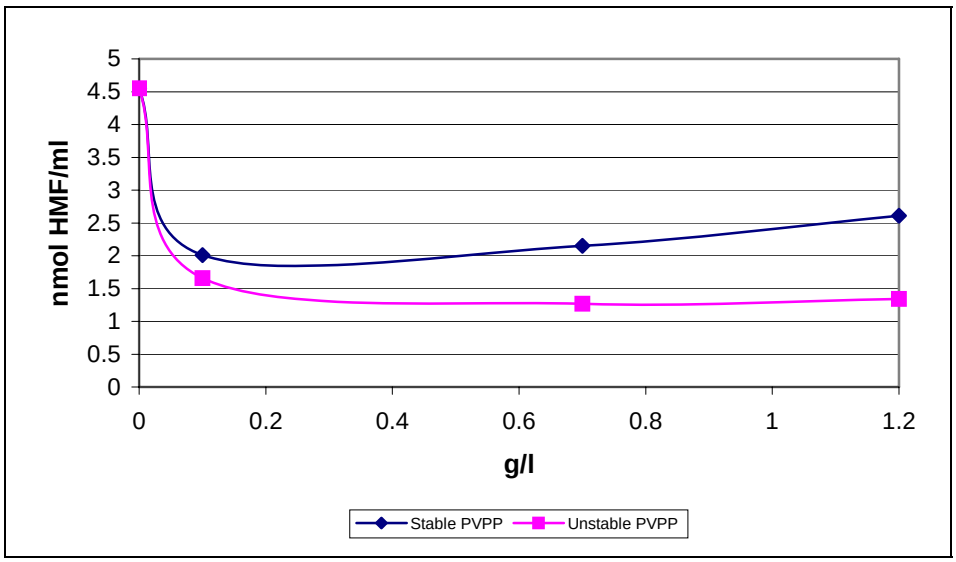


FIGURE 5-7 EFFECT OF PVPP DOSAGE ON 5-HMF IN BOTH STABLE AND UNSTABLE JUICE AT 20°C AND SIX HOURS CONTACT TIME

The following observations can be made from Figure 5-5 to Figure 5-7:

- Protein stability has a significant effect on the adsorption efficiency of CA1, especially at lower dosage volumes. This can be ascribed to the fact that activated carbon is a non-specific adsorbent, and that amines will block active sites on the carbon surface. However, at higher dosage volumes, the effect of protein stability becomes negligible due to the increase of available active sites. To reduce cost without under dosing CA1, the minimum required dosage for CA1 is in the region of 5 g/L;
- The effect of protein stability on the adsorption efficiency of SA4 is not as great as compared to CA1. However, the efficiency of HMF removal is much higher as compared to CA1 (Refer to Table 5-4 for efficiency comparison). To reduce cost without under dosing SA4, the minimum required dosage for SA4 is in the region of 2 g/L;
- An interesting observation regarding PVPP is that protein stability decreases the HMF adsorption efficiency of PVPP. This could be

an indication that the adsorption efficiency of PVPP is enhanced by the presence of amines, i.e. that the amines co-precipitate with the PVPP-polyphenol molecule. It would thus prefer to react with larger amine-polyphenol combination molecules. No conclusion regarding the reaction mechanism (as indicated in Figure 2-9 and Figure 2-10) can be made. To reduce cost without under dosing PVPP, the minimum required PVPP dosage is approximately 0.25 g/L.

TABLE 5-4 COMPARISON OF HMF REMOVAL EFFICIENCY

Dosage	CA1		SA4		Dosage	PVPP	
g/L	Stable (%)	Unstable (%)	Stable (%)	Unstable (%)	g/L	Stable (%)	Unstable (%)
0	0%	0%	0%	0%	0	0%	0%
1	44%	24%	83%	75%	0.1	56%	64%
2	42%	20%	89%	88%	0.7	53%	72%
5	46%	46%	87%	85%	1.2	43%	71%

5.7 INVESTIGATING THE EFFECT OF THREE ADSORPTION PRODUCTS ON THE RELATIVE CONCENTRATION OF HMF

The results for this section were obtained from two sets of experiments. These experiments were chosen to determine the following for both protein (and tartrate) stable and unstable juice:

- The effect of contact time and contact temperature on the HMF removal efficiency of the three adsorption products;
- The effect of dosage volume on the HMF removal efficiency of the three adsorption products at a set time and temperature (similar to the time and temperature on the GJC manufacturing plant).

5.7.1 The effect of contact time and contact temperature on the relative HMF concentration

Figure 5-8 to Figure 5-10 show the effect of contact time and temperature on the adsorption efficiency of CA carbon, SA carbon and PVPP,

respectively. Both carbons were dosed at 4 g/L and the PVPP dosed at 0.5 g/L during these experiments.

Refer to Figure 5-8. The following observations can be made:

- The optimum contact temperature for CA carbon is in the range of 35 to 50^{o}C irrespective of the contact time;
- The optimum contact time for CA carbon is 30 minutes, since this contact time yields the lowest HMF concentration at high contact temperatures;
- At temperatures above 60^{o}C the HMF removal efficiency seemingly decreases as temperature increases. The contact temperature should thus be kept at or below 60^{o}C;
- No conclusions could be drawn regarding protein and tartrate stability, except that it has little effect on CA1's adsorption capacity at a dosage of 4 g/L;
- High contact temperatures and long contact times show a significant increase in HMF.

Refer to Figure 5-9. The following observations can be made:

- The optimum contact temperature for SA carbon is in the range of 20 to 45^{o}C, irrespective of the contact time;
- The optimum contact time for SA carbon is between 30 and 60 minutes, since these contact times yield the lowest HMF concentration at high contact temperatures;
- At temperatures above 50^{o}C the HMF removal efficiency decreases as temperature increases. The contact temperature should thus be kept at or below 50^{o}C;

- No conclusions could be drawn regarding protein and tartrate stability, except that it has little effect on SA4's adsorption capacity at a dosage of 4 g/L;
- High contact temperatures and long contact times show a significant increase in HMF.

Refer to Figure 5-10. The following observations could be made:

- The optimum contact temperature for PVPP is in the range of 20 to 50^{o}C, irrespective of the contact time;
- The optimum contact time for PVPP is between 30 and 60 minutes, since these contact times yield the lowest HMF concentration at high contact temperatures;
- At temperatures above 60^{o}C the HMF removal efficiency decreases as temperature increases. The contact temperature should thus be kept at or below 60^{o}C;
- At 70^{o}C protein stability seems to affect adsorption capacity of PVPP (refer to 3-hours and 6-hours contact times). This might indicate that the amines start to react with reducing sugars to form HMF (However, this statement should be investigated). No other conclusions could be drawn regarding protein and tartrate stability, except that it has little effect on PVPP's adsorption capacity at a dosage of 0.5 g/L;
- High contact temperatures and long contact times show a significant increase in HMF.

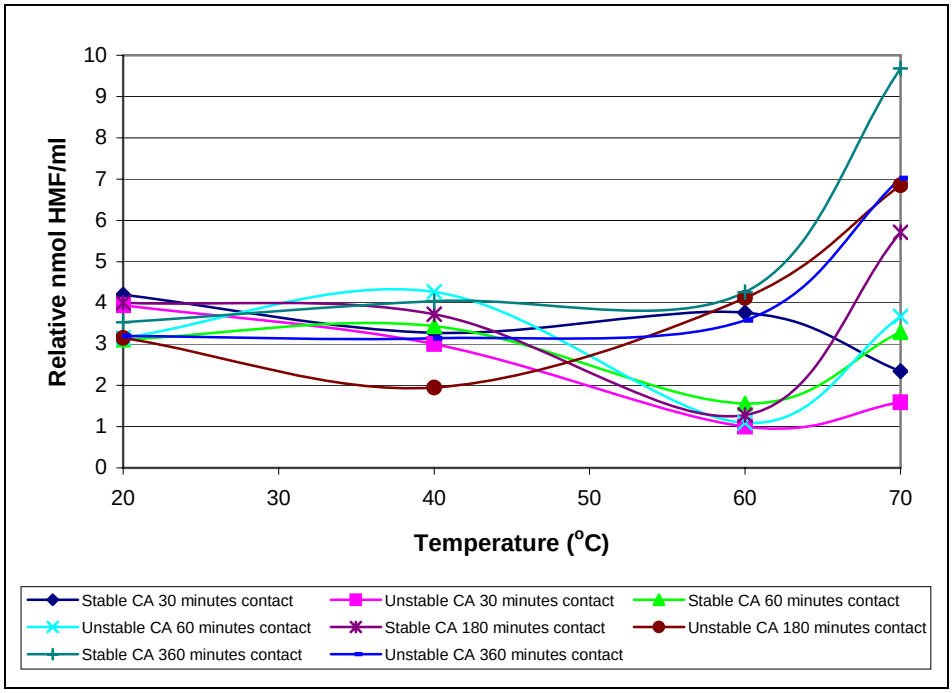


FIGURE 5-8 EFFECT OF TEMPERATURE ON CA CARBON AT VARIOUS CONTACT TIMES AND DOSAGE OF 4 G/L

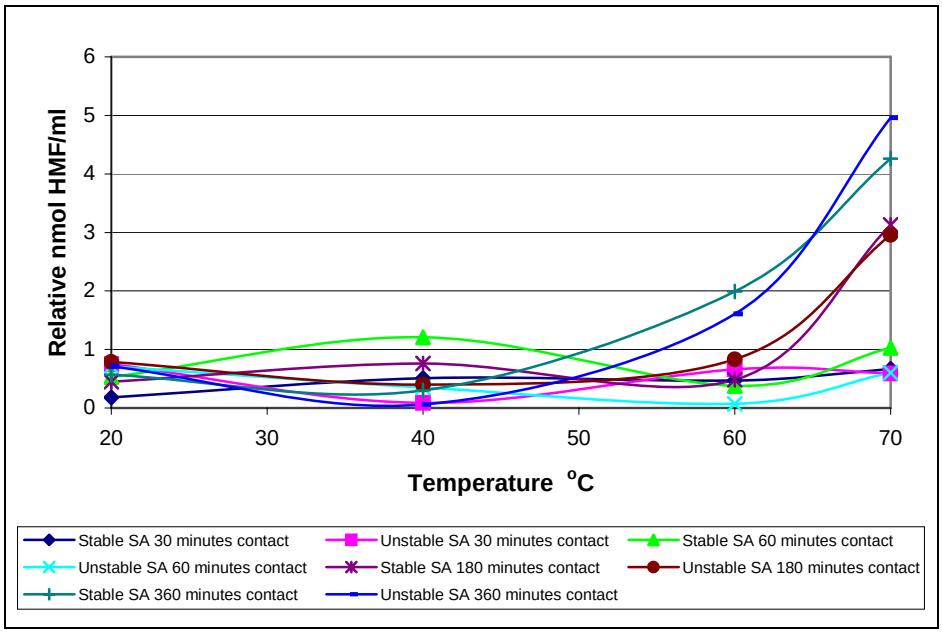


FIGURE 5-9 EFFECT OF TEMPERATURE ON SA CARBON AT VARIOUS CONTACT TIMES AND DOSAGE OF 4 G/L

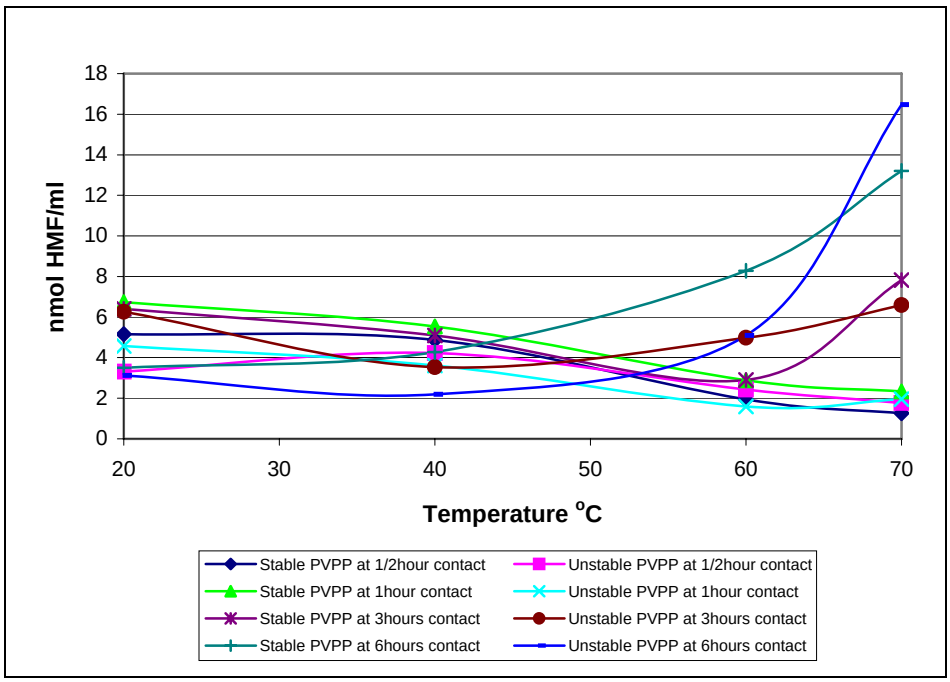


FIGURE 5-10 EFFECT OF TEMPERATURE ON PVPP AT VARIOUS CONTACT TIMES AND DOSAGE OF 0.5 G/L

5.8 SUMMARY

Direct concentration and storage of fresh juice is favourable for both Maillard- and/or non-enzymatic browning reactions. This is confirmed when comparing results from a diluted fresh juice concentrate (containing all natural occurring amines), sampled at the various stages of the conventional process, with results of a SO_2-juice (sampled similarly). Refer to Figure 5-1. This confirms a statement made regarding the effect of sulphur dioxide on non-enzymatic colour development by Bostan and Boyacioglu, 1997.

Protein stability affects CA1 at low dosages by decreasing HMF adsorption. SA4 is less affected by protein stability at similar dosages. PVPP requires protein to be effective, however at high temperatures (70°C) and long contact periods (3 hours and 6 hours) the efficiency seems to increase with protein stable juice and decrease with protein unstable juice.

However, all conclusions regarding protein stability cannot be statistically confirmed, since too little data were collected.

Heat treatment (combined with long exposure times) has a definite effect on the concentration of HMF and thus the browning potential of GJC. No conclusive difference was observed between protein stable and unstable juice. From the heat experiment results it can be derived that heat is the factor contributing most to an increase in HMF. This is in line with the findings of Bozkurt et al., 1999 implying that non-enzymatic oxidative browning is the ruling reaction during GJC browning (or HMF formation).

It was furthermore determined that a lag phase exists in the reaction(s) involving HMF formation during the heat experiments. This confirms the findings of Quintas et al. (2003) and might indicate that some of the precursors of HMF have to reach a critical concentration before the HMF-forming reactions are "initiated". At 100^{o}C the lag phase is approximately one hour, corresponding to zero increase in HMF. After one hour the rate of HMF-formation follows approximately first-order kinetics with a reaction rate constant (k) of 0.04452 s^{-1}.

The overall results seem to indicate that less heat treatment, protein stability, shorter times of storage and/or preservation with SO_2 will increase product quality.

6 EVALUATION OF THE CONVENTIONAL PROCESS

Following the results and discussion in Chapter 5, this chapter serves to suggest the following:

- Possible alterations to the conventional process to reduce the effects of heat on GJC in terms of browning potential (i.e. minimize increase in HMF concentration);
- Possible alternative adsorption products that can be used instead of CA1 during the decolourisation stage of the conventional process;
- Possible alternative concentration technologies that can be implemented (instead of the existing technologies e.g. falling-film evaporation, etc.).

A cost comparison (of basic operating expenditure) between the existing process and the alternative options serves as conclusion.

6.1 POSSIBLE ALTERATIONS TO THE CONVENTIONAL PROCESS

From Chapter 5 there seems to be one major area where the conventional process can be improved, namely: A reduction in heat treatment. Heat treatment can possibly be reduced in the following ways:

- Protein stabilisation before concentration, possibly reducing time required to decolourise a diluted direct concentrate of a fresh juice (and thus heat treatment);
- Storage of SO_2-juice instead of making direct concentrate of the fresh juice (implying a once-off concentration when required).

6.1.1 Protein stabilisation

Protein stabilisation is currently performed after the first concentration stage for SO_2-juice and after dilution of direct concentrate juice.

Decolourisation is performed during this step by addition of CA1 (activated carbon). Possible ways of minimising solid waste production and saving on operating cost include the following:

- Performing protein stabilisation prior to delivery to GJC plant;
- Performing protein stabilisation at GJC plant but prior to direct concentration.

If protein stabilisation is done at the wine cellars, a rebate for the bentonite used can be issued. This rebate can be coupled to the volume of juice undergoing stabilisation. Both of these options involve certain advantages and disadvantages.

The following possible advantages exist for the above alteration in the GJC manufacturing sequence:

- A reduction in the required CA1 (possibly half, refer to Figure 5-5);
- A reduction in the required CA1 will imply a reduction in the solid waste produced;
- Contracted companies can more easily reactivate bentonite due to the fact that it is not polluted with activated carbon. Thus an even greater reduction in solid waste is possible;
- A possible reduction in filtration media, since less solids is to be removed;
- Reduced exposure to high temperatures due to a shorter contact time required for decolourisation, namely a maximum of 2 hours (as opposed to seven hours for joined protein stabilisation and decolourisation in the conventional process at $55^{o}C$);
- A shorter GJC manufacture cycle and reduced boiler operating cost per cycle, i.e. an overall energy saving (An estimated heating cost saving of at least 20% should be attainable due to the drastic

reduction in the manufacturing time. Possible operating cost saving will not be taken into account for the purpose of this study.).

A few possible disadvantages/shortcomings include the following:

- A possible shortage of protein stabilisation capacity at wine cellars or GJC-plant (i.e. available tanks);
- Possible settling difficulty of suspended CA1 during the decolourisation of GJC.

6.2 DECOLOURISATION PRODUCT

Without changing the sequence of the conventional process, a change in decolourisation product might involve possible operating cost reduction. Both SA4 and PVPP indicate superior HMF adsorption capacity when compared to CA1. Again a reduction in solid waste produced is a possible advantage.

6.3 ALTERNATIVE CONCENTRATION TECHNOLOGIES

Falling-film evaporation (as was used in the conventional process) is quite harsh with regards to exposure to heat. Newer technologies have the capability to reduce heat treatment by up to 95% with the added advantage of significant savings in operating costs. These technologies include:

- Reverse osmosis (RO) – capable of concentrating grape juice to approximately 50 ^{o}Balling (High pressures are however required to accomplish this i.e. > 60bar(g));
- Centrifugal evaporation (CE) – capable of concentrating the juice to 70+ ^{o}Balling in one stage and in a tenth of the time when compared to falling-film evaporation;
- A combination of RO and CE – capable of concentrating the juice to 70+ ^{o}Balling with even less heat treatment.

Capital costs for these technologies are significant and will typically reach R10,000,000 to R12,000,000 to cater for the requirements of this plant.

6.4 COMPARING RUNNING COSTS OF THE CONVENTIONAL PROCESS AND THE CONVENTIONAL PROCESS WITH SUGGESTED CHANGES

Capital expenditure is ignored in this section, and only simplified operating costs are taken into account. Although there may be even greater savings in operating cost (e.g. boiler fuel savings and water savings), the simplification adds to the conservative approach to determining these costs.

6.4.1 Protein stabilisation

The following assumptions were made:

- Wherever protein stabilisation is performed, there is sufficient stabilisation capacity;
- The rebate for bentonite used during the protein stabilisation at the wine cellars is equal to the cost of bentonite used during the conventional process. This will not be the case, since less bentonite will be required. This assumption is thus conservative;
- 2 g/L will be required instead of 4 g/L (as per the conventional process), although experiments indicate that 1 g/L (refer to Figure 5-5) will be sufficient if protein stabilisation is performed prior to decolourisation;
- Possible filter media saving is negligible (although this will probably not be the case);
- Heating cost savings is negligible (although this will probably not be the case).

With the above assumptions the estimated possible operating cost saving amounts to approximately 50% of the activated carbon cost of the conventional process. The cost (at time of study) was R13.60/kg CA1 at a dosage of 4 g/L. The GJC manufacturing plant of this study produced approximately 20 million litres of GJC per annum, the annual CA1 cost for the conventional process would amount to R2,160,000. This would imply an operating cost saving of roughly R1,080,000 per annum.

6.4.2 Decolourisation product

It is conservatively assumed that the following dosages of SA4 and PVPP will replace CA1 in the conventional process: 2g/L and 0.5g/L, respectively. The costs for these two products (at time of study) were R22.50/kg SA4 and R260.00/kg PVPP. The estimated operating cost comparison (only taking into account adsorption product cost) is summarised in Table 6-1.

TABLE 6-1 OPERATING COST COMPARISON O DIFFERENT ADSORPTION PRODUCTS

	CA1	SA4	PVPP
Cost/kg	R13.60	R22.50	R260.00
Dosage/L	4g	2g	0.5g
Annual dosage	160,000kg	80,000kg	20,000kg
Annual cost	R2,160,000	R1.800,000	R5,200,000

From Table 6-1 the observation can be made that PVPP will not be an economically viable replacement for CA1. However, replacing CA1 with SA4 will imply an annual operating cost saving of approximately R360,000.

7 CONCLUSIONS AND RECOMMENDATIONS

7.1 BACKGROUND AND LITERATURE STUDY

The literature study showed that extensive work has been done in studying browning reactions and ways of preventing and curing the effects of such reactions. Four possible browning reaction pathways in grape juice were observed, namely:

- Enzymatic oxidative browning;
- Non-enzymatic oxidative browning;
- Non-enzymatic browning (the Maillard reaction);
- Caramelisation.

Enzymatic oxidative browning involves enzymatic catalysts such as Polyphenol Oxidase (Phenolase, PPO), or Peroxidase to assist in the formation of o-quinones from phenolics in the juice, which polymerises to form brown-coloured polymers. Enzymes present in grape juice are deactivated with mild heat treatment.

Non-enzymatic oxidative browning is similar to enzymatic oxidative browning except that an acidic media acts as catalyst.

Non-enzymatic browning, otherwise known as the Maillard reaction, is quite complex. It involves three stages, namely:

- The condensation of an amino acid with a reducing sugar to form Amadori or Heyns rearrangement products via an N-substituted glucosylamine;

- The degradation of the Amadori and Heyns rearrangement products (this stage can follow four to five pathways of which this study focussed on the those pathways involving the formation of HMF);
- The final stage of the Maillard reaction is characterised by the formation of brown nitrogenous polymers and co-polymers.

Caramelisation was discarded as a possible browning reaction pathway, since this would involve little to no water content in combination with exposure to very high temperatures for a significant period.

When investigating the manufacturing sequence of the conventional process the following observations were made:

- The direct concentrate of the fresh juice still contains all the natural occurring amines (proteins, amino acids and enzymes);
- The media (concentrate) is acidic;
- The media is exposed to relatively high temperatures during the concentration process for relatively long periods of time.

It can be derived that the browning pathway is not enzymatic of nature, since enzymes present in grape juice are deactivated with mild heat treatments.

From the literature study HMF was seen to be indicative of the browning potential of grape juice or GJC. The two remaining pathways to browning, namely the Maillard reaction and non-enzymatic oxidative browning, both involve the formation of HMF as an intermediate product.

7.2 ANALYSIS: METHOD DEVELOPMENT

A method of analysis was developed to qualify and quantify HMF. The method chosen was positive electron-spray ionisation with mass spectrometry. A possible shortcoming of this method is the extraction used

prior to analysis. The extraction method was not optimised. If a higher HMF yield for the analysis is required this method of extraction should be optimised. However, the method of analysis showed good repeatability when observing the results of the heat experiments. The extraction method thus served the purposes of this study.

7.3 EXPERIMENTAL

The following conclusions regarding experimental work done, can be made:

- The formation of HMF is strongly dependent on temperature and contact time. A lag phase of approximately one hour was observed during the heat experiments. This could be an indication that the precursors to HMF are required to reach a certain critical concentration before the HMF-forming reactions are "initiated". It is recommended that this phenomenon be further investigated.
- It was found that the rate of HMF formation start to follow roughly first order kinetics after an hour's exposure to 100^{o}C. The rate of formation of HMF was estimated as: $r_{HMF} = 0$ for t < 1hour and $r_{HMF} = 0.04452 C_{HMF}$ for t > 1hour. The high temperatures and excessively long periods of exposure (to these temperatures) during the conventional process will thus assist in the formation of unwanted HMF. Protein stability had little to no effect on the rate of formation of HMF;
- Protein instability has a negative effect on the adsorption capacity of CA1 (chemically activated PAC) below a dosage volume of approximately 4g/L. The effect of protein stability on SA4 (steam activated PAC) and PVPP (Polyclar V) was not that significant. However, it seems that PVPP has an increased adsorption capacity in the presence of natural occurring amines. Furthermore,

significantly better HMF adsorption characteristics were observed with SA4 and PVPP than with CA1. This leads to the conclusion that another adsorption media (e.g. SA4 or PVPP) will produce less solid waste due to less adsorption media required for the same degree of decolourisation;

7.4 EVALUATION OF THE CONVENTIONAL PROCESS

The conventional process favours the formation of HMF due to relatively high temperatures and long exposure to these temperatures. Based on the experimental results and the availability of more advanced concentration technologies, the following recommendations are made (in order of preference):

- Although high initial capital costs are required, it is recommended that the existing rising film evaporators be replaced by either membrane concentration (RO) followed by centrifugal evaporation (CE) or CE in two stages. The advantages should include significant operating cost saving and a superior product. However, before such an investment is made a detailed risk study should be conducted to evaluate the economic viability;
- Investing in additional SO_2-juice storage tanks should also be considered. Less activated carbon will be required and again a superior product will be produced due to less heat treatment than is required for direct concentrate juice. As with the above recommendation a detailed risk study to evaluate economic viability should be performed. Alternatively SO_2-juice can be stored at other cellars should they have the capacity;
- Protein stabilisation should be done at the wine cellars prior to delivery to the GJC manufacturing plant to reduce the required dosage of activated carbon (CA1). Furthermore, SA4 should be considered as replacement for CA1. However, it is recommended

that more detailed testing be done to confirm the viability of this replacement.

7.5 ADDITIONAL RECOMMENDATIONS

The phenomenon that SA4 adsorbs 5-HMF better than CA1 despite the fact that CA1 has a far greater pore volume capacity should be investigated. The formation of the hydrogen sulphide like odour on the addition of SA4 to heated juice might be an indication that chemisorption (instead of adsorption only) contributes to the removal of 5-HMF.

It is recommended that steam activated carbon *versus* chemically activated carbon from more than three suppliers be compared in terms of 5-HMF removal, pore volumes and surface reactions to shed some light on the above.

8 REFERENCES

Arena, E., Fallico, B. & Maccarone, E., 2000, "Influence of carotenoids and pulps on the color modification of blood orange juice", Journal of Food Science, Volume 65, No.3, pp458-460

Bickler, B., 2001, "High speed flash fractionation of natural products – polyphenols", Biotage, Technical Publication

Borneman, Z., Gökmen, V. & Nijhuis, H.H., 2001, "Selective removal of polyphenols and brown colour in apple juices using PES/PVP membranes in a single ultr-filtration process", Separation and Purification Technology, Volume 22-23, pp. 53-61

Bostan, A. & Boyacioglu, D., 1997, "Kinetics of non-enzymatic colour development in glucose syrups during storage", Food Chemistry, Volume 50, No. 4, pp. 581-585

Bozkurt, H., Gögüs, F. & Eren, S., 1999, "Nonenzymatic browning reactions in boiled grape juice and its models during storage", Food Chemistry, Volume 64, pp. 89-93

Bru, E., Barroso, C.G. & Bustamante, J.A., 1995, "Study of treatment with different adsorbants on sherry dry white wine", Acta Horticulturae, Volume 388, pp. 255-259

Buedo, A.P., Elustondo, M.P. & Urbicain, M.J., 2001, "Non-enzymatic browning of peach juice concentrate during storage", Innovative Food Science and Emerging Technologies, Volume 1, pp. 255-260

Cantarelli, C., Pompei, C., Peri, C. & Montedoro, G., 1971, "Formaldehyde for prevention of oxidative browning of white wines", Am. J. Enol. Vit., pp. 59-64

Caputi, A. JR, Brown, T., Ueda, M., 1969, Residual polyvinylpyrrolidone in wine", Am. J. Enol. Vit., pp. 152-154

Daume, J., 2001, "Cellar chores to do this month (April)", www.lafn.org/community/cellarmasters/chores2.htm

Dutson, T.R. & Orcutt, M.W., 1984, "Chemical changes in proteins produced by thermal processing", Journal of Chemical Education, Volume 61, No. 4, pp. 303-307

Eisenman, L., 1991, "Fining and fining materials", The Home Winemakers Manual

Escolar, D., Haro, M.R., Saucedo, A. & Ayuso, J., 1995, "Discolouring process in sherry wines studied by means of chromatic parameters", Am. J. Enol. Vit., Volume 46, No.1, pp. 138-142

Feather, M.S., 1982, "Sugar dehydration reactions", Food Carbohydrates, pp. 113-133

Flores, J.H., Heatherbell, D.A., Hsu, J.C. & Watson, B.T., 1988, "Ultrafiltration (UF) of white Riesling juice: effect of oxidation and pre-uf juice treatment on flux, composition, and stability", Am. J. Enol. Vit., Volume 39, No. 2, pp. 180-187

Fogler, H.S., 1999, "Elements of chemical reaction engineering", Volume 3, pp. 655-662

Garza, S., Ibarz, A., Pagán, J. & Giner, J., 1999, "Non-enzymatic browning in peach puree during heating", Food Research International, Volume 35, pp.335-343

Gomis, D.B., Alvarez, M.D.G., Naredo, L.S. & Alonso, J.J.M., 1991, "High performance liquid chromatographic determination of furfural and

hydroxymethylfurfural in apple juices and concentrates", Chromatographia, Volume 32, No. 1, pp. 45-48

Hassler, J.W., 1974, "Purification with Activated Carbon Industrial Commercial Environmental", Purification with Activated Carbon: Industrial Commercial Environmental

Kelly, S.H. & Finkle, B.J., 1969, "Action of ring-cleaving enzyme in preventing oxidative darkening of juices", Am. J. Enol. Vit., pp. 221-226

Kermasha, S., Goetghebeur, M., Dumont, J. & Couture, R., 1995, "Analysis of phenolic and furfural compounds in concentrated and non-concentrated apple juices", Food Research International, Volume 28, No. 3, pp. 245-252

Kime, R.W. & Lee, C.Y., 1987, "The use of honey in apple wine making", American Bee Journal, Volume 127, No. 4, pp. 270-271

Kramling, T.E. & Singleton, V.L., 1965, "An estimate of the non-flavonoid phenols in wines", Am. J. Enol. Vit., Volume 16, pp. 144-158

Lee, C.Y., Kime, R.W. & Gavitt, B., 1990, "The use of honey in wine making", American Bee Journal, Volume 130, No. 8, pp. 535-536

Lee, G.C. & Lee, C.Y., 1997, "Inhibitory effect of caramelisation products on enzymatic browning", Food Chemistry, Volume 60, No. 2, pp. 231-235

Lee, C.Y., Smith, N.L., Kime, R.W. & Morse, R.A., 1985, "Source of the honey protein responsible for apple juice clarification", Journal of Apicultural Research, Volume 24, No. 3, pp. 190-194

Lee, C.Y. & Whitaker, J.R. (Editors), 1994, "Enzymatic browning and its prevention", ACS Symposium Series 600

Litchfield, J.E., Thorpe, S.R. & Baynes, J.W., 1999, "Oxygen is not required for the browning and crosslinking of protein by pentoses: Relevance to Maillard reactions *in vivo*", The International Journal of Biochemistry and Cell Biology, Volume 31, pp. 1297-1305

Mayén, M., Barón, R., Mérida, J. & Medina, M., 1997, "Changes in phenolic compounds during accelerated browning in white wines from *cv.* Pedro Ximenez and *cv.* Baladi grapes", Food Chemistry, Volume 58, No. 1-2, pp. 89-95

McLellan, M.R., Kime, R.W., Lee, C.Y. & Long, T.M., 1995, "Effect of honey as an antibrowning agent in light raisin processing", Journal of Food Processing and Preservation, Volume 19, No. 1, pp. 1-8

McMurry, J., 1996, "Organic chemistry", 4th Edition, BROOKS/COLE, pp.1087-1089

Mennett, R.H. & Nakayama, T.O.M., 1969, "The adsorption of hydroxybenzoic acids by poly-n-vinyl pyrrolidinone", Am. J. Enol. Vit., pp.169-175

Nagaraj, R.H. & Monnier, V.M., 1995, "Protein modification by the degradation products of ascorbate: Formation of a novel pyrrole from the Maillard reaction of L-threose with proteins", Biochimiea et Biophysica Acta, Volume 1253, pp. 75-84

Nagel, C.W. & Wulf, L.W., 1979, " Changes in the anthocyanins, flavonoids, and hydroxycinnamic esters during fermentation and aging of Merlot and Cabernet Sauvignon", Am. J. Enol. Vit., Volume 30, No. 2, pp. 111-116

Oszmianski, J. & Lee, C.Y., 1990, “Inhibition of polyphenol oxidase activity and browning by honey”, Journal of Agricultural Food Chemistry, Volume 38, No. 10, pp. 1892-1895

Palmer, J.K. & Brandes, W.B., 1974, “Determination of sucrose, glucose, and fructose by liquid chromatography”, Journal of Agricultural Food Chemistry, Volume 22, No. 4, pp. 709-712

Panagiotakopoulou, V. & Morris, J.R., 1991, “Chemical additives to reduce browning in white wines”, Am. J. Enol. Vit., Volume 42, No. 3, pp. 255-260

Peterson, R.G. & Caputi, A., 1967, “The browning problem in wines II. Ion exchange effects”, Am. J. Enol. Vit., pp. 105-112

Petriella, C., Resnik, S.L., Lozano, R.D. & Chirife, J., 1985, “Kinetics of deteriorative reactions in model food systems of high water activity: Color changes due to nonenzymatic browning”, Journal of Food Science, Volume 50, pp. 622-630

Pilon, G.J., 1977, “Determination of tartaric acid in wine”, Am. J. Enol. Vit., Volume 28, No. 2, pp. 104-107

Polyclar website, www.iscorp.com, 2001, “Polyclar V and Polyclar VT, Stabilisers for wine”

QUINTAS, M., Mota, A.L., Brandão, T.R.S., Cruz, R.M. and Silva, C.L.M. (Escola Superior de Biotecnologia, Universidade Católica Portuguesa), Dr. António Bernardino de Almeida, Porto, Portugal “Products of sucrose caramelisation kinetics: Fructose, glucose and aromatic and coloring agents”, Annual meeting - Chicago, Session 29D - Food engineering: Thermal processing, 2003

Resnik, S. & Chirife, J., 1979, “Effect of moisture content and temperature on some aspects of nonenzymatic browning in dehydrated apple”, Journal of Food Science, Volume 44, No. 2, pp. 601-605

Saitta, M., Lo Curto, S., Dugo, G. & Salvo, F., 2001, “Identification of phenolic compounds in olive oils by HGC-MS/MS”, Elsevier, NY, www.elsevier.com

Siebert, K.J. & Lynn, P.Y., 1998, “Comparison of polyphenol interactions with polyvinylpoly-pyrrolidone and haze-active proteins”, Food Science and Technology Dept., Cornell University, pp. 24 - 31

US Army Corps of Engineers, 2001, “Engineering and design: Adsorption design guide”, Design Guide, pp. 3.1 - 3.36

Vanhoenacker, G., De Villiers, A., De Keukeleire, D. & Sandra, P., 2001, “Comparison of LC-MS and CE-MS for the analysis of polyphenols in red wines”, Elsevier, NY, www.elsevier.com

www.ionsource.com, 2002

www.srsmaldi.com, 2002

Yaun, JP. & Chen, F., 1999, “Simultaneous separation and determination of sugars, ascorbic acid, and furanic compounds by HPLC – dual detection”, Food Chemistry, Volume 64, pp. 423-427

Yeo, H. & Shibamoto, T., 1991, “Effects of moisture content on the Maillard browning model system upon microwave irradiation”, Journal of Agricultural Food Chemistry, Volume 39, No. 10, pp. 1860-1862

Zoecklein, B.W., Fugelsang, K.C., Gump, B.H. & Nury, F.S., 1997, “Wine analysis and production”, Wine Analysis and Production, pp. 253-254

9 NOMENCLATURE

9.1 GENERAL NOMENCLATURE

Unit	*Description*
^{o}Balling	Degrees Balling
^{o}Brix	Degrees Brix
GJC	Grape juice concentrate
hL	hectoliter
μL	microliter

9.2 SAMPLE NOMENCLATURE

A list of samples generated during the experiments can be found in Appendix E. In order to understand what each sample's name denotes, it is necessary to give some explanation.

9.2.1 General samples:

CA - NORIT CA1 Powdered Activated Carbon

SA - NORIT SA4 Powdered Activated Carbon

PVPP - Polyclar V, Polyvinylpolypyrollidone

H - Heat experiment sample

U - After H, CA, SA, or PVPP denotes protein unstable

S - After H, CA, SA, or PVPP denotes protein stable

9.2.2 Robertson sampling:

RA - 1st batch of samples taken at Robertson

RB - 2nd batch of samples taken at Robertson

RC - 3rd batch of samples taken at Robertson

1, 2, 3, 4… - After RA, RB, or RC, denotes after each manufacturing stage

9.2.3 Heat experiments:

1, 2, 3, 4… - Denotes 20 minute intervals for a period of three hours

9.2.4 Optimum conditions:

a - After CA, SA, or PVPP, followed by S or U, denotes 20^{o}C

b - After CA, SA, or PVPP, followed by S or U, denotes 40^{o}C

c - After CA, SA, or PVPP, followed by S or U, denotes 60^{o}C

d - After CA, SA, or PVPP, followed by S or U, denotes 70^{o}C

1 - After a, b, c, or d, denotes half an hour

2 - After a, b, c, or d, denotes one hour

3 - After a, b, c, or d, denotes three hours

4 - After a, b, c, or d, denotes six hour

9.2.5 Product Profiles:

1 - After CA, SA, or PVPP, followed by S or U, denotes 0.5g, 0.5g, and 0.05g respectively.

2 - After CA, SA, or PVPP, followed by S or U, denotes 1.0g, 1.0g, and 0.35g respectively.

3 - After CA, SA, or PVPP, followed by S or U, denotes 2.5g, 2.5g, and 0.60g respectively.

10 APPENDICES

- Appendix A - Technical data: Adsorption products
    - NORIT CA1 Specification sheet and in-house analysis report
    - NORIT SA4 Specification sheet and in-house analysis report
    - Polyclar V Specification sheet, and other information
- Appendix B - Typical calculations
- Appendix C - Samples generated during experiments and HMF concentrations

APPENDIX A – TECHNICAL DATA: ADSORPTION PRODUCTS

Intentionally left blank 1

Intentionally left blank 2

Intentionally left blank 3

Intentionally left blank 4

Intentionally left blank 5

Intentionally left blank 6

Intentionally left blank 7

Intentionally left blank 8

POLYVINYLPOLYPYRROLIDONE (PVPP)

Polyvinylpolypyrrolidone	
Properties	Typical data
Physical form	Free flowing powder
Chemical description	Polyvinylpolypyrrolidone
Colour	White to off-white
Moisture (Karl-Fischer)	3.5%
PH (1g/100mi water)	7.0
Adsorptive Capacity (Catechin removal)	55%
Solubility	Insoluble in water, acids, alkali and all organic solvents

Polyclar® V offers enhanced **absorption** characteristics and is easily removed by filtration.

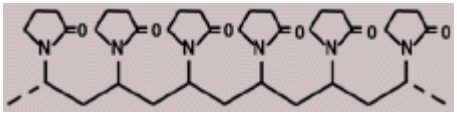


FIGURE A-1 CHEMICAL STRUCTURE OF POLYCLAR® STABILIZERS

Intentionally left blank 9

APPENDIX B – TYPICAL CALCULATIONS

B.1 RELATIVE SUGAR CONCENTRATION

TABLE B-1 RELATIVE SUGAR CONCENTRATION OF THE ROBERTSON SAMPLES

Sample Code	RSC	Sample Code	RSC	Sample Code	RSC
RA1	3.22	*RB1*	2.77	*RC1*	0.85
RA2	0.90	*No sample*		*RC2*	0.85
RA3	0.78	*No sample*		*RC3*	0.54
RA4	1.69	*RB4*	1.62	*RC4*	1.61
RA5	1.41	*RB5*	1.57	*RC5*	1.54
No sample		*RB6*	1.54	*RC6*	1.53
RA7	1.18	*RB7*	1.48	*RC7*	1.47
RA8	1.41	*RB8*	1.49	*RC8*	1.41
RA9	3.47	*RB9*	3.56	*RC9*	3.51
RA10	3.39	*RB10*	3.43	*No sample*	
RA11	3.48	*RB11*	3.43	*No sample*	

B.2 RATE OF HMF FORMATION

For calculation of the rate of formation of HMF it was assumed that the following reaction takes place:

$$Compounds: X, Y, Z \rightarrow HMF \tag{B-1}$$

Mol balance:

Since compounds X, Y and Z are not known, a mol balance based on HMF was developed as follows:

$$\frac{dC_{HMF}}{dt} = r_{HMF} \tag{B-2}$$

Where C_{HMF} is the concentration of HMF in mol/L and r_{HMF} is the rate of formation in mol/L/s. It is therefore known that r_{HMF} is a function of the concentration of HMF:

$$r_{HMF} = k\left[fn\left(C_{HMF}\right)\right] \tag{B-3}$$

Or:

$$\frac{dC_{HMF}}{dt} = k\left[fn\left(C_{HMF}\right)\right] \tag{B-4}$$

Manipulating the equation slightly and integrating on both sides yields:

$$\frac{1}{k}\int_{C_{HMF,0}}^{C_{HMF}} \frac{dC_{HMF}}{\left[fn\left(C_{HMF}\right)\right]} = \int_{o}^{t} dt \tag{B-5}$$

Rate law:

The following step is to determine the reaction order of the rate of formation of HMF. Reaction orders of 1 and 2 were tested and compared as follows:

1st Order		2nd Order	
$r_{HMF} = kC_{HMF}$	(B-6)	$r_{HMF} = kC_{HMF}^2$	(B-7)

Combining (B-5) with (B-6) and (B-7), respectively:

$$\frac{1}{k}\int_{C_{HMF,0}}^{C_{HMF}} \frac{dC_{HMF}}{C_{HMF}} = \int_0^t dt \qquad \text{(B-8)} \qquad \frac{1}{k}\int_{C_{HMF,0}}^{C_{HMF}} \frac{dC_{HMF}}{C_{HMF}^2} = \int_0^t dt \qquad \text{(B-9)}$$

Integrating on both sides of (B-8) and (B-9), respectively:

$$\frac{1}{k}\ln\left[\frac{C_{HMF}}{C_{HMF,0}}\right] = t \qquad \text{(B-10)} \qquad \frac{1}{k}\left[\frac{1}{C_{HMF,0}} - \frac{1}{C_{HMF}}\right] = t \qquad \text{(B-11)}$$

Plotting the factors on the left hand sides of (B-10) and (B-11) *versus* t, yields Figure 5-3 and Figure 5-4, respectively. A linear plot indicates a good estimate of the reaction order. The slope of the line is equal to 1/k.

APPENDIX C – SAMPLES GENERATED AND RELATIVE HMF CONCENTRATION

TABLE C-1 SAMPLES GENERATED DURING EXPERIMENTS WITH CORRESPONDING HMF CONCENTRATIONS

No	ID	nmol/ml
1	2CASa1	0.94
2	2CASa3	0.52
3	2CASb2	0.88
4	2CASc2	1.68
5	2CASc4	2.06
6	2CASd3	2.94
7	2CAUa1	1.16
8	2CAUa3	0.89
9	2CAUb2	1.12
10	2CAUb3	4.92
11	2CAUc2	2.82
12	2CAUc4	3.57
13	2HS1	5.35
14	2HS10	79.35
15	2HS2	4.33
16	2HS3	3.08
17	2HS4	3.46
18	2HS5	12.64
19	2HS6	14.18
20	2HS7	32.69
21	2HS8	39.3
22	2HS9	51.56
23	2HU1	4.6
24	2HU10	93.03
25	2HU2	2.85
26	2HU3	2.93
27	2HU4	5.03
28	2HU5	7.99
29	2HU6	17.83
30	2HU7	36.36
31	2HU8	44.16
32	2HU9	62.21
33	2PVPPSa1	1.51
34	2PVPPSa3	1.26
35	2PVPPSb1	0.91
36	2PVPPSc2	2.86
37	2PVPPSc4	2.39
38	2PVPPSd3	4.26
39	2PVPPUa1	1.76
40	2PVPPUa3	2.14
41	2PVPPUb2	1.4
42	2PVPPUC2	5.4
43	2PVPPUc4	3.71
44	2PVPPUd3	4.17
45	2SASa1	0.23
46	2SASa3	0.32
47	2SASb2	0.27
48	2SASc2	0.21
49	2SASc4	1.26
50	2SASd3	2.82
51	2SAUa1	0.42
52	2SAUa3	0.21

No	ID	nmol/ml
53	2SAUb2	0.46
54	2SAUc2	0.73
55	2SAUc4	0.93
56	2SAUd3	2.71
57	BLANK	0.07
58	BLANK	0.05
59	BLANK	0
60	BLANK	0
61	BLANK1	0.17
62	BLANK2	0.14
63	CAS1	2.53
64	CAS2	2.64
65	CAS3	2.44
66	CASA1	2.61
67	CASa2	6.39
68	CASa2	1.59
69	CASa4	3.94
70	CASb1	3.27
71	CASb2	3.44
72	CASB3	3.72
73	CASb4	4.04
74	CASc1	3.76
75	CASc2	1.56
76	CASc3	1.28
77	CASc4	4.26
78	CASd1	2.34
79	CASd2	3.29
80	CASd3	5.71
81	CASd4	9.68
82	CAU1	3.47
83	CAU2	3.64
84	CAU3	2.45
85	CAUa1	1.75
86	CAUa2	0.99
87	CAUa3	5.43
88	CAUa4	4.4
89	CAUb1	3
90	CAUb2	4.27
91	CAUb3	1.95
92	CAUb4	3.14
93	CAUc1	1
94	CAUc2	1.09
95	CAUc3	4.12
96	CAUc4	3.58
97	CAUd1	1.59
98	CAUd2	3.67
99	CAUd3	6.85
100	CAUd4	7.02
101	HS1	4.19
102	HS10	78.45
103	HS2	5.89
104	HS3	5.98
105	HS4	5.38
106	HS5	12.49

No	ID	nmol/ml
107	HS6	20.81
108	HS7	39.19
109	HS8	45.87
110	HU1	4.05
111	HU10	119.62
112	HU2	5.26
113	HU3	4.49
114	HU4	6.05
115	HU5	11.41
116	HU6	13.76
117	HU7	27.46
118	HU8	41.98
119	HU9	51.19
120	PVPPS1	2.01
121	PVPPS2	2.15
122	PVPPS3	2.61
123	PVPPSa1	5.16
124	PVPPSa1	1.26
125	PVPPSa2	6.73
126	PVPPSa3	7.68
127	PVPPSa4	3.5
128	PVPPSb1	8.75
129	PVPPSb2	5.53
130	PVPPSb3	6.72
131	PVPPSb4	4.27
132	PVPPSc1	1.94
133	PVPPSc2	2.89
134	PVPPSc3	2.9
135	PVPPSc4	8.28
136	PVPPSd2	2.34
137	PVPPSd3	7.83
138	PVPPSd4	13.21
139	PVPPU1	1.66
140	PVPPU2	1.27
141	PVPPU3	1.34
142	PVPPUa1	3.32
143	PVPPUa2	8.76
144	PVPPUa3	6.26
145	PVPPUa4	3.13
146	PVPPUb1	4.23
147	PVPPUb2	3.61
148	PVPPUb2	1.95
149	PVPPUb3	3.54
150	PVPPUb4	2.19
151	PVPPUc1	2.43
152	PVPPUc2	1.6
153	PVPPUc3	4.98
154	PVPPUc4	5.1
155	PVPPUd1	1.76
156	PVPPUd3	6.6
157	PVPPUd4	16.47
158	RA10	32.56
159	RA11	44.46
160	RA2	5.84

No	ID	nmol/ml
161	RA3	4.64
162	RA4	13.16
163	RA5	8.18
164	RA7	2.66
165	RA8	18.46
166	RA9	41.18
167	RB1	23.52
168	RB10	37.03
169	RB11	47.41
170	RB2	41.01
171	RB5	30.47
172	RB6	66.36
173	RB7	35.78
174	RB8	32.15
175	RB9	56.89
176	RC1	0.45
177	RC2	0.99
178	RC3	0.69
179	RC4	0.1
180	RC5	4.95
181	RC6	4.13
182	RC7	4.95
183	RC8	4.58
184	RC9	8.62
185	S1	2.18
186	S1	2.45
187	S1	2.16
188	S2	5.29
189	S2	4.59
190	S2	5.7
191	S3	9.65
192	S3	10.49
193	S3	10.03
194	S4	26.03
195	S4	25.67
196	S4	25.51
197	S5	51.51
198	S5	51.58
199	S5	51.69
200	S6	102.98
201	S6	102.98
202	S6	102.96
203	SAS1	0.77
204	SAS2	0.5
205	SAS3	0.6
206	SAsa1	0.18
207	SASa2	0.53
208	SASa3	0.45
209	SASa4	0.57
210	SASb1	0.51
211	SASb2	1.21
212	SASb3	0.76
213	SASb4	0.3
214	SASc1	0.47

No	ID	nmol/ml
215	SASc2	0.38
216	SASc3	0.48
217	SASc4	1.99
218	SASd1	0.67
219	SASd2	1.03
220	SASd3	3.13
221	SASd4	4.26
222	SAU1	1.16
223	SAU2	0.55
224	SAU3	0.69
225	SAUa2	0.71
226	SAUa3	0.79
227	SAUa4	0.71
228	SAUb1	0.75
229	SAUb1	0.09
230	SAUb2	0.35
231	SAUb3	0.4
232	SAUb4	0.06
233	SAUc1	0.66
234	SAUc2	0.07
235	SAUc3	0.83
236	SAUc4	1.61
237	SAUd1	0.59
238	SAUd2	0.6
239	SAUd3	2.96
240	SAUd4	4.96
241	Sb1	1.39
242	Sb2	4.23
243	Sb3	8.21
244	Sb4	20.5
245	Sb5	39.29
246	Sb6	90.53