GRAPE JUICE FILTRATION, THERMOPYHSICAL PROPERTIES OF CLEAR FRUIT JUICES AND PRESSURIZED LOW POLARITY WATER (PLPW) EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM GRAPE CANES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

ERKAN KARACABEY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
FOOD ENGINEERING

SEPTEMBER 2009

Approval of the thesis:

GRAPE JUICE FILTRATION, THERMOPYHSICAL PROPERTIES OF CLEAR FRUIT JUICES AND PRESSURIZED LOW POLARITY WATER (PLPW) EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM GRAPE CANES

submitted by **ERKAN KARACABEY** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Food Engineering Department, Middle East Technical University** by,

Prof. Dr. Canan Özgen _____
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Zümrüt B. Ögel _____
Head of Department, **Food Engineering**

Prof. Dr. A. Levent Bayındırlı _____
Supervisor, **Food Engineering Dept., METU**

Prof. Dr. Nevzat Artık _____
Co-Supervisor, **Food Engineering Dept., Ankara University**

Examining Committee Members:
Prof. Dr. Ali Esin _____
Food Engineering Dept., METU

Prof. Dr. A. Levent Bayındırlı _____
Food Engineering Dept., METU

Prof. Dr. Sedat Velioğlu _____
Food Engineering Dept., Ankara University

Assoc. Prof. Dr. Hami Alpas _____
Food Engineering Dept., METU

Assoc. Prof. Dr. Ender Poyrazoğlu _____
Food Engineering Dept., Ankara University

Date: September 4, 2009

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: Erkan Karacabey

Signature :

ABSTRACT

GRAPE JUICE FILTRATION, THERMOPYHSICAL PROPERTIES OF CLEAR FRUIT JUICES AND PRESSURIZED LOW POLARITY WATER (PLPW) EXTRACTION OF POLYPHENOLIC COMPOUNDS FROM GRAPE CANES

Karacabey, Erkan
Ph.D., Department of Food Engineering
Supervisor : Prof. Dr. Levent Bayındırlı
Co-supervisor : Prof. Dr. Nevzat Artık

September 2009, 140 pages

Filtration of grape juice was investigated. Effects of process parameters of filtration were examined. The usage of precoating material and filter aid were found to be necessary to prolong the filter life. Filter cake was found to be incompressible with the effect of increasing pressure. Increase in temperature caused increase in flow rate due to the reduction in viscosity. The influences of depectinization and clarification on filtration process were also examined. Depectinization and clarification decreased the resistance and increased the flow rate. Improvement in the quality of the grape juice was observed when these pretreatments were employed.

The effects of temperature and soluble solid concentration on physical properties of clarified fruit juices were investigated. High temperature and soluble solid content dependencies of density, viscosity and heat capacity were detected. Experimental data were fitted as a function of temperature and soluble solid content. Models being valid for studied clarified fruit juices were achieved for density and viscosity with the regression coefficients (R^2) higher than 0.90.

Optimization of the solid-liquid extraction conditions for *trans*-resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics from milled grape canes has been investigated. Temperature and ethanol concentration were found to be major process variables for all responses. Maximum yields of *trans*-resveratrol, t*rans*-ε-viniferin, ferulic acid, and total phenolics were predicted as 4.25 mg/g dw, 2.03 mg/g dw, 1.05 mg/g dw, and 9.28 mg/g dw, respectively.

Optimization of extraction conditions for antioxidant activity of grape cane extracts measured by the Trolox equivalent antioxidant capacity (TEAC) and the oxygen radical absorbance capacity ($ORAC_{FL}$) assays was carried out using solid-liquid extraction and response surface methodology. Ethanol concentration and temperature employed for the extraction of antioxidant agents from grape cane samples were found to be statistically significant process variables affecting antioxidant activity measured by the TEAC and ORAC methods.

t*rans*-Resveratrol and *trans*-ε-viniferin were extracted from milled grape canes using pressurized low polarity water (PLPW). The extraction temperature was significant for both compounds: extraction at 160^{o}C resulted in a 40% loss of *trans*-resveratrol compared to 95^{o}C while reduction of trans-ε-viniferin at both temperatures remained at 30%. Increasing ethanol concentration from 0 to 25% increased the extraction of total phenolics and *trans*-ε-viniferin by 44% and 489%, respectively. Solvent flow rate also influenced *trans*-ε-viniferin extraction. Effective diffusivities of *trans*-resveratrol increased by three times with increasing temperature. The modified Gompertz equation satisfactorily explained the extraction of the stilbenes investigated.

Keywords: Filtration, grape juice, optimization, grape cane, stilbene, resveratrol, pressurized low polarity water extraction, antioxidant activity, effective diffusivity, kinetic analysis of extraction.

ÖZ

ÜZÜM SUYU FİLTRASYONU, BERRAK MEYVE SULARININ TERMOFİZİKSEL ÖZELLİKLERİ ve ÜZÜM ASMA DALINDAN POLİFENOLİK BİLEŞİKLERİN BASINÇLANMIŞ DÜŞÜK POLARİTELİ SU (BDPS) ile ÖZÜTLENMESİ

Karacabey, Erkan
Doktora, Gıda Mühendisliği Bölümü
Tez Yöneticisi : Prof. Dr. Levent Bayındırlı
Ortak Tez Yöneticisi : Prof. Dr. Nevzat Artık

Eylül 2009, 140 sayfa

Üzüm suyu filtrasyonu incelenmiştir. Değişkenlerin filtrasyon işlemine etkileri değerlendirilmiştir. Ön kaplama ve filtre yardımcı maddelerinin kullanımının filtrenin ömrünü uzatmak için gerekli olduğu kanısına varılmıştır. Filtre kekinin artan basınç etkisiyle sıkıştırılamaz olduğu bulunmuştur. Artan sıcaklık üzüm suyunun akışmazlığındaki azalma nedeniyle akış hızının artışına neden olmuştur. Depektinizasyonun ve berraklaştırmanın filtrasyon işlemine etkileri araştırılmıştır. Depektinizasyon ve berraklaştırma direnci azaltmış ve akış hızını artıtmıştır. Bu ön işlemler gerçekleştirildiğinde üzüm suyunun kalitesinde gelişme gözlenmiştir.

Sıcaklık ve çözünür kuru maddenin berrak meyve sularının termofiziksel özellikleri üzerine etkileri incelenmiştir. Yoğunluk, akışmazlık ve ısı kapasitesinin yüksek sıcaklık ve çözünür kuru madde bağlılığı belirlenmiştir. Deneysel sonuçlar sıcaklık ve çözünür kuru maddenin fonksiyonu olarak ifade edilmiştir. Yoğunluk ve akışmazlık için, çalışılmış berrak meyve suları için geçerli olan modeler 0.90'dan yüksek değerlerdeki regresyon katsayıları ile elde edilmiştir.

Öğütülmüş üzüm asma dallarında *trans*-resveratrol, *trans*-ε-viniferin, ferulik asit ve toplam fenolikler için katı-sıvı özütlemesinin işlem koşullarının optimizasyonu incelenmiştir. Sıcaklık ve etanol derişiminin tüm koşullar için temel işlem değişkenleri oldukları bulunmuştur. *trans*-Resveratrol, t*rans*-ε-viniferin, ferulik asit, ve toplam fenolikler için en yüksek değerler cinsinden sırasıyla 4.25 mg/g dw, 2.03 mg/g dw, 1.05 mg/g dw, and 9.28 mg/g dw olarak belirlenmiştir.

Asma dalı özütlerinin Trolox eşdeğer antioksidan kapasitesi (TEAK) ve Oksijen radikali tutma kapasitesi ($ORTK_{FL}$) yöntemleriyle ölçülen antioksidan aktiviteleri için özütleme koşullarının optimizasyonları katı-sıvı özütlemesi ve yanıt yüzey metodu kullanılarak yapılmıştır. Antioksidan maddelerinin asma dalı örneklerinden özütlenmesi için kullanılan etanol konsantrasyonu ve sıcaklığın, TEAK ve $ORTK_{FL}$ yöntemleriyle ölçülen antioksidan aktivitesini istatistiksel olarak önemli düzeyde etkileyen işlem değişkenleri oldukları bulunmuştur.

t*rans*-Resveratrol and *trans*-ε-viniferin basınçlanmış düşük polariteli su (BDPS) yöntemi kullanılarak öğütülmüş asma dallarından özütlenmiştir. Özütleme sıcaklığı her iki madde içinde önemlidir: 160^{o}C de özütleme 95^{o}C'dekine göre %40'lık *trans*-resveratrol kaybıyla sonuçlanmışken, *trans*-ε-viniferindeki azalma bu iki sıcaklık değerinde %30 düzeyinde kalmıştır. Etanol konsantrasyonunun %0'den %25 kadar artması toplam fenolik ve *trans*-ε-viniferinin özütlenmesini sırasıyla %44 ve %489 arttırmıştır. Çözgen akış hızıda *trans*-ε-viniferin özütlenmesini etkilemiştir. *trans*-Resveratrolun efektif diffüzivitesi artan sıcaklıkla birlikte üç katına çıkmıştır. Modifiye Gompertz eşitliği incelenen stilbene maddelerinin özütlenmesini yeterli düzeyde açıklamıştır.

Anahtar kelimeler: Filtrasyon, üzüm suyu, optimizasyon, asma dalı, stilbene, resveratrol, basınçlanmış düşük polariteli su özütlemesi, antioksidan aktivitesi, etkin yayınganlık, özütlemenin kinetik analizi

To my family

&

To my grandfathers; Yusuf Karacabey & Kemal Göktürk

ACKNOWLEDGEMENTS

Completion of this study can not be accomplished without many people's contributions, help and encouragement. It is a pleasure for me to mention those who deserve my sincere acknowledgement.

I would like to express my sincere gratitude and respect to my supervisor Prof. Dr. Levent Bayındırlı, my co-supervisor Prof. Dr. Nevzat Artık for their valuate guidances in this study and their encouragement and supports.

I would like to specially thank to Prof. Dr. Giuseppe Mazza to give me an opportunity to utilize from his laboratory. I am grateful for sharing his knowledge and being willing to discuss my work and suggestions.

I would like to extend my thanks to reviewers of my thesis, Prof. Dr. Ali Esin and Prof. Dr. Sedat Velioğlu for their constructive criticisms, encouragements and suggestions for improvements that have greatly contributed to the making this thesis.

My thanks are extended to Prof. Dr. Şebnem Harsa and Doç. Dr. Metin Tanoğlu to give me an opportunity to carry my experiments out in their laboratories in Izmir High Technology Institute.

I would also like to thank Mrs. Lana Fukumoto, a technician in Dr. Mazza's laboratory for her help and suggestion.

I would like to express special thanks to my friends Cem and Hande Baltacıoğlu to make me felt their strong and continous supports, encouragements, and helps during not just this thesis but also my life since I met them. I would like to open another thanks sentence for my friends, B. Gökçen Mazı and Işıl Barutçu Mazı, Mete Çevik, Neslihan Altay Dede for helps and valuable encouragements. My thanks are

extended to Dr. Özge Şakıyan Demirkol, Dr. Mutlu Pilavtepe Çelik, Dr. Halil İbrahim Çetin, Dr. Sencer Buzrul, and Dr. Nadide Seyhun for sharing their knowledges and suggestions during my study.

I would like to extend my thanks to my friends, Eda Cilvez, Nalan Uysal and Oya Nihan Uncu to hold my moral at the highest level by their smiles even in the worst cases.

I would like to mention valuable friendship and help of my friends, Shawkat Ali and Tanja Dubric. Life would not be easy without their contributions when being away from home.

At last, I would like to express sincere thanks to my parents and my sister, without their patience and support it would not be easy for me to complete this study. I am gratefull to have my family.

This study was supported by the State Planing Organization (DPT) Grant No: BAP-08-11-DPT2002K120510-GT-4.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

LIST OF FIGURES

FIGURES

CHAPTER I

INTRODUCTION

Food production is under control of factors including; human health, product quality, accessibility, economical limitations, and official regulations.

Above mentioned factors have individual effects in food industry, but it is not easy to think their effects individually and also to evaluate them due to the possible integration influences. Because these factors so integrated each others that considerable influence of each one on others also appears and these ones should be also considered to plan of productions.

In the light of these facts, food industry have been searching new process techniques, technologies, products parallel to developments and changes in these factors.

During the development of a new technology or a new method related to any product requirements appear and researchers try to meet these requirements considering consumer demands, economical aspects, human health and official regulations.

Researchers, engineers, and/or any developers mainly focus on to explain the new technology and/or the new method being developed. Because this is crucial and basic step and directly shapes the future of target purpose.

Modeling is the one way to explain the developing system mathematically, since representing model gives information about the system and makes it possible to predict the results to evaluate system. The reason of importance of modeling can be explained that seeing the whole picture with possible predicted results opens the view of researcher and as a result opens the view of researcher and better prototypes appear. In addition as it is mentioned above, some factors determine the boundaries

of investments. Modeling of target purpose is also usable to evaluate the feasibility of investment.

Fruit juice industry having clear fruit juices as a subgroup, is one of the main group of food industry. Fruits are not easily transported in long distance for consumption due to sensitivity against environmental conditions and fast spoilage. This limitation creates the requirement of new products to provide similar advances like that in raw fruits including; taste, flavor, and nutrition. As a result, processes in the production of fruit juices are very important and should be carefully designed to meet requirements determined depending on consumer, official regulations, and technological facilities. One of the processes for fruit juice production is filtration. Filtration is extremely important for process especially meeting consumer demands for clear fruit juices. It directly affects product quality. The efficiency with possible high quality product of filtration process is determining step of clear fruit juice production. As a result it should be designed very well so that consumer, investigators, and regulations can be satisfied. At this point the importance of modeling appears once again due to reasons mentioned before.

Development of a new technology and/or a new method is not enough alone, because the physical properties are also operative parameters having great influence on the products. Thermophysical properties directly affect the process efficiencies and parameters of product quality, so they should be considered during the processes. On the other hand the literature data about thermophysical properties of food products are limited and it is required to carry out experiments to determine these properties for each case. This process is expensive and time consuming, as a result one equation as functions of environmental conditions and the composition of food products can be useful to approximately predict these values instead of experimental results.

In production industries another problem created in the pre-process and/or during processing is by product which is used in another purpose or it is just named as waste. Waste production can be a problem for environment and economy. The utilization of by products is preferable as if it is environmental benign and

economical. One of the wastes of grape production is grape canes produced during pruning of grape plants. This waste can be utilized as a potential source of bioactives. Because literature knowledge displayed that agricultural wastes have the possible great potential of phytochemicals. Grape canes have not been used for any purpose except usage of them as a combustion material. The possible utilization areas of grape canes will take the increasing attention of researchers.

1.1 Clear Fruit Juices

In food industry fruit juice production is the most important way of the utilization of fruits. There are general steps in fruit juice production. The main steps are as follows; washing, sorting, removal of unwanted particles (stems, leaves, etc.), pulp production, pressing of the mash, depectinization, clarification, filtration, concentration, pasteurization, and packaging or storage. These steps are generally applied during fruit juice production but some differences can occur.

1.1.1 Grape Juice Production

There are differences during production of red and white grape juices, but the main steps are same for both. After the preliminary steps of grape fruits like washing, sorting, removal of stems, and leaves, press of grape fruits is performed. Grape fruits are milled to obtain mash and mash fermentation is done at low temperature for white grape fruits and at high temperature for red grape fruits by adding enzyme. Obtained raw grape juice is depectinased by using pectolytic enzyme preparation. And then clarification of grape juice is done by addition of gelatin, kieselsol solution and bentonite. Before packaging or concentration of grape juice, tartrate stabilization is performed to prevent the formation of K-bitartrate crystals. For this purpose it is stored in a tank at low temperature for 3-4 days. The flow diagram of red grape juice production is in **Figure 1**.

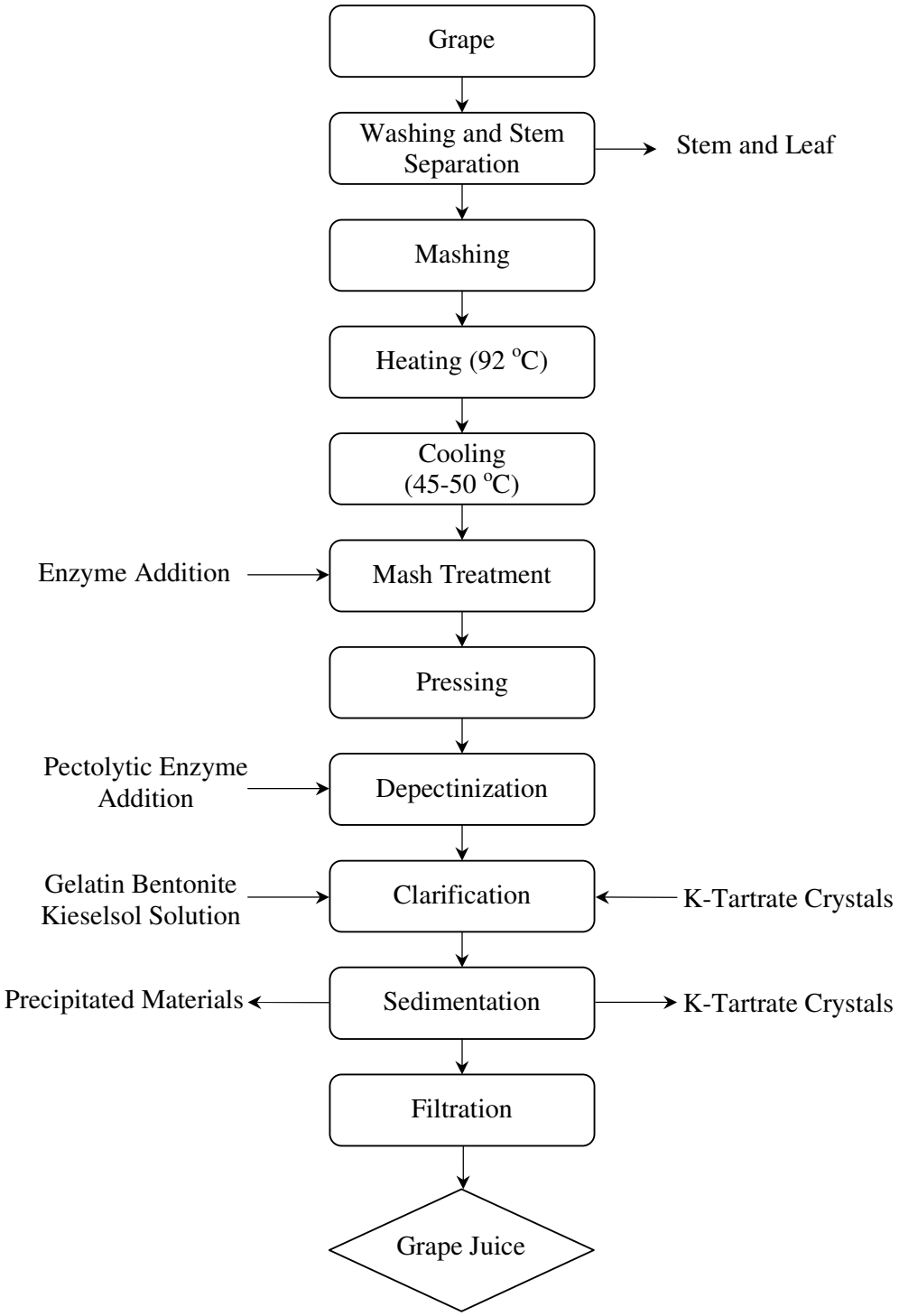

Figure 1 The flow chart of grape juice production

1.1.2 Filtration

Solid-liquid filtration may be defined as the unit operation in which the insoluble solid component of a solid-liquid suspension is separated from the liquid component by passing the latter through a porous membrane or septum which retains the solid particles on its upstream surface, or within its structure, or both. The solid-liquid suspension is known as the feed slurry or prefilt, the liquid component that passes through the membrane is called the filtrate and the membrane itself is referred to as the filter medium. The separated solids are known as the filter cake (Brennan, Butters, Cowell, and Lilley, 1990).

The flow of filtrate may be brought about by means of gravity alone, by the application of a pressure greater than atmospheric upstream of the medium (pressure filtration), by applying a vacuum downstream of the medium (vacuum filtration) or by means of centrifugal force (centrifugal filtration) (Brennan, Butters, Cowell, and Lilley, 1990).

The applications for filtration in the food industry may be considered to fall into three categories. The first category embraces all those applications wherein slurries containing appreciable amounts of insoluble solids, i.e. more than one or two percent by weight, are separated into their solid and liquid components. Either the liquid or solid component, or both, may be valuable. In such operations a cake is formed on the upstream surface of the medium and the process is known as cake filtration. The second category is termed clarification and involves removing small quantities of insoluble solid from a valuable liquid. Here the object usually is to produce a clear liquid and solids are generally unwanted. In such filtrations a cake may build up on the medium or alternatively, where the amount of solids is very small, they may become enmeshed within the structure of the medium. The third category, often referred to as microfiltration, involves the removal of very fine particles, of the order

of 1μm or less, and is generally directed at removing microorganisms from liquid foods (Brennan, Butters, Cowell, and Lilley, 1990).

As an alternative to altering the particle size distribution of a suspension by shifting the state of aggregation of the component particles, it is possible to bring about this change by adding solid material having a different size distribution. Such materials are known as filter aids. These materials are also used as filter media in precoat filtration processes (Brennan, Butters, Cowell, and Lilley, 1990).

The requirements of a filter aid are that it should have suitable particle size and shape characteristics for achieving the desired cake permeability, that it should be chemically inert under the conditions used, and that it should be inexpensive (Orr, 1977).

1.1.2.1 Filtration Method

The choice of a filter design for a given process depends on many factors, among which are the properties of the solid particles to be removed, i.e., particle size and shape distribution and state of aggregation; the properties of the fluid, i.e., viscosity, density, and interaction with structural materials; the quantity of material to be handled; whether the process should be batch wise, continuous, or either; the dryness of the cake produced, if applicable; the requirement of cake washing, the concentration of solids in the suspension; the value of the materials being processed; whether the material to be retained is the solid, the liquid, or both.

An important factor in the design of a filter is the source of the driving force, which may be gravity, suction, positive pressure, or the application of centrifugal force. This choice will in turn depend upon consideration of the factors listed above (Orr, 1977).

Filtration processes are conventionally divided into classes for convenience when considering the mathematical analysis of the factors involved. However in virtually all real filtration processes more than one of these mechanisms may take part, and either the mathematical analysis is adjusted to account for this or simplifying assumptions are made. These simplifying assumptions are often justified by the dominance of mechanisms.

In medium filtration the particles are retained because they are larger than the holes in the filter medium. In this sense the filter mdeium behaves as a sieve. This relatively uncommon mechanism is usually thought of in relation to the screening of large particles, but it also applies to the retention of fine particles on membrane filters and woven metal cloths (Orr, 1977).

In depth filtration the separation process occurs within the medium only, the particles being smaller than the pores of the medium.

In cake filtration the solid material accumulates on the surface of the medium, so that, after a short initial period, filtration is through the bed of deposited solid. This process will continue until the pressure drop across the cake exceeds the maximum permitted by economic or technical considerations or until the space available is filled. This method of filtration is the most widely employed in the process industries and is very well suited to the filtration of concentrated suspensions and the recovery of large quantities of solid. The most important factor in cake filtration is the permeability or resistance of the filter cake, and this may be controlled, more or less, by altering the particle size distribution of the material, sometimes by adding another solid to it, and also by altering the state of aggregation of the solid (Orr, 1977).

1.1.3 Thermophysical Properties of Fruit Juices

Clarified fruit juices are widely available on the beverage market of food industry. The requirements of this market lead the industry to investment for development of

new processes and technologies. The modeling and designing of equipment for the fluid flow and heat transfer operations involved in clarified fruit juice processing at different concentrations and temperatures require data on several engineering properties. These two important parameters, temperature and soluble solid content of products generally vary in the processes, which affect the physical and thermo-physical properties including density, viscosity, and heat capacity (Gratao, Junior, Polizelli, Telis-Romero, 2005). A knowledge of these properties is of particular significance for effective design of food processing equipment, such as evaporators, pumps, heat exchangers, filters and mixers (Nindo, Tang, Powers, and Bolland, 2004, Holdsworth, 1993, Telis-Romero, J., Telis, Gabas and Yamashita, 1998, de Moura, Germer, Jardim, and Sadahira, 1998, Zuritz et al., 2005, Zainal, Abdul Rahman, Ariff, Saari, and Asbi, 2000, Nindo, Tang, Powers, and Singh, 2005, Shamsudin, Mohamed, and Yaman, 2005, and Ramos and Ibarz, 1998). In addition, the knowledge of the physical and thermophysical properties varying with temperature and soluble solid content is very important in the food process control. Unfortunately, only a few process variables are available for online measurement because of the lack of suitable sensors. Thus, mathematical models that express the dependence of thermal properties on temperature and soluble solid content are very interesting alternatives to experimentation and useful tools for the implementation of computer-aided routines for equipment design and process automation. In addition, these properties are characteristic parameters in the optimization of productivity and quality of products. Clarified fruit juices and their physical properties have been received considerable attention due to their significance.

Thermo-physical properties of the different juices have been found to exhibit a close relationship with temperature and soluble solid content and mathematical models have been reported (Ali, Ramaswamy, & Awuah, 2002, Lau, 1991, Telis-Romero et al., 1998, Zuritz et al., 2005, Zainal et al., 2000, Nindo et al., 2004, Nindo et al., 2005, Ramos and Ibarz, 1998, Cepeda and Villarán, 1999, and Magerramov, Abdulagatov, Azizov, and Abdulagatov, 2007). The soluble solid content is under control of proposed product and handled raw material, including seasonal effects, harvesting time, and fruit species etc. (Poyrazoğlu, Gökmen, and Artık, 2002),

whereas temperature level is directly related to the process steps and storage conditions.

Studies have indicated that the change of the physical properties of the clarified fruit juices show parallel trends under same conditions in terms of soluble solid content and temperature (Constenla, Lozano, and Crapiste, 1989, Cepeda, and Villarán, 1999, Azoubel, Cipriani, El-Aouar, Antonio, and Murr, 2005, Zuritz, et al., 2005). These results encourage finding single models for prediction of thermo-physical properties being valid for all clarified fruit juices.

1.1.3.1 Density

Fruit juices are commonly treated as sugar-containing solutions. The official table of density values for sucrose at 20°C was published by Plato (1900) and is still commonly in use (Nagy, Chen & Shaw, 1993) in calculation of density for mechanization of fruit juice industrial applications. For more exact calculation, it is advantageous to use specific equations.

Aguado and Ibarz (1988) indicated that a linear equation fits very well the density variation with temperature at a fixed concentration, and that a second degree polynomial equation fits well the variation of density with temperature and concentration. This work has the goals of physicochemical characterization of different fruit derivatives and of determining the density of clarified peach juice and orange juice at several concentrations, and of apple and quince purees as a function of temperature.

1.1.3.2 Viscosity

In many food processing operations it is essential to know the viscosity of the fluid being processed, so that the most suitable equipment can be selected. During some

operations the viscosity may change considerably. This is particularly so in processes involving heating, cooling, homogenization and concentration as well as during many industrial fermentations; these viscosity changes need to be considered when designing these processes (Lewis, 1996).

Measurement of viscosity is often very important for quality control, particularly on products that's expected to be of a particular consistency in relation to appearance or mouth feel, e.g. cream, yoghurt, tomato paste and custards (Lewis, 1996).

Viscosity is defined as the internal friction of a fluid or its tendency to resist flow (Bourne, 1982). Viscosity is also a measure of the rate of flow (Lewis, 1996).

- *Temperature:* There is an inverse relationship between viscosity and temperature (Bourne, 1982).
- *Concentration of Solute:* There is usually a direct nonlinear relationship between the concentration of a solute and viscosity at constant temperature (Bourne, 1982).
- *Pressure:* The viscosity of most liquids is essentially constant over a pressure range of 0-100 atm (Bourne, 1982). Hence the pressure effect can usually be ignored on the viscosities of liquid foods in the range (0-100 atm) of pressure.

Temperature influence on viscosity has been found to be related to soluble solids content, and experimental data for fixed concentrations can be related to the temperature using the Arrhenius-Guzman equation (Alvarado and Romero, 1989). The rheological behavior of apple juice obtained from Malus floribunda has been studied for cloudy and clarified juices (Cepeda, Villaran and Ibarz, 1998), and no references has been found for clarified and depectinised juice.

1.1.3.3 Heat Capacity

Heat measurements are often made by means of a calorimeter (Riedel, 1951; Hwang and Hayakawa, 1979), which is a simple technique although requiring a careful calibration as a result of the heat capacity of the apparatus. The differential scanning calorimeter is the best alternative for experimentally determining the heat of foods, but has the disadvantage of being expensive (Constenla et al., 1989; Sweat, 1995). Some empirical equations have been proposed for the estimation of heat of various food products as a function of composition (Miles, Van Beek, & Veerkamp, 1983; Lamb, 1976). In these equations one can easily verify that heat of foods depends strongly on the water content, since water has the highest heat of all food components (Saravacos and Kostaropoulos, 1995). Experimental values of heat are available for some food products and food processing materials (Lewis, 1987; Jowitt, Escher, Hallsrom, Meffert, Spiess, and Vos, 1983) but most of them are restricted to a certain temperature and/or water content.

1.1.4 Mathematical Modeling

A mathematical model, as a tool to control and predict results, means an approximate representation of a process in mathematical terms. Mathematical models are widely used in food area for simulating whole industrial process as well as small laboratory experiment. They are used as a general approximation and also as a way to understand the process in detail.

A good mathematical model should be general (apply to a wide variety situations), realistic (based on correct assumptions), precise (its estimates should be finite numbers, or definite mathematical entities), accurate (its estimates should be correct or very to correct) and there should be no trend in the deviations of the model from the experimental data. A good model should be robust (relatively immune to errors in

the input data), and fruitful (its conclusions are useful or point the way to other good models) (Özilgen, 1998).

1.1.4.1 Filtration Theory

Particle filtration techniques are employed for the micro and macro particles. In particle filtration process the solids are separated from a liquid by means of a medium which retains the solids, but allows the liquid to pass through. Accumulation of the particles on the medium clogs its holes and prevents the flow. Filter aids, i.e., inert particles of diatomaceous earth, cellulose, etc., are used in filtration processes to keep these holes open. In a cake filtration process filtration actually occurs through the cake (**Figure 2**). The filter medium is precoated with a layer of filter aid before the beginning of filtration. The liquid food is also mixed with the filter aid and fed in to the filter. Filtration occurs through the cake, the food particles and filter aid are retained in the filter, while the liquid passes through. The cake builds up continuously through the process, the food particles are dispersed among the filter aid particles, therefore clogging is prevented (Özilgen, 1998).

From a study of the flow of liquids through beds of sand, Darcy proposed the empirical relation, known as Darcy's law (Darcy, H., 1856).

$$u = K' \frac{\Delta P}{L} \qquad (\mathbf{1})$$

where u is the overall fluid velocity, L the thickness of the bed, ΔP the pressure drop across the bed, K′ a constant characteristic of the bed and fluid properties.

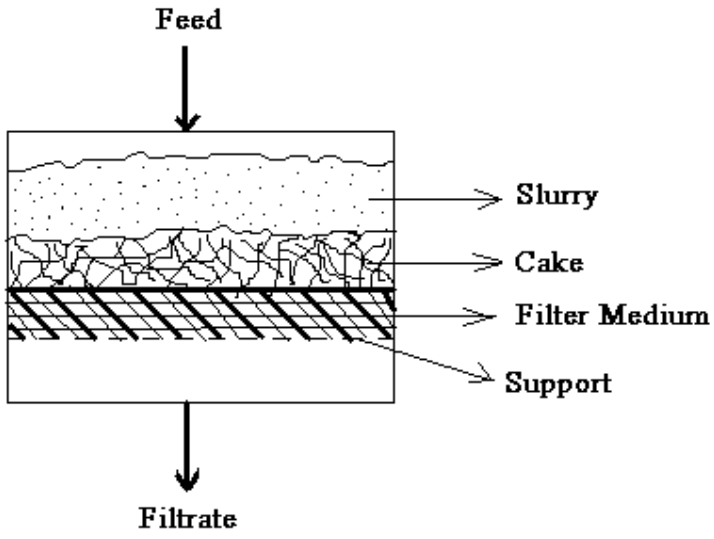

Figure 2 Cake filtration

Before Darcy's work was published, Poiseuille presented the equation for the flow of liquid through a capillary of circular cross section

$$\frac{dV}{dt} = \frac{\Delta P.\Pi.r^4}{8\mu L} \qquad (2)$$

where r is the capillary radius, and μ the viscosity of the fluid.

The Poiseuille equation states that the rate of flow of fluid through a capillary is inversely proportional to the viscosity of that fluid. If this relationship is assumed for packed beds, the Darcy equation may be rewritten

$$u = \frac{1}{A}\frac{dV}{dt} = \frac{K.\Delta P}{\mu L} \qquad (3)$$

this defines K, the permeability of the bed.

Although there are various methods of filtration, i.e. high pressure systems, vacuum filtration with scraped surface, ultrafiltration, etc, operational parameters which control filtration rates are generally the same. Generally the same filtration theories apply to these equipments. Effect of the operational parameters on filtration rates can be described easily with mathematical equations. Pressure, accumulation of the retentate or the filter aids, and composition of the retentate are among the important operating parameters. Specific process units, i.e. high pressure filtration units, are designed to achieve filtration with selected combination of these parameters. Model studies on filtration of the beverages are extremely limited (Peleg and Brown, 1976; De La Garza and Boulton, 1984).

Sperry's model has formed the basis of the filtration theory (Orr, 1977). In the one of the most widely used models Sperry (1917) described the filtration rates as:

$$\frac{dV}{dt} = \frac{\Delta PA}{\mu} \frac{1}{[R_m + \alpha c V/A]} \qquad \textbf{(4)}$$

where A is the filtration area, c is the concentration of solids being collected, ΔP is the pressure difference across the filter, R_m is the filter medium resistance, t is time, and V is the volume of the filtrate collected. The parameters α and μ are specific cake resistance and viscosity of the filtrate, respectively. Equation (4) was found to be completely unsatisfactory for describing wine filtrations because of filter fouling; therefore, it was modified by De La Garza and Boulton (1984) to include higher powers of cake resistance such as power model and exponential model respectively:

$$\frac{dV}{dt} = \frac{\Delta PA}{\mu} \frac{1}{[R_m + \alpha c (V/A)^n]} \qquad \textbf{(5)}$$

or

$$\frac{dV}{dt} = \frac{\Delta PA}{\mu} \frac{1}{[R_m \exp(kV/A)]} \qquad \textbf{(6)}$$

where n and k are positive constant.

Modelings of apple juice filtration and sourcherry juice filtration have been studied and exponential model has been found to be valid to describe the filtration of apple juice and sourcherry juice (Bayındırlı et al., 1989, Şahin and Bayındırlı, 1993).

De La Garza and Boulton's power model has not been used since a constant value could not be found for parameter n, to describe all data (Bayındırlı, Özilgen, and Ungan, 1989).

According to exponential model total filtration resistance is equal to:

$$R_{total} = \frac{dt}{dV}\frac{\Delta P A}{\mu} \qquad \textbf{(7)}$$

so that Equation (6) can be rewritten as;

$$R_{total} = R_m \exp(\beta V) \qquad \textbf{(8)}$$

where the exponential fouling coefficient, $\beta = k / A$

If the natural logarithm of both side of Equation (8) is taken;

$$\ln R_{total} = \ln R_m + \beta V \qquad \textbf{(9)}$$

If a semi log plot of the total resistance, R_{total} vs filtrate volume, V is plotted, the intercept will be medium resistance, R_m and the slope will be the exponential fouling coefficient, β.

1.1.4.2 Modeling of Thermophysical Properties

Food industry today is very rapidly developing. There are many parameters that have importance in the food industry. The thermophysical properties are of importance and used in the clarified fruit juice industry.

The main items in a clarified juice production plant, the pipes, the chillers, the mixers, the agitators, the heat exchangers and all the others, require this thermophysical information to be well constructed and well operated for optimum production.

This necessary information can be available experimentally, but this is not always convenient. The best way is to use already available information as data for determination of the required information by using the appropriate model derived for the necessary objectives (Bayındırlı, 1992).

1.1.4.2.1 Density

The statistical analysis for the modeling of density of the clear fruit juices depends on the available data obtained from the experiments and is done by use of statistical software, which allows the determination of the proper fit for multiparametric model of the density of clear fruit juices and regression analysis is performed to analyze the experimental data (Bayındırlı and Erşan, 1998).

1.1.4.2.2 Viscosity

The Arrhenius equation to a great extent explains the relationship between the temperature and viscosity.

$$\mu = \mu_o \exp(Ea/RT) \quad \textbf{(10)}$$

where μ (cP) is the viscosity at temperature T (K) and μ_o is the viscosity of water at that temperature. Ea (kcal/gmol) is the activation energy of flow and R is the gas constant (Bayındırlı, 1993).

The concentration effect in viscosity can be identified by the following equation (Mooney, 1951):

$$\ln(\mu/\mu_o) = 2.5\phi/(1 - K\phi) \quad \textbf{(11)}$$

where ϕ denotes the volume fraction of solids and K is a coefficient that takes into account the interaction between particles (Bayındırlı, 1993).

In order to express on a weight basis, as °Brix, and to take into account the effect of temperature, the above equation was modified to the following expression (Bayındırlı, 1993):

$$\ln(\mu/\mu_o) = AX/(100 - B*X) \quad \textbf{(12)}$$

1.1.4.2.3 Heat Capacity

In modeling of heat capacity of food materials, temperature and water content are important parameters. Water content is selected as a parameter since water has the highest heat capacity and food materials contain high amount of water. To model heat capacity of fruit juices the statistical analysis can be used by use of the statistical software.

1.1.5 Aims of the Studies about Filtration of Grape Juice and Modeling of Thermophysical Properties

In fruit juice industry, filtration is one of the most important steps of the process due to its direct effect on process time and also its effect on the quality of product. Filtration of any fruit juice is possible by modeling of filtration of that fruit juice. The results rising from model are used in plant design, management of production planning and process time. The most consumed clear fruit juices are apple juice, sourcherry juice and grape juice. Modeling of the filtration of two of them, apple juice and sourcherry juice, have been studied. The last one, grape juice will be studied in this study.

Knowledge about thermophysical properties is fundamental to analyze the unit operations. They are important in plant and process design in food industry. They are also quality indicators of food products. In literature limited data related to thermophysical properties of food materials are available and they are limited in very narrow ranges. Although these data can be determined experimentally when they are required, this method is inconvenient for food industry since experimental works are expensive and time consuming procedures. To overcome these problems and achieve the data easily prediction of the thermophysical properties can be done by use of appropriate models.

The aims of this study;

- to model the filtration of the grape juice,
- to investigate the influences of temperature and soluble solid concentration of three different clarified fruit juices, including apple, grape and sourcherry, on their density, viscosity, and heat capacity values,
- to get single models for each physical properties being valid for studied fruit juices.

1.2 Extraction of Grape Cane Phenolics

1.2.1 Grape Cane Phenolics

Phytoalexins are low molecular weight secondary metabolites produced in response to stress conditions such as fungal attack, injury, heavy metal ions or UV light in plants such as hop cones, grapevine, cocoa, peanut and blueberry (Jerkovic & Collin 2007; Bailey, 1982; Frémont 2000; King, Bomser & Min, 2006; Counet, Callemien & Collin, 2006, and Lyons, Yu, Toma, Cho, Reiboldt, Lee & van Bremen, 2003).

Recent interest in the health-beneficial properties of bioactive compounds has encouraged researchers to screen potential new sources of those compounds (Ju and Howard, 2003). Agricultural byproducts have gained increasing importance due to their potential health benefits (Makris et al., 2007).

Rayne et al (2008) has presented the considerable potential of grape canes (*Vitis vinifera*), a byproduct of the wine industry as a source of bioactive compounds, especially stilbene compounds, resveratrol and viniferin, and other phenolic compounds. Grapes and their derivatives are considered as important dietary sources of stilbene compounds included in the phytoalexin group. In grapevine, grape berry, leave, stem and skin, the response to stresses includes the synthesis of stilbenes, resveratrol (*trans*-3,4',5-trihydroxystilbene), its glycoside (piceid), its dimer, ε-viniferin and isomers of these compounds (Aggarwal, Bhardway, Aggarwal, Seeram, Shishodia & Takada, 2004; Pezet, Gindro, Viret & Spring, 2004; Jeandet et al., 1997; Adrian, Jeandet, Breuil, Levite, Debord & Bessis, 2000, and Romero-Pérez, Lamuela-Raventós, Andrés-Lacueva & Carmen de la Torre-Boronat, 2001).

Resveratrol (*trans*-3-5-4' trihydroxy stilbene) is a polyphenol demonstrated to elicit a broad spectrum of biological effects including antioxidant capacity, cardio protection, anticancer activity, anti-inflammatory effects, estrogenic/anti-estrogenic

properties (King, Bomser, Min, 2006, Wolter, Stein, 2002, Roemer, Mahyar-Roemer, 2002, Bhat, Kosmeder, Pezzuto, 2001). Likewise, *trans*-ε-viniferin, a dimer of *trans*-resveratrol has been demonstrated to be toxic to fungal parasites (Langcake, 1981), and has been found to have inhibitory effects on human cytochrome P450 enzymes (Piver, Berthou, Dreano, Lucas, 2003), and antioxidant activity in aqueous and nonaqueous mediums (Privat, Telo, Bernardes-Genisson, Vieira, Souchard, Nepveu, 2002).

Ferulic acid (4-hydroxy-3-methoxycinnamic acid), another abundant phenolic compound in grape canes, has received much attention in the study of traditional medicines since it was found to be one of the effective components in medicine herbs such as *Angelica sinensis, Cimicifuga heracleifolia* and *Lignsticum chuangxiong* (Sakai, Kawamata, Kogure, Mantani, Terasawa, Umatake, Ochiai, 1999). The reported physiological functions of ferulic acid and its derivatives include: antioxidant activity, cholesterol-lowering activity, antimicrobial and anti-inflammatory activity and anti-cancer effects (*10–13* Ou, Kwok, 2004, Ketsawatsakul, Whiteman, Halliwell, 2000, Lo, Chung, 1999, Mori, Kawabata, Yoshimi, Tanaka, Murakami, Okada, Murai, 1999).

The broad-spectrum of bioactivities of these compounds is due in part to their antioxidant capacities (Privat et al., 2002, Wolter & Stein, 2002, Ou & Kwok, 2004, King et al., 2006, Velioglu et al., 1998). While synthetic antioxidants are widely used food additives, some arguments exist about the safety and adverse effects of these substances (Shahidi & Wanasundra, 1992; Aruoma, Murcia, Butler, & Halliwell, 1993). Halliwell and Gutteridge (2000) concluded that natural antioxidants such as vitamins and polyphenols may have crucial effects in the prevention of diseases attributed to damage caused by free radicals. As a result, there is increasing demand for the utilization of phytochemicals in the preparation of dietary supplements, nutraceuticals, functional food ingredients, and food additives, pharmaceutical and cosmetic products. At this point, the determination of the antioxidant activities of these compounds has gained increasing importance (Kinsella, Frankel, German, Kanner, 1993; Castelluccio et al., 1995; Belguendouz, Fremont, & Linard, 1997;

Cacace, Mazza, 2003a; Lucas-Abellán, Mercaderros, Zafrilla, Fortea, Gabaldón, & Núñez-Delicado, 2008). The relationship between antioxidant activity and the structure of phenolic compounds has been presented (Rice-Evans, Miller, & Paganga, 1996; Fukumoto, & Mazza, 2000; Burda & Oleszek, 2001; Caruso, Tanski, Villegas-Estrada, & Rossi, 2004) and several authors including Fukumoto and Mazza (2000), Kähkönen, Hopia, and Heinonen (2001), and Moyer, Hummer, Finn, Frei, and Wrolstad (2002) have reported that antioxidant activity of berries is due primarily to anthocyanins and total phenolic contents.

1.2.2 Extraction

Extraction and purification of bioactive compounds from natural sources has become important for the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, functional food ingredients, and additives to food, pharmaceutical and cosmetic products (Cacace, Mazza, 2003a).

Many factors such as temperature, time, solvent composition, and solvent to solid ratio can influence the extraction process (Cacace, Mazza, 2002, 2003a, 2003b, and Liyana-Pathirana, Shahidi, 2005). Also, different variables can impact the process differently and there may be interactions of analyzed factors. In addition, it was hypothesized that extraction conditions may have significant impact on the antioxidant activity of extracts obtained from different plant sources, and processed under different conditions.

Statistical analysis is often used to optimize the extraction of bioactives. Response surface methodology (RSM), which enables evaluation of the effects of independent process variables and their interactions on dependent variables, is a package of statistical and mathematical techniques employed for developing, improving, and optimizing processes (Myers, Montgomery, 2002).

Similar to the solvent employed in the solid-liquid extraction, uses of organic solvents such as ethanol, methanol, ethyl acetate, and acetone have commonly reported for extraction of the bioactive compounds from plant material (Merken and Beecher, 2000, Cacace, Mazza, 2003b). Disadvantages of long time, labor intensive procedure and toxic waste generation have been reported for solvent extraction process (Choi, Chan, Leung, and Huie, 2003). Environmental regulations have also prompted the industry to reduce organic solvent consumption. In addition, there is an increasing public sensitivity for food security, safety and quality, and consumers have started demanding more information about food and food processing (Colin, Cacace, Mazza, 2007).

In recent years, there has been an increasing demand to develop novel processes to overcome some of the major drawbacks encountered in conventional extraction methods (Cacace, Mazza, 2006, Herrero et al., 2005, Ju and Howard, 2005; Herrero, M.; Cifuentes, A.; & Ibanez, E., 2006). Pressurized low polarity water extraction (PLPW), which is known as subcritical water extraction, has shown important promise to take in place of conventional ones due to its using low-cost, environmentally benign, and non-toxic solvent. Pressurized low polarity water extraction (PLPW), which is a technology using hot water under pressure sufficient to maintain water in the liquid state well above its boiling point, modifies the properties of water by increasing the temperature above 100^{o}C to improve its extraction efficiency (Ibeñaz et al., 2003, Ju and Howard, 2005, Kim, Mazza, 2006). At ambient pressure and temperature, water is a polar solvent with a high dielectric constant (ε = 78) being relatively high to extract nonpolar compounds but at 300^{o}C and P = 23 MPa this value decreases to 21, which is similar to the value for ethanol (ε=24 at 25^{o}C) or acetone (ε = 20.7 at 25^{o}C). This means that the polarity, viscosity, surface tension, and dissociation constant of water can be significantly lowered to values similar to those of organic solvents by elevated temperatures under pressure, thus allowing extraction of low-polarity and non-polar compounds (Richter, Jones, Ezzell, and Porter, 1996, Fernandez et al., 1997, Yang et al., 1998, Carabias-Martínez, Rodríguez-Gonzalo, Revilla-Ruiz, & Hernández-Méndez, 2005).

The efficiency of PLPW is affected by temperature, time as well as the presence of small quantities of organic solvents and surfactants. In most applications, pure water is the extractant used; however, it has been shown that water modified with some reagents such as acids, complexing agents, and organic solvents etc., produces better results compared to those obtained by pure water (Carabias-Martínez et al., 2005, Morales-Muñoz, Luque-García and Luque de Castro, 2006, Naczk and Shahidi, 2006).

How fast the compound will dissolve and reach the equilibrium concentration in the liquid is one of the determinative factors of the extraction process. The interaction between the solute containing particle and the solvent during extraction process includes a series of phenomenological steps. During the extraction of plant components diffusion mass transfer of solute in the solid phase is usually the rate controlling step (Schwartzberg, Chao, 1982, Gertenbach, 2002). Thus, the modeling of effective diffusivity of solute in solid matrix may be useful to have a better knowledge about the extraction of bioactive compounds from plant materials. Mathematical modelings involving mass transfer parameters (effective diffusivity) are received much attention in the advancement in PLPW extraction.

1.2.3 Aim of the Studies about the Extraction of Grape Cane Phenolics

The objectives of the study were to optimize the solid-liquid extraction of the major phenolics present in grape canes and the antioxidant activity of extracts using response surface methodology (RSM). The correlation between antioxidant activity of extracts and their total phenolic content was also determined. Another objective was to figure out the effectiveness of the novel technology, “Pressurized low polarity water extraction” on the extraction of the major phenolics present in grape canes, and the determination of the antioxidant activity of extracts obtained by this method.

Mass transfer of *trans*-resveratrol from milled grape cane particles by solid-liquid extraction in an agitated vessel was also studied, and effective diffusivities in the

solid phase were determined. Effective diffusivities of stilbene compounds were determined to clearly understand the mass transfer occupied in the PLPW extraction. Kinetic analysis was performed to explain the extraction process.

CHAPTER II

MATERIAL AND METHODS

2.1 Materials

Red raw grape samples supplied from Plant of Research and Application of Viticulture of Agricultural Faculty of Ankara University in Kalecik, Ankara, Turkey has been used to model the filtration of grape juice. Different clear fruit juices provided from a local market (Apple juices, sour cherry juices and grape juices in 1L packages produced by DİMES inTokat, Turkey) have been purchased to investigate the thermophysical properties (viscosity and density) of these fruit juices as a function of temperature and sugar contents. Clarified fruit juices were concentrated in a rotary evaporator under vacuum (Rotavapor-R, Büchi Swiss). The fruit juice samples at the specified ^{o}Brix values for trials were made by reconstituting the main concentrates with distilled water. Refractometers, ranging from 0 ^{o}Bx to 32 ^{o}Bx (Cole-Parmer Inst. Co., USA) and from 28 ^{o}Bx to 62 ^{o}Bx (N2-E Atago, Japan) were used to measure the soluble solid content of sample in ^{o}Brix at constant temperature.

2.2 Methods

2.2.1 Filtration

Raw red grapes delivered from vineyard were processed to raw grape juice following industrial procedure explained as below:

Raw grapes were washed with water and their stems were removed and transferred to a vessel. The weight of vessel and grapes together was measured to determine the

amount of grape processed. Grapes were crush in a vessel and heated up to 85^{o}C. Heating to higher temperature provides better color development for grape juice, but temperature values above 85^{o}C were not employed to avoid thermal degradation and cooked taste in grape juice. After heating, crashed grapes in the vessel were left to cool down to 45^{o}C. Reason of this specific temperature level was due to enzyme application. Pectolitic enzyme preparation provided by pilot plant for fruit juice production in Agricultural Faculty of Ankara University was reported to have the highest activity at this temperature level. To achieve desired activity, crashed grapes were left for at least 2 hours for enzyme activity after addition of preparation (0.1 mL enzyme preparation/kg crashed grape). Pectolictic enzyme preparation was used to break down dissolved pectin in raw grape juice. Pectin attaches to positively charged protein molecules. Pectin–protein complexes were stable and inhibited precipitation of declarification constituents. Breaking down dissolved pectin by pectolytic enzyme preparation provides better clarification for the production of clear fruit juice by releasing protein molecules attached with pectin molecules. Crashed grapes were manually pressed by screwing grapes in clothes. Bentonite and gelatin having negative and positive charges, respectively, were added to raw grape juice for clarification following press step. Preliminary experiments were carried out for amounts of bentonite and gelatin and determined as 0.75 and 0.03 g/L raw grape juice, respectively. Bentonite removed polyphenols and pectin carrying negative charges, whereas proteins having negative charges attached to gelatin. Raw grape juices were stored in a refrigerator at 4°C for precipitation. During storage destabilized colloidal particles including polysaccharides, pectin, pheolics, glycoproteins, proteins, and enzyme were precipitated by flocculating agents (bentonite and gelatin).

Filtration of depectinase and clarified grape juice was carried out using laboratory scale filtration unit. Cake filtration was aimed. The experimental setup will be composed of mainly three parts:

- A pressure supply, nitrogen gas,
- A reservoir which will be 2.5 L stainless steel storage vessel,

- A standard filtration cell holder.

The experimental apparatus is given in **Figure 3**.

Diatomaceous earth mixed with water and filled into storage vessel was forced by the help of nitrogen supply creating adjusted pressure with a pressure regulator placed on top of supply to pass through the filtration cell to achieve the precoating of the filter medium. Then diatomaceous earth as a filter aid and grape juice were mixed and fed to the storage vessel. The grape juice was forced to pass through precoated medium in the filtration cell to separate unwanted particles and to get clear grape juice. During filtration cake formation also occurred.

Clarified and non-clarified grape juices were filtered to show the effect of clarification on filtration process without using any precoating material and filter aid at 21°C and 0.65 atm gage pressure. Experiments were carried out under varying pressure levels; 0.3, 0.65, 1, 1.5 and 2 atm gage pressures at 21°C using 0.1 g/cm^2 filter medium and 0.005 g/mL grape juice to figure out the influence of pressure applied. Diatomaceous earth was employed as a precoating material and a filter aid. Amount of diatomaceous earth was used at three different levels (0.05, 0.1, and 0.25 g/cm^2) to investigate precoating effect on filtration. 0.002, 0.005, and 0.01 g diatomaceous earth/mL grape juice were added to determine the change of filtration trend with a filter aid. Pressure level of 0.65 atm gage and 21°C were kept constant during the trials for precoating and filter aid studies. Three different temperature levels (8, 21, and 34°C) were selected to determine temperature influence on filtration. Amounts of precoating and filter aid were 0.1 g/cm^2 filter medium and 0.005 g/mL grape juice were used for temperature trials in which pressure was adjusted as 0.65 atm gage pressure. In addition perlite was used to investigate the influence of different precoating material and filter aid. Soluble solid content of clarified raw grape juice was adjusted to two level being 16 and 28^{o}Bx.

An applied pressure difference during filtration experiments was kept constant by the help of pressure regulator. Filter paper (Whatman 41, Diameter of 90 mm) having specific filter area of 33.2 cm^2 was used as a filter medium for each trial.

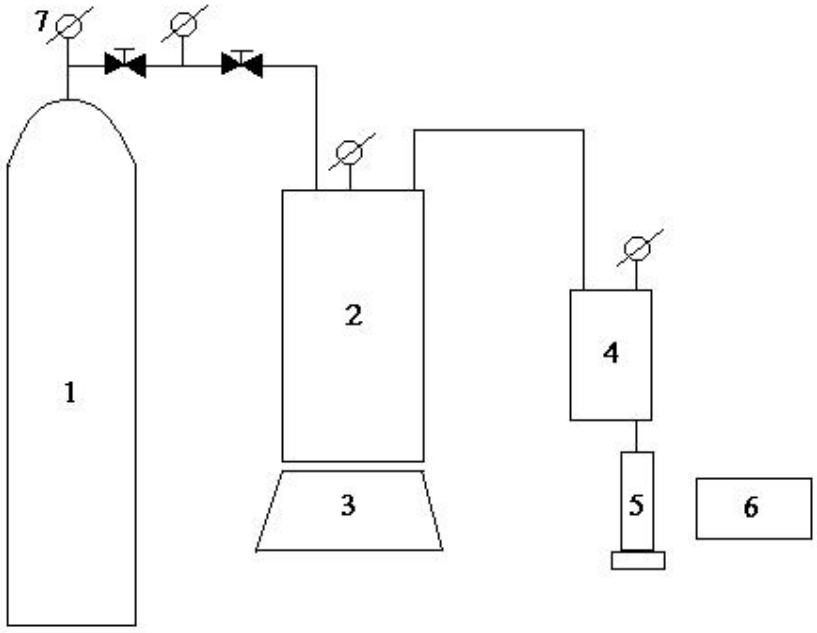

Figure 3 Experimental apparatus for filtration

1. Pressure Supply (Nitrogen Gas)
2. Stainless Steel Storage Vessel
3. Magnetic Stirrer
4. Filtration Cell Holder
5. Graduated Cylinder
6. Timer
7. Pressure Regulator

Filtrate volume versus time was measured to calculate the filtration rate and the resistance.

2.2.2 Density

Fruit juice density, ρ (g/ml) was measured with gravimetric method. The sample contained in a standard volumetric pycnometer (25 mL and 50 mL, Isolab, Turkey) was weighed in an analytical balance (UFO-3000, UFOTECH, Turkey). Sample temperature was varied by equilibration in the thermostatic water bath (ST 402, NÜVE, Turkey). Temperature values ranged from 25°C up to 60°C with 5°C increments. Soluble solid content of samples varying from 10°Bx to 60°Bx were changed at 10°Bx increments. The density results of three different fruit juices were evaluated individually, and also the same results were used to get a generalized model as a function of temperature and soluble solid content of clear fruit juices.

2.2.3 Viscosity

This thermophysical property of clear fruit juices were measured with Cannon-Fenske capillary viscometer. Because Rao (1999) stated that single strength juices and concentrated clear fruit juices generally exhibit Newtonian flow behavior or close to it and capillary tube viscometers are applicable for Newtonian fluids to measure their viscosities. Kinematic viscosities of three clear fruit juices were determined under varying levels of temperature and soluble solid content of samples. Temperature values varied from 25°C up to 60°C. Temperature was increased at 5°C intervals. A water bath and submerged heater were used to adjust temperature of the samples. Refractometers (0-32°Bx Cole-Parmer Inst. Co., USA and 28-62° Bx N2-E Atago, Japan) were used to adjust soluble solid content of the samples in terms of brix (soluble sugar amount in 100 g solution). Their brix values were in between 10° to 60° Brix and it was increased at 10° intervals. To measure the kinematic viscosity of the samples, efflux time values required to pass through the certain distance on the capillary tube were measured for each clear fruit juices at certain temperatures and soluble solid contents. The kinematic viscosities of the samples were determined by

multiplying the efflux time value by viscometer constant of that capillary tube. In literature apparent viscosities of materials are generally given at specified conditions. In this study, kinematic viscosities of clear fruit juices at specified conditions were given in terms of apparent viscosity at the same conditions to provide appropriateness with literature. The kinematic viscosity value was multiplied with density value at the same conditions to get the apparent viscosity. Density value calculated by corresponded model of clear fruit juice was used for determination of apparent viscosity. Experimental results were tried to fit a well known model, Arrhenius Equation (10);

Arrhenius Equation represents the viscosity change of any fluid according to temperature change, so the effect of soluble solid contents of clear fruit juices should be considered. To add the effect of soluble solid content in terms of brix value, its effect on activation energy of clear fruit juice, which is the term of Arrhenius Equation, was investigated. The model of activation energy of clear fruit juice representing the effect of soluble solid content of clear fruit juice was obtained to put it into Arrhenius Equation. The viscosity results of three different fruit juices were evaluated individually, and also the same results were used to get a generalized model as a function of temperature and soluble solid content of clear fruit juices.

2.2.4 Heat Capacity

Heat capacities (Cp) of clear fruit juices and distilled water were measured with Differential Scanning Calorimeter, DSC (DSC Q 10, TA Instruments, USA). First of all samples were prepared to measure their heat capacities. During preparation of samples important parameter considered was soluble solid content of clear fruit juices. For each three different clear fruit juices six samples were prepared for each parallel. Differences between samples were their soluble solid contents. Their soluble solid contents changed in the range of 10^{o} - 60^{o}Bx, and increased at 10^{o}Bx intervals. Refractometers (0-32^{o} Bx Cole-Parmer Inst. Co., USA and 28-62^{o} Bx N2-E Atago, Japan) were used to adjust the soluble solid contents of fruit juices. Samples were

prepared from previously concentrated fruit juices stored in refrigerator. Distilled water was added to adjust the soluble solid content. Following the preparation of samples, samples were weighed into aluminum pans using sensible balance (APX-200, Denver Instrument, USA) and plastic pipettes before the settlement of pans into sample holder of DSC. Weights of samples were determined in to the advised literature range that is between 5 to 15 mg. Prepared pans were put into sample holder of DSC one by one. Sample holder had two places, one of them was for sample pan and other one was for empty pan as a blank. Cooler apparatus was attached to holder unit to cool down the temperature of samples and empty pan below the required temperature value. It was 25^{o}C for the present study. Temperature of sample pan and empty one can be visualized following computer software that was compatible with differential scanning calorimeter. This software was provided by TA Inst. Company with computer system and DSC. DSC was performed adjusting different methods. In this study RAMP method was used. In this method temperature of samples was increased at constant temperature change rate. Rate of temperature change was selected as 5^{o}C/min., being compatible with literature values for food materials. This rate should not be chosen at higher level. Because as possible and reasonable as lower rates give more accurate results in DSC studies. This procedure was performed two times for each clear fruit juice samples at each adjusted soluble solid content values. TA Inst. Company provided software (Universal Analysis 2000, TA Instrument. USA) to analyze the results. This software gave heat rate vs. temperature graph and data. Using these data heat capacity of sample can be calculated according to below Equation (13);

$$Cp = \frac{E \cdot (dQ/dt)}{(dT/dt) \cdot m} \qquad \textbf{(13)}$$

In this equation (1/m)·(dQ/dt) data were taken from software at different time and temperature values and (dT/dt) data was constant and determined as mentioned before. E was a function of temperature and it was determined using reference material. Generally sapphire is used for this purpose because of its stability at wide temperature range without any phase change. But in the present study distilled water

was chosen as a reference material. Because one of the main component of clear fruit juice samples was water, and it follows trend similar to food stuffs in temperature range of 25 to 60^{o}C. Another important point is that water does not show any phase change at those temperature levels at atmospheric pressure. In addition to above advantages, water is widely available. To model E as a function of temperature, water was used. Comparison of literature value of heat capacity of water with experimental results achieved from differential scanning calorimeter measurement was performed and model was obtained to use in the evaluations of heat capacities of clear fruit juices.

The heat capacities of clear fruit juices were evaluated for each clear fruit juices and models representing the change of heat capacities of fruit juices with respect to temperature and soluble solid contents were achieved by analysis of calculated heat capacities of clear fruit juices at different temperature values and soluble solid contents.

2.2.5 Statistical Analysis

Statistical analyses were performed using Minitab Statistical Software. The suitabilities of the fitted equations were evaluated by the regression coefficient (R^2), the significance level (p), mean square error (MSE) and residual analysis.

2.2.6 Sample Preparation

Grape cane samples of the *Vitis viniferia* variety Pinot noir (one of the most renowned red grapes in the world) were collected from a private vineyard, near Penticton, B.C. Canada in February, 2007 and freeze dried. Dried grape canes were ground in Wiley mill (Thomas® - Wiley® Mill Model ED-5, Arthur H. Thomas Co., Philadelphia, P.A., USA), using screen having 1mm mesh size and 1 mm gap between blades, and then stored in sealed plastic bags in humidity-controlled storage

room at 23-25°C until extraction. Average particle diameter (d_p) (255.5 μm) of milled grape cane was calculated by sieve analysis (Cacace, Mazza, 2003b).

2.2.7 Extractions

2.2.7.1 Solid-Liquid Extraction

Ground samples were extracted in an agitated 4 L glass beaker of 15.3 cm ID with 2.5 L of solvent for optimization experiment (**Figure 4**). Ethanol/water mixtures (v/v) at different concentrations were used as solvents. An airfoil axial impeller (Lightnin model A 310, Mixing Equipment Co. Inc., Rochester, NY) with a 6.35 cm diameter was used for mixing. The extraction tank was set in a thermostatic water bath set at the desired temperature. A cap was attached on the top of the beaker to avoid solvent loss, condensing vaporized solvent during the extraction. Shape factors were adjusted to $S_1 = D_a/D_t = 0.41$; $S_2 = B/D_a = 0.61$; $S_3 = L/D_a = 0.35$; $S_4 = W/D_a = 0.092$; $S_6 = H/D_t = 0.84$ to ensure uniform mixing and to minimize their effect on mass transfer. Grape cane samples were mixed with solvents after desired temperature and flow regime were built up. Liquid samples were periodically taken from the extractor to determine the equilibrium in terms of mass transfer. Extractions were ended when extracts and pomace reached equilibrium indicated by no further change of absorbance readings of liquid samples at 280 and 320 nm.

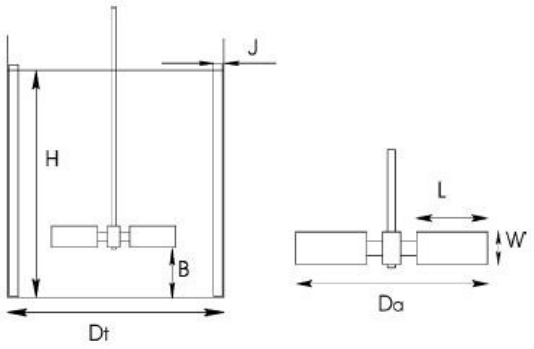

Figure 4 Characteristic dimensions of the vessel and impeller

2.2.7.1.1 Mixing Conditions

The axial impeller was located at a distance of $D_t/4$ above the tank bottom and at 15° angle in the tank without baffles. Mixing rate was adjusted in the range of 1150-1750 rpm for dispersion of particles in solvents and for rapid initial mixing of liquid reactants (Perry, Greene, and Maloney, 1997). The rotational speed, N was fixed at 1250 rpm to ensure that the flow was in the turbulent regime ($Re > 10^4$), avoiding variation of the effect of the rotational speed on mass transfer of a given compound.

Solvent height in the tank was kept constant at 14 cm so that the vessel solvent-height to diameter ratio was 0.92. The geometry of the vessel and impeller, shape factors, rotational speed, and location of shaft and impeller were fixed to avoid variation in the mixing system for all extractions carried in this study.

As a reference method, solvent extraction using an ethanol/water 80:20 (v/v) solution was carried out for 30 min at 60°C with gentle stirring (Romero-Pérez, Lamuela-Raventós, Andrés-Lacueva, Carmen de la Torre-Boronat, 2001). Solvent to solid ratio was 125 mL/g.

2.2.7.2 Pressurized Low Polarity Water Extraction

The pressurized low polarity water (PLPW) extractor, described by Cacace and Mazza (2006) and Kim and Mazza (2006), was used for the extraction of bioactive compounds from milled grape canes. PLPW system can be seen in **Figure 5**. The solvent used in PLPW system was water. Ethanol was employed adjusting to the concentration levels of 7.4, 15, and 25% (v/v) as a modifier. Six different temperature levels (85, 95, 105, 120, 140, and 160^{o}C) were evaluated using 7.4% ethanol/water mixture at a flow rate of 1 mL/s. The flow rates of 0.5, 1, 2, 3, and 5 mL/s were tried to analyze its effect on the PLPW extraction using 7.4% ethanol/water mixture at 105^{o}C. Key components of the PLPW extractor used were: an HPLC pump (510 model, Waters, Milford, MA), a 1.0 m preheating coil (1/16 inch o.d.), extraction cell, a temperature-controlled oven (5700A series, Hewlett-Packard, Palo Alto, CA), a 2.0 m cooling coil (1/16 inch o.d.), a back pressure regulator with a cartridge of 5.2 MPa (750 psi) (Upchurch Scientific, Oak Harbor, WA), and a collection vessel. The extraction cell manufactured in the mechanical work shop of the research center was 10 cm in length and 19.3 mm internal diameter (1.0 inch o.d.). All experiments were conducted using the same extraction cell. Connections, fittings, extraction cell, and tubing (1/16 inch o.d.) were made of stainless steel and adequate for pressures up to 34 MPa (4900 psi).

To keep the milled grape cane sample inside the extraction cell, glass wool (5 mm thick) was placed at both ends of the extraction cell. The extraction cell was fitted with 100 μm frits at the inlet and outlet and was mounted in the oven. The system was tested for leaks and the temperature was increased to the required value for each experiment. Extraction was started by pumping the solvent at the desired flow rate and at 5.2 MPa (750 psi). The extractions were dynamic and were performed to collect a certain extract volume, which depended on the time and flow rate of process. The extract was collected into the opaque plastic container under nitrogen gas stream to avoid the photodegradation and oxidation of bioactive compounds. At

the end of each extraction, the extraction cell was removed and the system was washed by pumping through 50 mL of 50:50 (v/v) ethanol/tetrahydrofuran solvent mixture and then rinsed with 100 mL of Milli-Q water. Extracts collected from each experiment were stored in a refrigerator at 4 °C, and the solid residues were removed from the extraction cell, weighed, dried in a vacuum oven at 60 °C for 24 h, and stored at -25 °C.

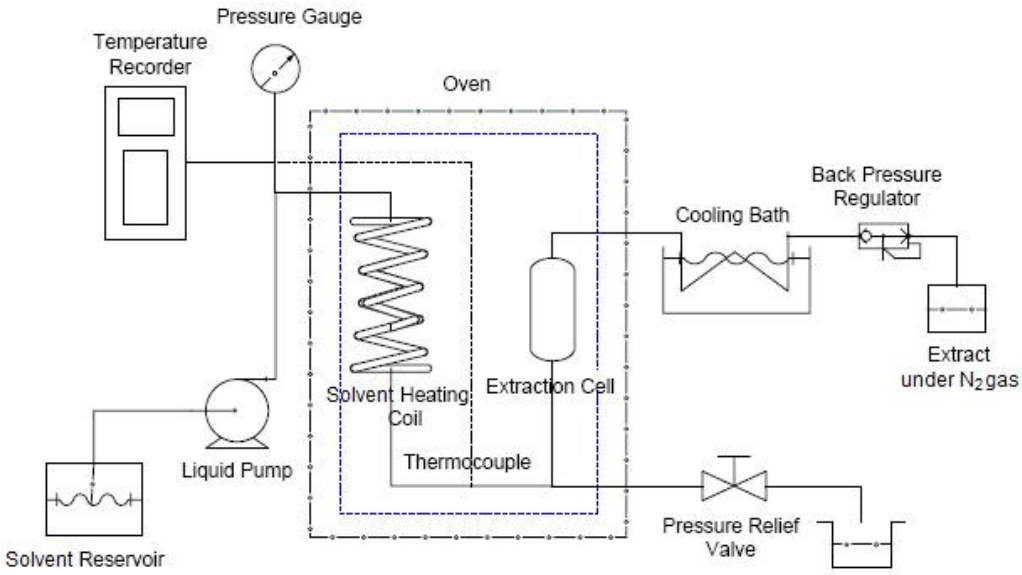

Figure 5 Pressurized low polarity water extraction system (PLPW)

2.2.8 Analysis

For the determination of free phenolics, and total phenolics all extracts were filtered through a 0.2 µm PVDF membrane disc held in 13 mm diameter syringe filter holders (Chromatographic Specialties Inc., Brockville, Ontario, Canada) and stored in a refrigerator at 4^{o}C till their analysis. LC-DAD analysis was carried out using a liquid chromatograph system (Agilent 1100 series, Agilent Technologies Inc., Palo

Alto, CA) equipped with a photodiode array detector, an auto sampler, and a control module. Samples of 5 μL were injected into a reversed-phase C_{18} column (Zorbax SB, 5 μm, 250 × 4.6 mm, ID Agilent Technologies Inc.) preceded by a guard column (Inertsil 5 ODS, 5 μm, 30 × 4.6 mm, ID Phenomenex, Torrance, CA). A gradient solvent system was used with solvent A being phosphoric acid (50 mM) and solvent B being pure methanol at HPLC grade. The elution profile had the following proportions (v/v) of solvent B: 0 min, 5%; 0-5 min, 5%; 5-51 min, 5-55%; 51-61 min, 55-100%; 61-68 min, 100%; 68-73 min, 100-5%; and 73-83 min, 5%. The solvent flow rate was 0.4 mL/min.

trans-Resveratrol (detection at 320 nm) and ferulic acid (detection at 320 nm) were analyzed qualitatively comparing their retention times and UV spectras with authentic standards (Sigma-Aldrich; Oakville, ON, Canada). Concentrations of resveratrol and ferulic acid were calculated using their peak areas and standard curves. ε-Viniferin (detection at 320 nm) was identified by comparing its UV spectra to those reported in the literature (Jean-Denis, Pezet, & Tabacchi, 2006). ε-Viniferin was quantified by assuming to have an identical molar extinction coefficient with *trans*-resveratrol, as described previously (Adrian, Jeandet, Breuil, Levite, Debord, & Bessis, 2000). The concentration of total phenolics was calculated using total area of peaks at 280 nm and expressed as an equivalent of *trans*-resveratrol using standard curve of *trans*-resveratrol.

2.2.9 Experimental Design

Optimization of extraction conditions for yields of *trans*-resveratrol (Z_1), *trans*-ε-viniferin (Z_2), ferulic acid (Z_3), total phenolics (Z_4), effective diffusivity of *trans*-resveratrol (Z_5), antioxidant activity measured by TEAC method (Z_5), and measured by ORAC method (Z_6) were carried on using response surface methodology. The independent variables were temperature (X_1), solvent to solid ratio (X_2), and ethanol concentration (X_3). A central composite design was selected for optimization of process variables each at 5 levels with 18 runs including four replicates at the central

point. The range and levels of independent variables and code values are presented in **Table 1**.

Experimental data were analyzed using Minitab (Minitab 15.1.0.0.) and SAS v. 9.1.3 (2002-2003) (SAS Ins. Inc., NC, USA) statistical software and fitted to a second order polynomial regression model containing the coefficient of linear, quadratic, and two factors interaction effects. The model equation of response (Z) of the three independent variables (X_1, X_2, and X_3) is:

$$Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \qquad \textbf{(14)}$$

where Z is the dependent variable, β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient.

The response surface graphs of predicted values by models were plotted using Sigma Plot v. 8.02 (2002) (SPSS Inc., Chicago, Illinois, USA).

The values of R^2 and adjusted-R^2 of models were evaluated to check the model adequacies (Myers, Montgomery, 2002).

Table 1 Three-factor, five level central composite design used for RSM

standard order[a]	run order[b]	factor 1 (X_1)	factor 2 (X_2)	factor 3 (X_3)
		temperature (°C)	solvent to solid ratio (mL/g)	ethanol concentration (%, v/v)
1	4	30 (-1)	50 (-1)	36 (-1)
2	15	70 (1)	50 (-1)	36 (-1)
3	11	30 (-1)	90 (1)	36 (-1)
4	10	70 (1)	90 (1)	36 (-1)
5	1	30 (-1)	50 (-1)	80 (1)
6	6	70 (1)	50 (-1)	80 (1)
7	14	30 (-1)	90 (1)	80 (1)
8	2	70 (1)	90 (1)	80 (1)
9	3	16.4 (-1.68)	70 (0)	58 (0)
10	9	83.6 (1.68)	70 (0)	58 (0)
11	16	50 (0)	36.8 (-1.68)	58 (0)
12	5	50 (0)	103.6 (1.68)	58 (0)
13	7	50 (0)	70 (0)	21 (-1.68)
14	18	50 (0)	70 (0)	95 (1.68)
15	12	50 (0)	70 (0)	58 (0)
16	13	50 (0)	70 (0)	58 (0)
17	17	50 (0)	70 (0)	58 (0)
18	8	50 (0)	70 (0)	58 (0)

[a] No randomized, [b] randomized.

2.2.10 Antioxidant Activity

The Trolox equivalent antioxidant capacity assay, (TEAC) and the oxygen radical absorbing capacity ($ORAC_{FL}$) were employed to measure total antioxidant activity (TAA) of grape cane extracts. The TEAC method was modified from the procedure of Rice-Evans, Miller, and Paganga (1997) and Pellegrini, Re, Yang, and Rice-Evans

(1999) for use in microplates (Fukumoto, & Mazza, 2000). Briefly, a mixture of 5 mL of 7 mM ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (prepared with MilliQ-water) and 88 μL of 140 mM potassium persulfate ($K_2O_8S_2$) (prepared with MilliQ-water) was prepared. This concentrated ABTS reagent was stored in the dark for 12-16 hrs. On the day of the analysis, 50 μL of Trolox standard (0-50 mg/L) or diluted extract sample (from 0 up to 6.79 g/L) was added in triplicate into wells of 96 well-microplates. To each well containing standard or sample, 250 μL of diluted ABTS solution (the mixture of 0.51 mL of concentrated ABTS reagent and 30 mL of 80% MeOH/MilliQ-water) was added to each well of microplate and the plate was read after 5 min using a Spectramax 384 Plus microplate reader (Molecular Devices, Sunnyvale, CA) at 734 nm. The slopes for Trolox and samples were calculated from graphs of absorbance change [A734 nm at Conc. 0 – A734 nm at Conc. X] versus concentration as can be seen in Figure 6 for Trolox standard. The TEAC of the sample was calculated by dividing the slope of the sample by the slope of Trolox. The antioxidant activity was expressed as mg of Trolox equivalents per g of dry sample.

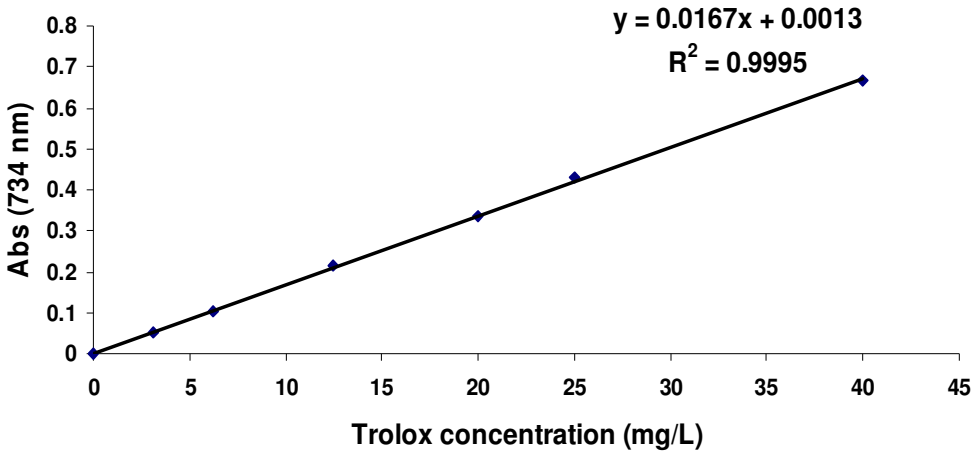

Figure 6 Absorbance change with concentration of Trolox standard

The oxygen radical absorbing capacity ($ORAC_{FL}$) method was modified from the procedure of Ou, Hampsch-Woodill, and Prior (2001) for use in microplates (Fukumoto, & Mazza, 2000). Briefly, 87 μM fluorescein stock solution was prepared in 75 mM phosphate buffer at pH 7.4 and stored at 4°C. On the day of the analysis, 20 μL of stock solution of fluorescein was diluted to 25 mL with 75 mM phosphate buffer at pH 7.4 to make up 87 nM fluorescein solution and heated to 37°C in a waterbath. A 70 mM of AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride) solution was prepared in 75 mM phosphate buffer as the peroxyl radical generator. This solution was also heated up to 37°C. Trolox standard (0-50 mg/L) was diluted with 75 mM phosphate buffer at pH 7.4. Samples were also diluted with buffer at appropriate dilutions to fit within the standard curve. The wells of an opaque fluorescent plate were filled with 25 μL of sample and standard in triplicate at various concentrations. Wells around the outside edges of the plate were not used. To all wells, 200 μL of 87 nM fluorescein solution was added followed by the addition of 50 μL of 70 mM AAPH solution. Immediately the plate was put into a Spectramax Gemini EM microplate fluorometer (Molecular Devices, Sunnyvale, CA) and read. An excitation of 485 nm and emission of 530 nm with cutoff filter of 515 nm was used and readings were taken every 2 minutes for 150 min. SoftMax Pro (version 5.3) was used to calculate the area under the curve. A standard curve for Trolox was made from the data. The Trolox equivalent for a sample was found using the standard curve. The average of three replicates was used to calculate the ORAC value for each sample. The results obtained by the ORAC method were correlated with the results measured by the TEAC method. Correlation coefficients between antioxidant activities determined by both methods and total phenolics of extracts were also determined using SAS v. 9.1.3 (2002-2003) (SAS Ins. Inc., NC, USA) statistical software.

2.2.11 Modeling of Extraction Systems of Bioactives from Grape Cane Samples

2.2.11.1 Effective Diffusivity in the Solid-Liquid Extraction

Convection, molecular diffusion, and eddy diffusion are the different mechanisms involved in solute transfer in solid-liquid extraction. However, generally it is diffusion due to random molecular motion in solid that dominates the extraction (Gertenbach, 2002). Convection and eddy diffusion are fast compared to molecular diffusion; and in the leaching of foods, diffusion in the solid is usually the rate-controlling step (Schwartzberg, Chao, 1982). The extent of control is indicated by the Biot number defined by Schwartberg and Chao (Schwartzberg, Chao, 1982):

$$Bi = \frac{k_c\, m\, a}{D_{eff}} \qquad \textbf{(15)}$$

where k_c is the external mass transfer coefficient in the extract, a is the characteristic dimension of the solid, D_{eff} is the diffusivity in the solid, and m is the partition coefficient for a solvent and it was calculated using the following equation for each extraction trial:

$$m = \frac{y_e}{x_{dm}} \qquad \textbf{(16)}$$

where y_e is weight fraction of a given compound in the liquid phase at equilibrium whereas x_{dm} is the weight fraction of the same compound in dry solid phase at equilibrium and it was calculated from the mass balance Eq. (16) (Gertenbach, 2002):

$$x_{dm} = \frac{x_m - (1 - DM_p)\cdot y_e}{DM_p} \qquad \textbf{(17)}$$

where x_m and y_e are the weight fractions of the marker in the wet pomace, and liquid extract, respectively. The factor DM_p is the weight fraction of dry matter in the wet pomace.

Extraction process for grape cane samples can be assumed to progress under the following conditions:

- Solid sample material is spherical particles with radius of a, characteristic dimension of solid,
- The solvent in extractor is well-mixed,
- There is no interaction between diffusion of given compound and other compounds in sample,
- The diffusivity of a given compound in the solid phase is constant,
- The initial given compound concentration in the solid phase is uniform,
- The controlling stage of extraction is the diffusion of a given compound in the solid phase,
- There is no chemical reaction.

The extraction of a phenolic compound from spherical plant particles was assumed to obey to second Fick's law (Cacace, Mazza, 2003a, Schwartzberg, Chao, 1982, Gertenbach, 2002, Franco, Sineiro, Pinelo, Nuñez, 2007), and to determine the values of τ corresponding to experimental dimensionless extract concentration (Y), the analytical solution of Fick's law for finite volume ratio (α), and very short time (τ), the method described by Schwartzberg, and Chao (1982) was used.

The Dimensionless time value (τ), a dimensionless time was defined as:

$$\tau = \frac{D_{eff}\, t}{a^2} \qquad \textbf{(18)}$$

The value of D_{eff} was evaluated by multiplying the slope of the model predicted by linear regression of τ versus time, t by a^2.

2.2.11.2 Effective Diffusivity in the PLPW Extraction

Extraction process for grape cane samples can be assumed to progress under the following conditions:

- Solid sample material is spherical particles with radius of *a*, characteristic dimension of solid,
- There is no interaction between diffusion of given compound and other compounds in sample,
- The diffusivity of a given compound in the solid phase is constant,
- The controlling stage of extraction is the diffusion of a given compound in the solid phase,
- There is no chemical reaction.

Diffusion rate of the limiting step of a phenolic compound extraction from spherical plant particles can be described by Fick's second law (Schwartzberg, & Chao, 1982, Gertenbach, 2002, Cacace, Mazza, 2003b, and Pinelo, Sineiro, & Núñez, 2006):

$$\frac{\partial C}{\partial t} = D_{eff}\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \qquad \textbf{(19)}$$

with the initial and the boundary conditions assuming the negligible external mass transfer resistance,

$$C_{(t=0)} = C_i \qquad \textbf{(20)}$$

$$\left.\frac{\partial C}{\partial r}\right|_{(r=0)} = 0 \qquad \textbf{(21)}$$

$$C_{(r=R)} = 0 \quad \textbf{(22)}$$

where C is the solute concentrations (mg/mL) at any location in the particle at time t (s); C_i is the initial solute concentration (mg/mL); D_{eff} is the effective diffusivity (m^2/s); t is extraction time (s); r is radial distance coordinate from centre of spherical particle (m); R is the average particle radius (m).

The series solution of Eq. (19) is given by Crank (1975) and Cussler (1984);

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp\left[\frac{-D_{eff}n^2\pi^2 t}{R^2}\right] \quad \textbf{(23)}$$

where M_t is the total amount of solute (mg/g dw) removed from grape cane at time t; M_∞ is maximum amount of solute (mg/g dw) extracted at equilibrium.

It is generally assumed that, for extraction from plant matrixes where external resistance is negligible, the first term of the series solution can usually be used with little error (Schwartzberg, 1975).

$$1 - \frac{M_t}{M_\infty} = \frac{6}{\pi^2}\exp\left(-\frac{D_{eff}\pi^2 t}{R^2}\right) \quad \textbf{(24)}$$

The linear (graphical) solution plotting $\ln(1 - M_t/M_\infty)$ vs. time can be used to determine the values of D_{eff} corresponding to ratio of total migration to the maximum migration concentration (M_t/M_∞).

2.2.11.3 Kinetic Model

For the PLPW extraction of *trans*-resveratrol, *trans*-ε-viniferin from grape cane samples, modified Gompertz Equation as a kinetic model has been proposed to explain the extraction mechanism.

$$y = y_{\infty} \cdot \exp\left\{-\exp\left[\frac{m \cdot e}{y_{\infty}} \cdot (\lambda - t) + 1\right]\right\} \qquad \textbf{(25)}$$

where y is total amount of phenolic compound (mg/g dw) extracted from grape cane sample at time t; y_{∞} is the maximum amount of phenolic compound (mg/g dw) extractable at equilibrium; m is the fastest extraction rate (mg/g.min); and λ is time period required to reach the beginning of the maximum extraction rate (min).

SigmaPlot 2000 Version 6.00 (Chicago, IL, USA) was used for linear and nonlinear regression analysis. The goodness-of-fit of the models was assessed using adjusted regression coefficient (R^2 *adj*) and mean square error (*MSE*) values.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Clear Fruit Juices

3.1.1 Filtration of Grape Juice

De La Garza and Boultons' "Exponential model" of wine filtration was evaluated to represent the cake filtration of grape juice. Results indicated that the selected model was not found to satisfy to explain the cake filtration process except non-clarified grape juice filtration. Bayındırlı (1989) and Şahin (1992) have reported satisfactory model fitting of the cake filtration of apple juice and sourcherry juice, respectively, employing the same model which was proposed for grape juice. Different results of filtrations of grape juice and apple and sourcherry juice may be attributed to the methodologies applied to achieve clear fruit juices in these studies. When the studies were compared, the process of grape juice production was found to be different from apple and sourcherry juices. Apple juice and sourcherry juice studies included particles causing fast clogging of the filter unit when they were introduced to filtration unit, whereas in grape juice filtration, those particles were already removed before filtration process and achieved more clear and colloids free grape juice compared to apple and sourcherry juices. As a result it was observed that the capacity of the system was not enough to see the clogging of filter medium except non-clarified trial, when the filtration experiment was carried out. Linear regression analysis indicated that total resistance linearly decreased whereas flow rate increased with increasing filtration time (**Figure 7 and Figure 8**).

This can be attributed to washing effect of flow of raw grape juice into filtration cell. Because the grape juice washed out the precoating material over the filter paper and

created a precoating material free zone at the very beginning of filtration process, when filtration was started up. This area increased with continuing process; as a result total resistance occurred in the process decreased. Reduction of total resistance due to removal of the precoating material was dominant effect compared to increase in total resistance due to plugging particles at the studied capacity of filtration units. In other words, observed trend of the grape juice filtration was similar to the very beginning of apple and sourcherry juices. Experimental conditions, measured flow rate data and calculated total resistance of filtration clarified grape juice without the filter aid and the precoating material were summarized in **Table 2**. All experiment conditions were represented in the Tables in Appendix A.

Table 2 Experimental conditions of clarified grape juice filtration and calculated resistance parameters

Labelled as Table A1 in Appendix A.

Experiment Conditions	
Pressure (atm.gauge) (Bar)	0.65
Filter aid material	-
Amount of precoating (g)	-
Amount of filter aid (g)	-
Temperature (^{o}C)	21
Brix of grape juice	28.6
pH of grape juice	3.66
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.002709
Pressure difference (Pa or N/m^2)	65000
Filtration area (m^2)	0.00332
Filter medium resistance, R_m, (1/m)	15504.08
Exponential fouling coefficient, β, (1/m^3)	-0.00238

CLF, clarified grape juice.

Filtration Process					
filtrate (mL)	time (s)	dt/dV	V (m^3)	R_{Total} (m^{-1})	ln(R_{Total})
0					
100	18	0.18	50	14340.46	9.57084
200	30	0.12	150	9560.308	9.165375
300	41	0.11	250	8763.616	9.078364
400	51	0.1	350	7966.923	8.983054
500	57	0.06	450	4780.154	8.472228

R_{Total}, total resistance.

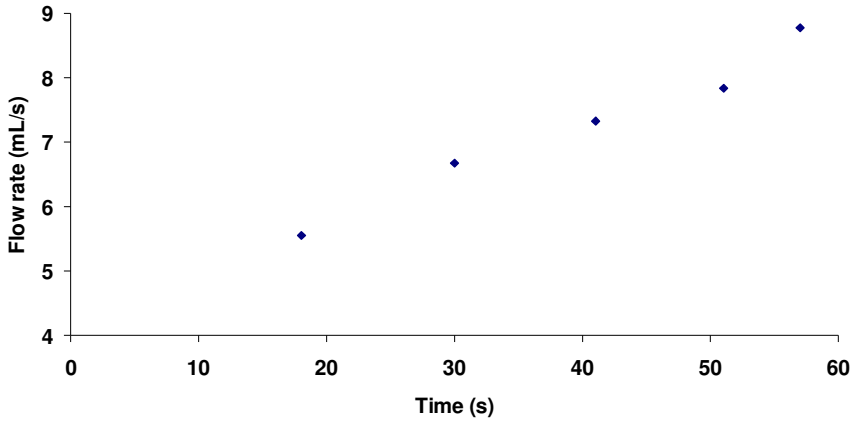

Figure 7 Change of flow rate of clarified grape juice filtration

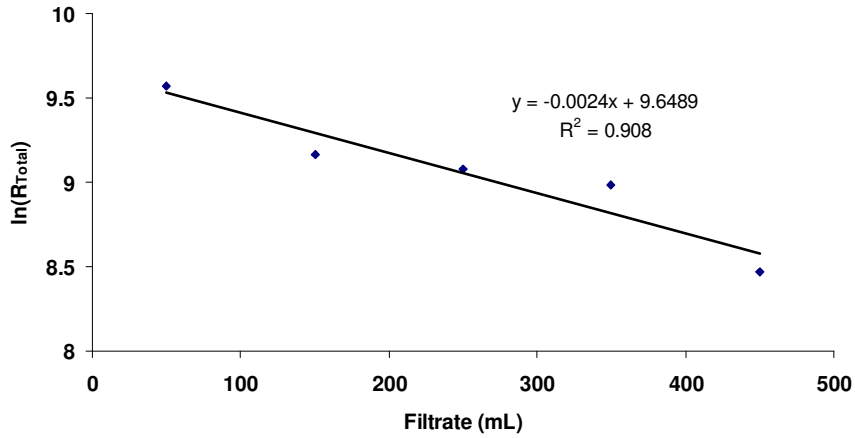

Figure 8 Change of total resistance of clarified grape juice filtration.

In contrast to the trials of clarified grape juice filtration, increase in total resistance and decrease in flow rate of filtration of non-clarified grape juice was observed due to present of plugging colloids (**Figure 9 and Figure 10**) even the washing out of the precoating materials over filter paper at the specific zone occurred. As a result, non-clarified grape juice filtration was fitted to the "Exponential model" and represented

by **Figure 10**. Except one set of trial to see the filtration of non-clarified grape juice, all trials were carried out using clarified juice. Because of this, the effects of the remaining filtration conditions were evaluated without the model fitting in the following section.

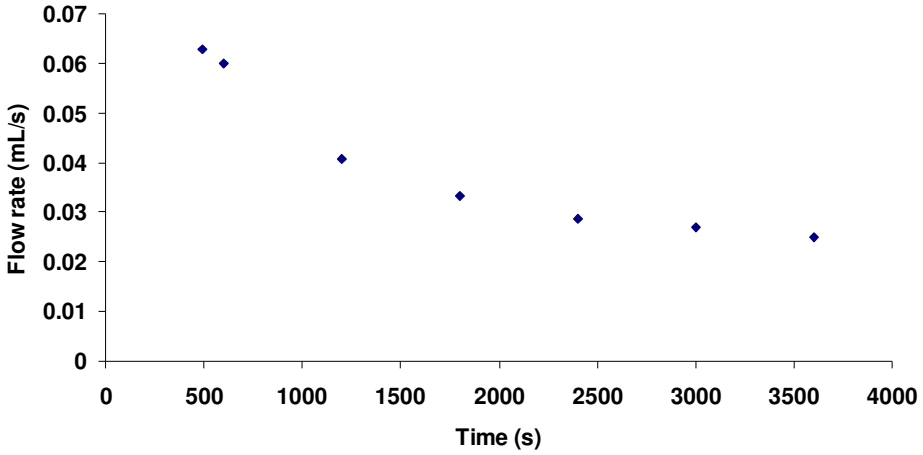

Figure 9 Change of flow rate of non-clarified grape juice filtration

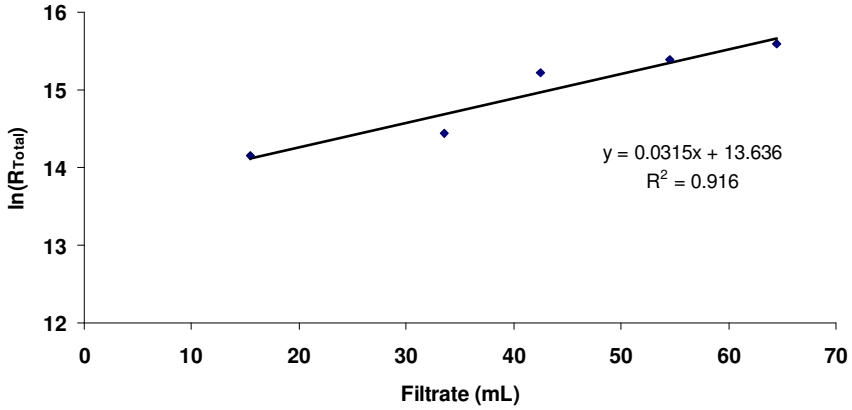

Figure 10 Total resistance change of non-clarified grape juice filtration

3.1.1.1 Effect of the Addition of the Precoating Material

Preliminary trials indicated the valuable effect of using the precoating material on the efficiency of filtration process in terms of available operation time. Three different amounts of the precoating were employed to visualize its effect on filtration process. Amounts of the precoating material of 0.05, 0.1, and 0.25 g/cm^2 filter area resulted in the decrease in the filtration rate as amount was increased. As filtration rate decreased, the life of filter unit increased by retarding plugging of filter medium using the precoating material. **Figure 11** displayed the lowering filtration rate with increasing the amount of the precoating material. Although the usage of the precoating negatively affects the flow rate, plugging of filter medium by slimy particles was retarded. Therefore, the precoating is necessary to achieve long operating life of filtration unit and higher quality clear grape juice.

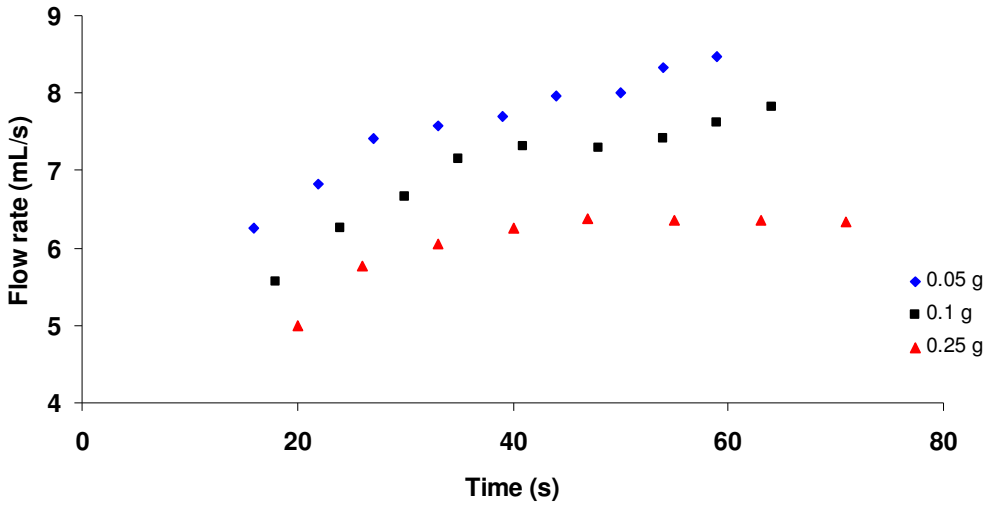

Figure 11 Effect of the precoating material (g/cm^2 filter area) on the filtration of grape juice

3.1.1.2 Effect of the Addition of the Filter Aid Material

Another parameter of the filtration process was filter aid which was used to improve the porosity of filter cake and to prevent the contact of slimy particles to each other and to retard plugging and to increase flow rate. Addition of the filter aid increased the filter medium resistance but decreased the cake resistance, since cake resistance was inversely proportional to the porosity of cake. Three different levels of the filter aid (0.002, 0.005, and 0.01 g/mL raw fruit juice) were employed. **Figure 12** displayed that the flow rate increased with increasing amount of the filter aid from 0.002 g/mL to 0.005 g/mL whereas further increase in the amount up to 0.01 g/mL resulted in reduction of flow rate.

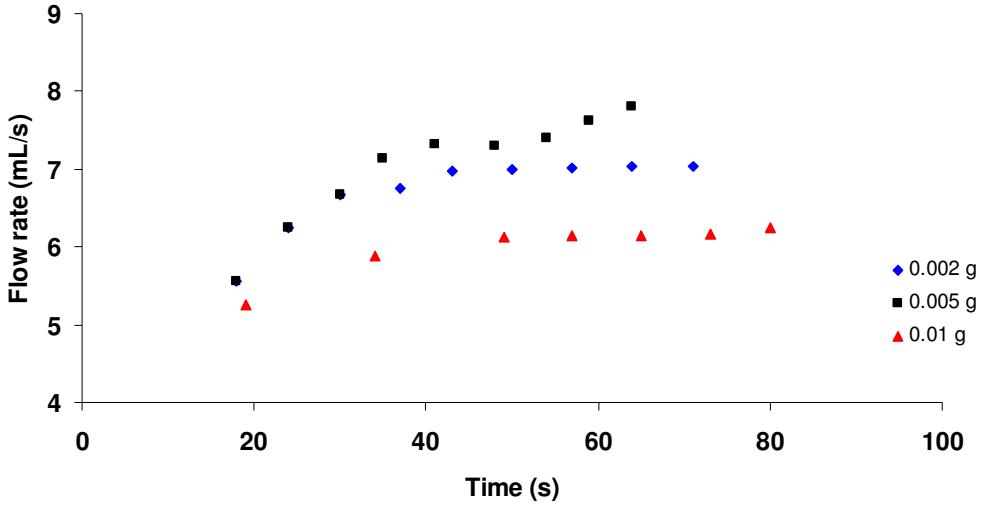

Figure 12 Effect of the filter aid material (g/mL grape juice) on the filtration of grape juice

3.1.1.3 Effect of Temperature of Fruit Juice on the Filtration Process

Three different temperatures, 8°C, 21°C, and 34°C, were tried during filtration of grape juice to evaluate its effect on the flow rate (**Figure 13**). Increasing temperature increased the flow rate of filtration process from 8°C to 34°C, but the improvement in the flow rate from 8°C to 21°C was found to be higher than the increase from 21°C to 34°C. Improvement of flow rate with temperature may be attributed to the effect of temperature on the viscosity of fruit juice filtered.

Viscosities of liquids decrease as temperature rise up. Decrease in temperature caused an increase in viscosity; as a result flow rate decreased meaning higher filtration resistance. In addition to the above effect of temperature, solubility of colloidal materials decreased at lower temperatures and those materials contributed to the plugging of filter medium.

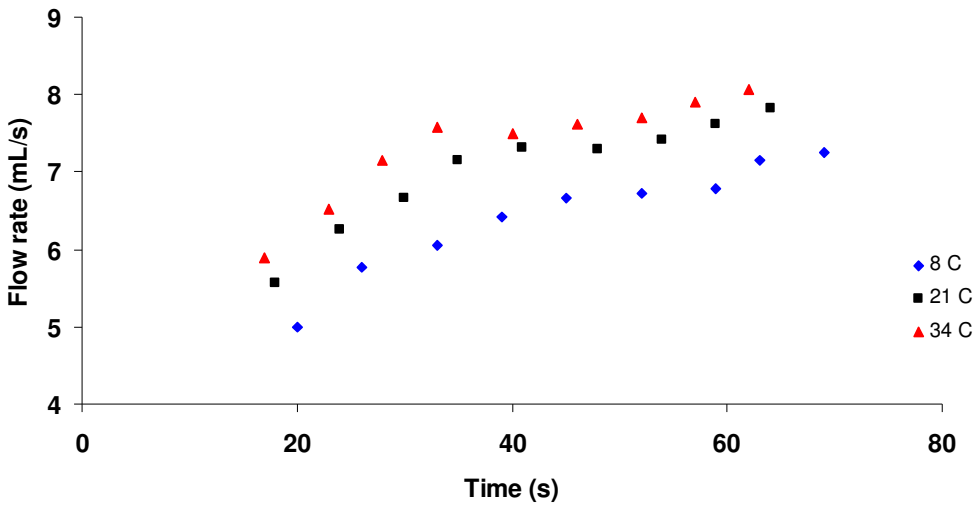

Figure 13 Effect of temperature on the filtration of grape juice

During the selection of operating temperature, possible adverse effect of higher levels should be considered besides of its favorable effect on flow rate. High temperatures may produce lower quality products in terms of color, flavor and/or nutritive components and means extra operating costs due to the requirement of preheating of grape juice, since clarification process of raw grape juice is carried out at low temperature, about 4^{o}C.

3.1.1.4 Effect of Operating Pressure

Increase in the flow rate of filtration process was observed with increasing pressure level applied (**Figure 14**). Pressure difference is the driving force for flow rate. In addition, resistance of filter medium is also increased with pressure. Five pressure levels (0.3, 0.65, 1, 1.5, and 2 bar) were used to investigate its effect on filtration, so filter cake was concluded as incompressible. From 0.3 bar to 2 bar continuous increase of flow rate was observed. Because pressure decreased the exponential filter cake resistance whereas it caused increase in filter medium. In addition, pressure also increases viscosities of liquids due to reduction of number of holes according to "hole theory". **Figure 14** indicated that the effect of pressure on flow rate was dominant compared to its effects on medium resistance and viscosity of grape juice. The reason of the different effects of pressure on the filtration of grape juice in contrast to apple and sourcherry juices reported by Bayındırlı (1989) and Şahin (1992), respectively, was the compressible particles removed from grape juice during the clarification process.

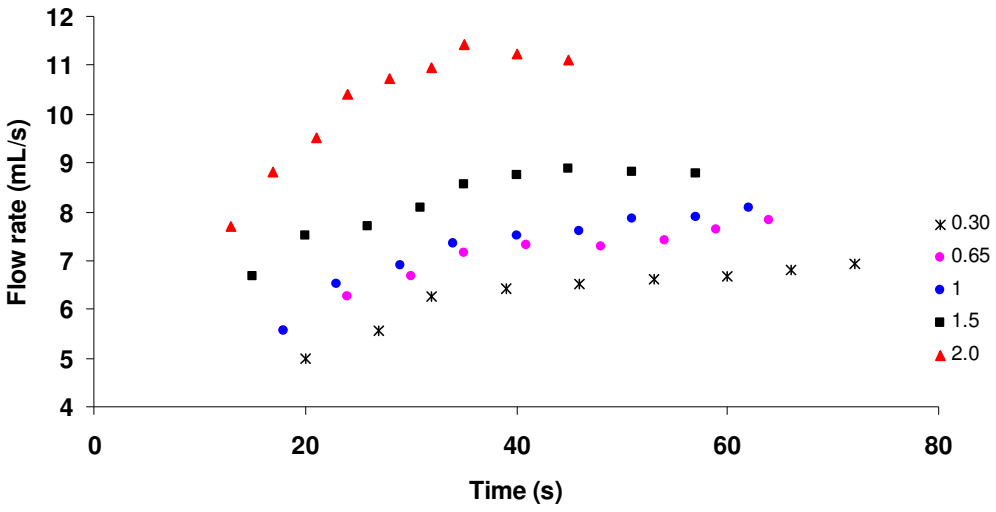

Figure 14 Effect of operating pressure on the filtration of grape juice

3.1.1.5 Effect of Soluble Solid Content

Figure 15 showed significant difference in the flow rates of filtration of grape juices adjusted to 16°Bx and 28°Bx, respectively. Soluble solid content directly affects density and viscosity of fruit juices. Increasing soluble solid content increases viscosity and density. Change of viscosity and density due to the change of soluble solid content of grape juice reflected to the flow rate of filtration process. Increasing soluble solid content caused decrease in flow rate (**Figure 15**).

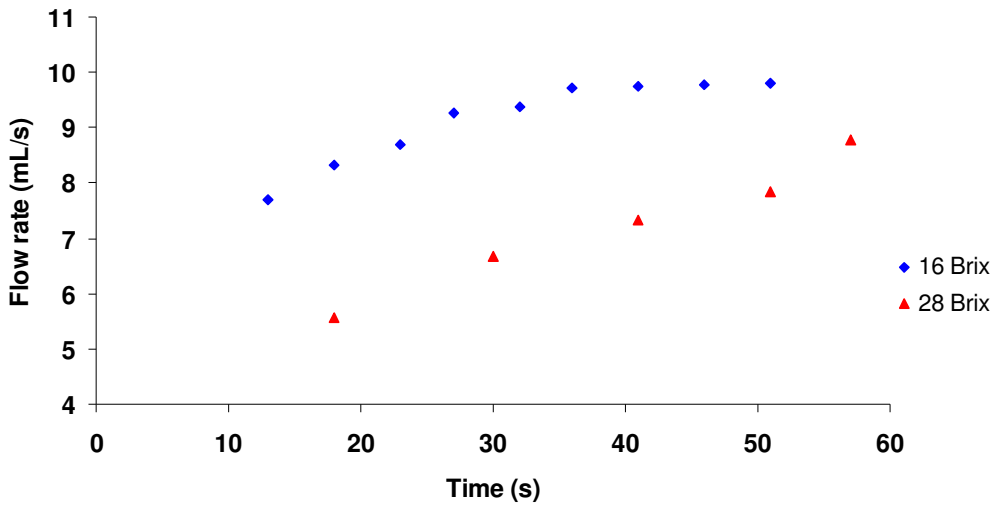

Figure 15 Effect of soluble solid content of grape juice on filtration process

3.1.2 Thermophysical Properties

The results demonstrates that the temperature and soluble solid content of fruit juices are significant characteristics to estimate their physical properties; density, viscosity and heat capacity.

3.1.2.1 Effect of Soluble Solid Content and Temperature on Density of Clarified Fruit Juices

Densities of clarified fruit juices presented very strong dependencies of temperature and soluble solid content ($p \leq 0.0001$). As can be seen from **Figure 16-Figure 21**, the effect of increasing soluble solid content of studied fruit juices caused increase in density values, whereas increasing temperature resulted in decrease in density.

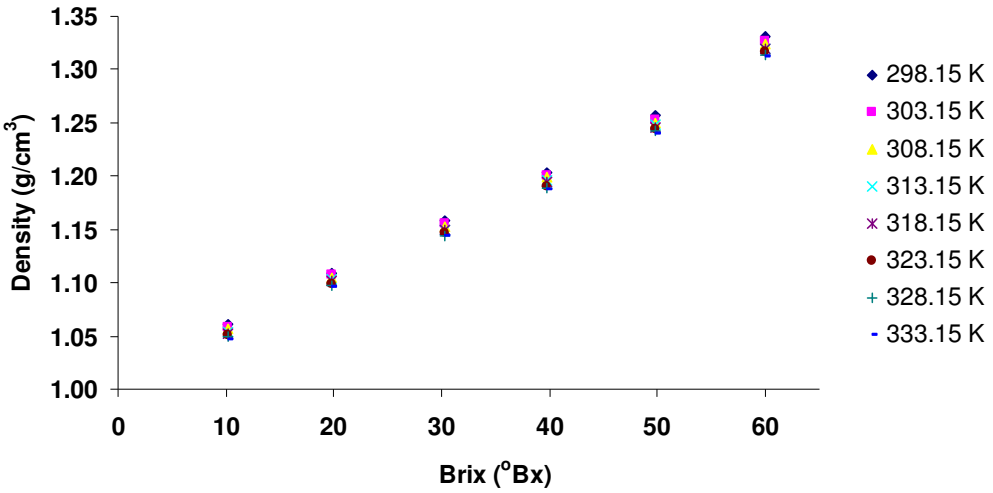

Figure 16 Density change of apple juice w.r.t. sugar concentration (Brix) at constant temperature (K)

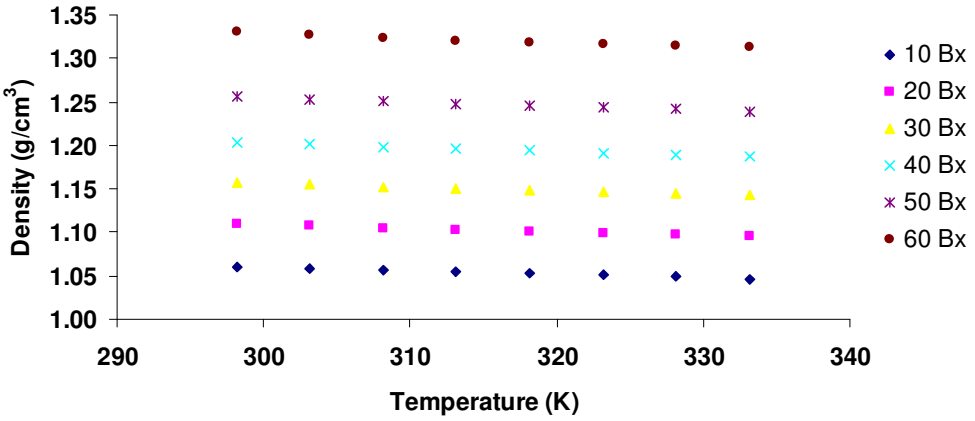

Figure 17 Density change of apple juice w.r.t. temperature (K) at constant sugar concentration (Brix)

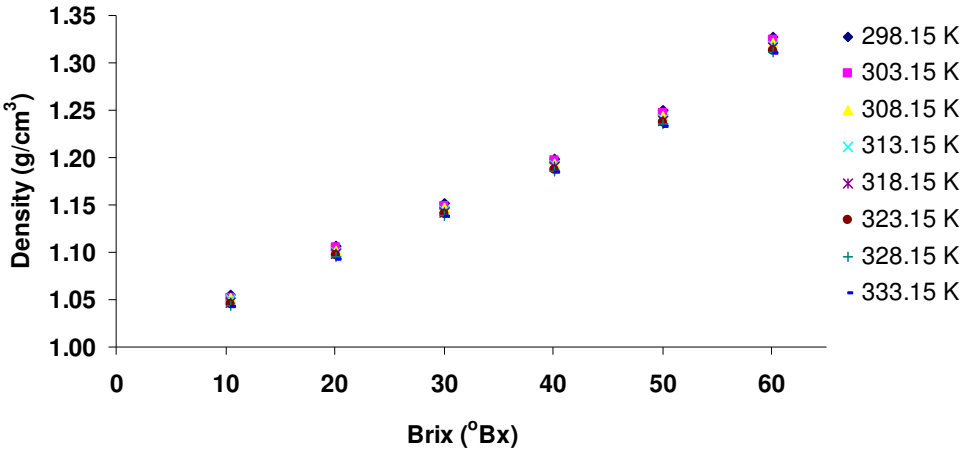

Figure 18 Density change of grape juice w.r.t. sugar concentration (Brix) at constant temperature (K)

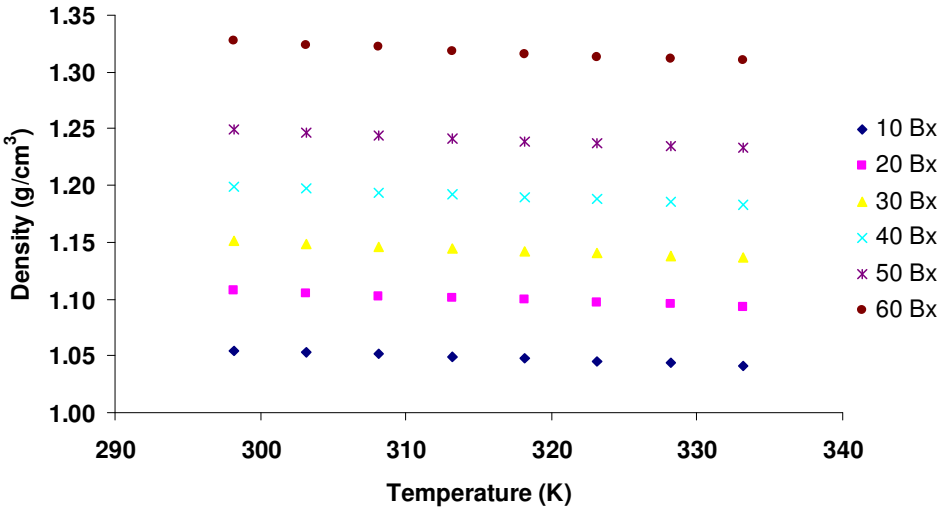

Figure 19 Density change of grape juice w.r.t. temperature (K) at constant sugar concentration (Brix)

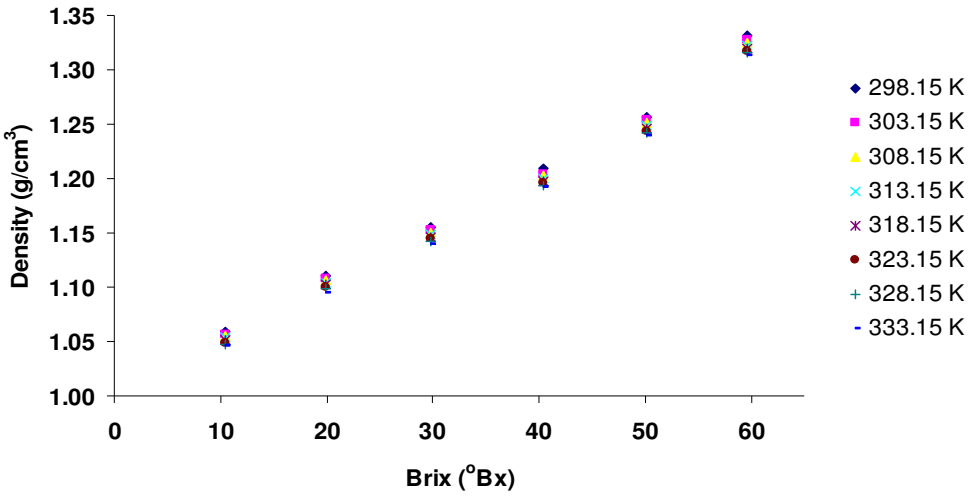

Figure 20 Density change of sour cherry juice w.r.t. sugar concentration (Brix) at constant temperature (K)

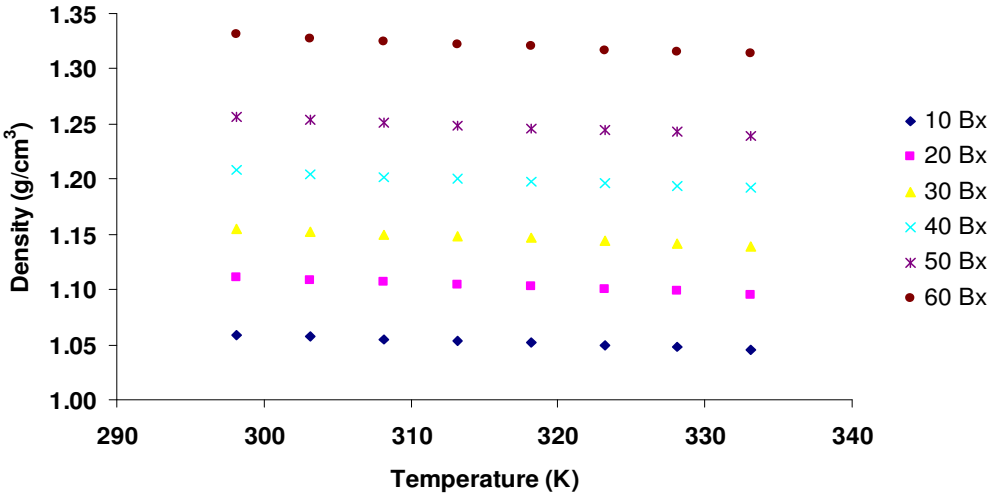

Figure 21 Density change of sour cherry juice w.r.t. temperature (K) at constant sugar concentration (Brix)

The similar trends have been reported in the previous studies (Alvarado and Romero, 1989, Constenla et al., 1989, Ramos and Ibarz, 1998, Telis-Romero et al., 1998,

Cepeda and Villarán, 1999, Zainal, Abdul Rahman, Ariff, Saari, and Asbi, 2000, Gratão, Júnior, Polizelli, and Telis-Romero, 2005, and Shamsudin et al., 2005). Three models that fitted best with the experimental values for three clear fruit juices were obtained by multiple regression analysis of the experimental results individually Eqs. (26), (27), and (28). These models represent the density as functions of temperature and sugar concentration;

For apple juice,

$$\rho = 1.15834 + 0.0035118 \cdot B + 0.00002419 \cdot B^2 - 0.00044298 \cdot T \quad (\boldsymbol{R^2 = 0.998}) \quad \textbf{(26)}$$

For grape juice,

$$\rho = 1.15150 + 0.0033929 \cdot B + 0.00002580 \cdot B^2 - 0.00043320 \cdot T \quad (\boldsymbol{R^2 = 0.996}) \quad \textbf{(27)}$$

For sour cherry juice,

$$\rho = 1.15808 + 0.0034995 \cdot B + 0.00002522 \cdot B^2 - 0.00044492 \cdot T \quad (\boldsymbol{R^2 = 0.996}) \quad \textbf{(28)}$$

The following equation (Eq. 29) as the best fit for the experimental values was a polynomial type with a regression coefficient of *0.996* and a *MSE* of $3*10^{-5}$.

$$\rho = 1.1560 + 3.4687 \times 10^{-3} \cdot B + 2.503 \times 10^{-5} \cdot B^2 - 4.4037 \times 10^{-4} \cdot T \quad (\boldsymbol{R^2 = 0.996}) \quad \textbf{(29)}$$

, where ρ is a density of studied clarified fruit juices (g/cm^3) and temperature (T) and soluble solid content (B) units of sample are K and ^{o}Brix, respectively.

The data predicted using the equations reported by Ramos and Ibarz (1998) and Gratao et al (2005) are nearly coincident with the experimental results of clarified fruit juices, whereas the calculated data from the equation of Constenla et al (1989) are different (**Figure 22**). Constenla et al (1989) have reported the effect of sugar

content and type on the thermophysical properties of clarified apple juice, accounting for more than 90% of soluble solids. Sugar concentrations of studied fruit juices are similar and correspond the higher than 90% of their soluble solid contents (Muramatsu, Tagawa, and Kasai 2005). Thus, the fitted model can be used to predict the densities of clarified apple, grape and sour cherry juices in the studied temperature and soluble solid content varying from 25 ^{o}C to 60 ^{o}C and from 10 ^{o}Brix to 60 ^{o}Brix, respectively.

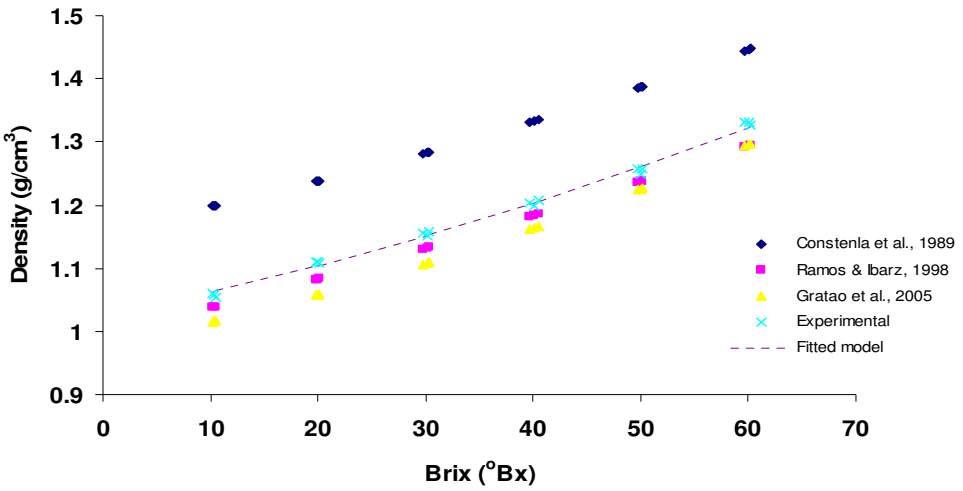

Figure 22 Comparison of fitted model and literature data of density with change of soluble solid content

3.1.2.2 Effect of Temperature and Soluble Solid Content on Viscosity of Clarified Fruit Juices

Temperature and soluble solid content of fruit juices were found to be highly significant factors ($p \leq 0.001$). Their effects on fruit juices are shown in **Figure 23-Figure 28** for apple juice, grape juice and sour cherry juice respectively.

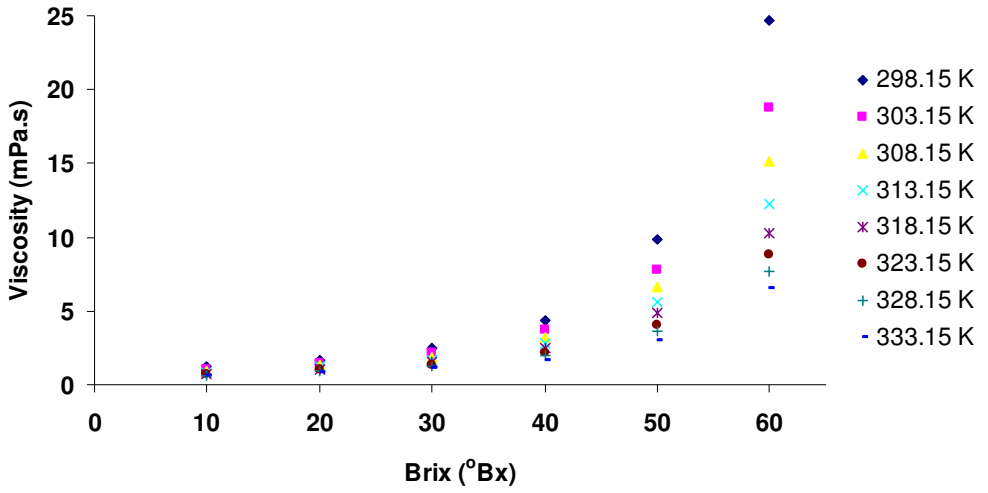

Figure 23 Viscosity change of apple juice w.r.t. sugar concentration (Brix) at constant temperature (K)

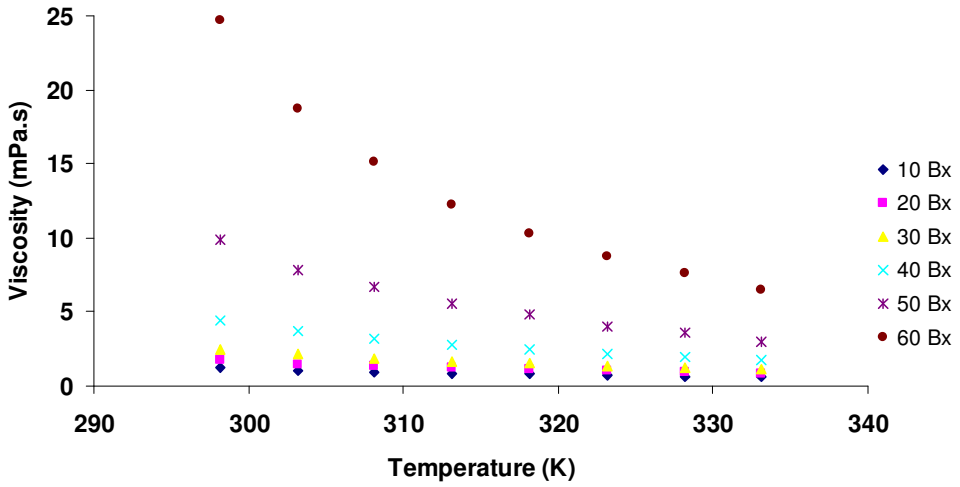

Figure 24 Viscosity change of apple juice w.r.t. temperature (K) at constant sugar concentration (Brix)

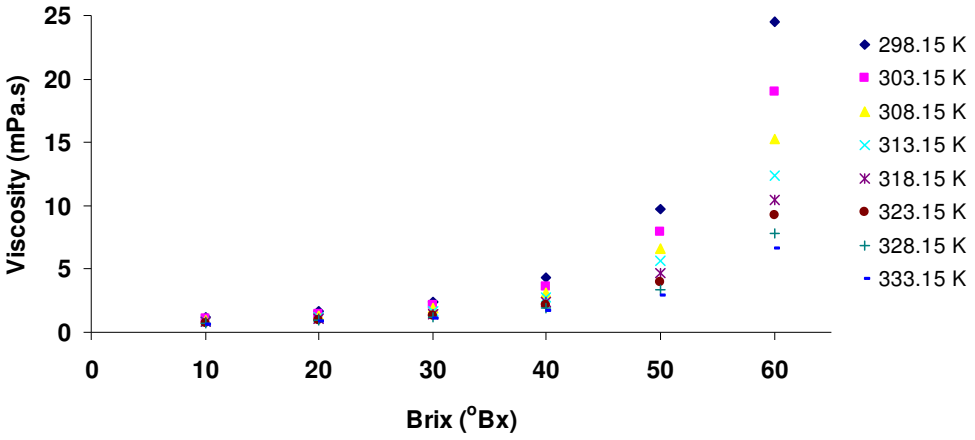

Figure 25 Viscosity change of grape juice w.r.t. sugar concentration (Brix) at constant temperature (K)

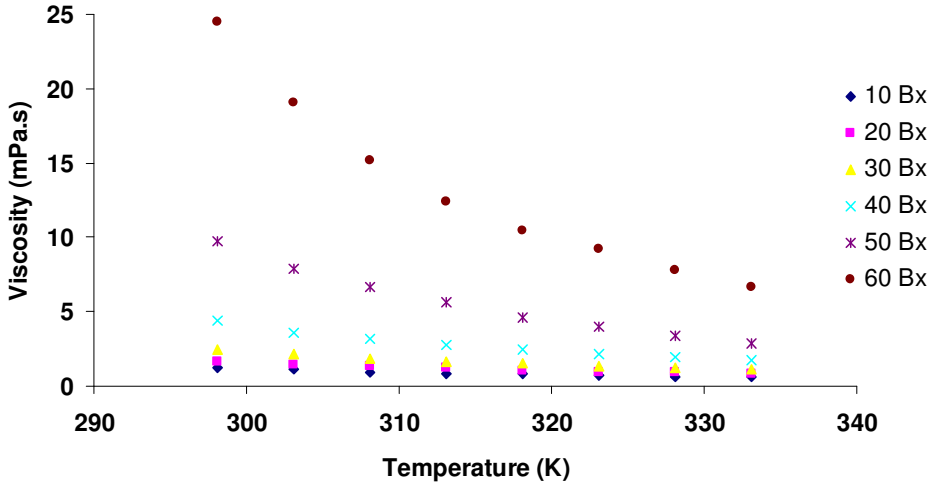

Figure 26 Viscosity change of grape juice w.r.t. temperature (K) at constant sugar concentration (Brix)

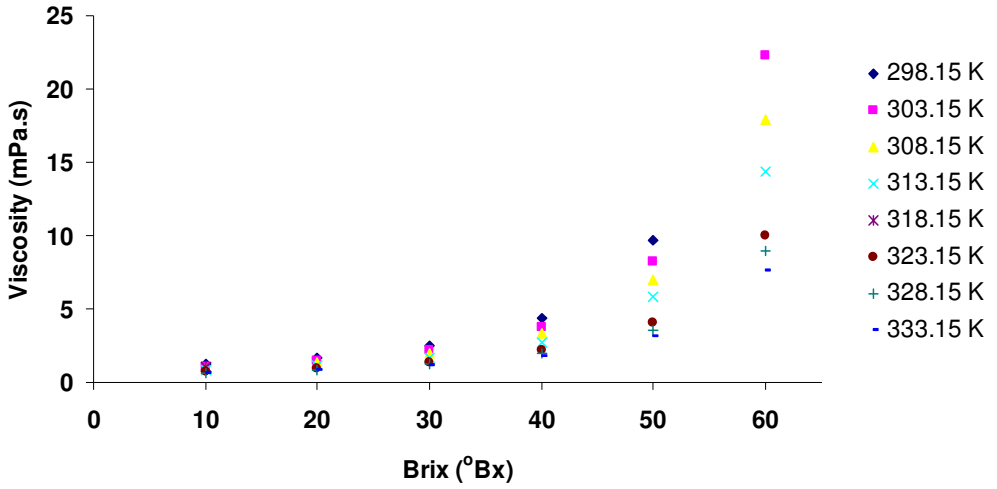

Figure 27 Viscosity change of sour cherry juice w.r.t. sugar concentration (Brix) at constant temperature (K)

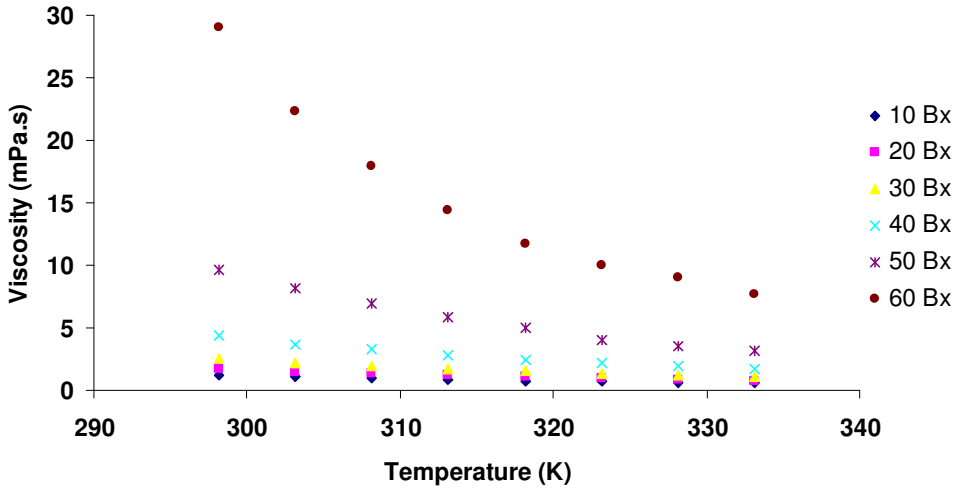

Figure 28 Viscosity change of sour cherry juice w.r.t. temperature (K) at constant sugar concentration (Brix)

The viscosities of clarified fruit juices were considerably reduced by increased temperature. As can be seen from **Figure 29**, viscosity change with temperature was relatively high at higher soluble solid contents (above 30°Bx), however, the weak temperature influence was observed at lower concentration (below 30°Bx). The changes of viscosities of clarified fruit juices at the soluble solid content range of 10° to 30°Bx followed parallel trends with increasing temperature, while at the higher levels of soluble solid contents; the measured viscosity values of sourcherry juice were higher than other two fruit juices at the lower temperature levels (**Figure 29**). Bayındırlı (1992 and 1993) has reported the effect of temperature on the viscosity due to intermolecular distances increasing as a result of thermal expansion. The positive influence of soluble solid concentration on the magnitude of the viscosity has reported by Rao (1999). The increasing soluble solid content enhanced the viscosity due to the increase in hydrogen bonding with hydroxyl groups and the distortion in the velocity pattern of the liquid by hydrated solute molecules (Constenla et al., 1989, and Azoubel et al., 2005).

Arrhenius type relation (10) can be used to describe the effects of temperature and soluble solid content on viscosity (Krokida et al., 2001, and Nindo et al., 2004). The activation energy term (Ea) and frequency factor term (μ_o) of Arrhenius Eq. (10) were explained as a function of soluble solid contents of clear fruit juices (Eqs. 30-35);

For apple juice,

$$Ea = 15364.3 + 4.3778 \cdot B^2 \qquad (\boldsymbol{R^2 = 0.982}) \qquad \textbf{(30)}$$

$$\mu_o = 2.03 \times 10^{-6} - 3 \times 10^{-8} \cdot B \qquad (\boldsymbol{R^2 = 0.954}) \qquad \textbf{(31)}$$

For grape juice,

$$Ea = 15274.0 + 4.3851 \cdot B^2 \qquad (\boldsymbol{R^2 = 0.961}) \qquad \textbf{(32)}$$

$$\mu_o = 2.07\times10^{-6} - 3\times10^{-8}\cdot B \qquad (\boldsymbol{R^2 = 0.909}) \qquad \textbf{(33)}$$

For sour cherry juice,

$$Ea = 15749.5 + 4.3374\cdot B^2 \qquad (\boldsymbol{R^2 = 0.988}) \qquad \textbf{(34)}$$

$$\mu_o = 1.93\times10^{-6} - 3\times10^{-8}\cdot B \qquad (\boldsymbol{R^2 = 0.971}) \qquad \textbf{(35)}$$

As a result the activation energy models and the frequency factor models of three different clear fruit juices were found to be so close to each other that the generalized models of these terms in Arrhenius Equation (10) representing all three clear fruit juice viscosities were obtained applying regression analysis of experimental data of fruit juices. The calculated activation energy (*Ea*) values of the clarified fruit juices were correlated with their soluble solid contents. The polynomial model (Eq.36) with regression coefficient of 0.982 presented the influence of soluble solid content. The natural logarithm of another calculated parameter (μ_o) of Arrhenius equation from experimental data was also correlated with soluble solid concentrations of fruit juices by least square method (Eq.37). As a result, the change of viscosity of the clarified fruit juices with respect to temperature and soluble solid content was described by Equation (38).

$$Ea = 15583.3225 + 4.3246\cdot B^2 \qquad (\boldsymbol{R^2 = 0.982}) \qquad \textbf{(36)}$$

$$\ln\mu_o = -13.0697 - 8.9\times10^{-4}\cdot B^2 \qquad (\boldsymbol{R^2 = 0.938}) \qquad \textbf{(37)}$$

$$\mu = 2.1082\times10^{-6}\exp\left(\left(\frac{15583.3225 + 4.3246\cdot B^2}{R\cdot T}\right) - 8.9\times10^{-4}\cdot B^2\right) \qquad \textbf{(38)}$$

, where μ was viscosity in the unit of Pa.s, *B* and *T* were soluble solid content and temperature in the units of ^{o}Bx and K, respectively.

Activation energy data calculated from experimental results of this study varied from 16.3 kj/mol upto 30.7 kj/mol. Krokida et al (2001) have reported that the activation energy (*Ea*) in Newtonian fluid foods increases from 14.4kJ/mol for water to more than 60kJ/mol for concentrated clarified juices and sugar solutions. The activation energy range in the present study was coincident with data reported by Krokida et al (2001). These equations would be suitable for predicting the rheological properties of studied clarified juices over a wider range of solids content.

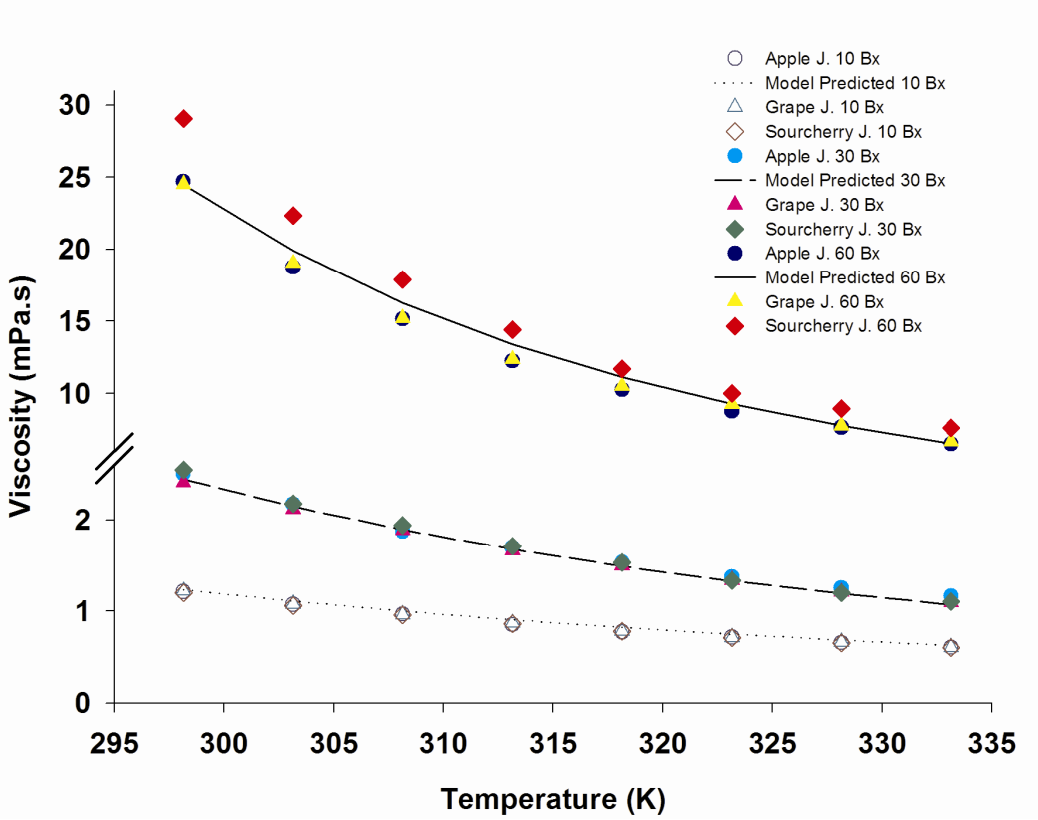

Figure 29 Comparison of viscosities predicted by model and experimental results

3.1.2.3 Effect of Temperature and Soluble Solid Content on Heat Capacities of Clarified Fruit Juices

Heat capacities of foodstuffs are related to their compositions (Sweat, 1995). Significant temperature and soluble solid content effects on heat capacities of clarified fruit juices were observed ($p \leq 0.001$). Soluble solid contents of clarified fruit juices adversely influenced the heat capacity, whereas increasing temperature enhanced the heat capacity of fruit juices (**Figure 30**-**Figure 32**).

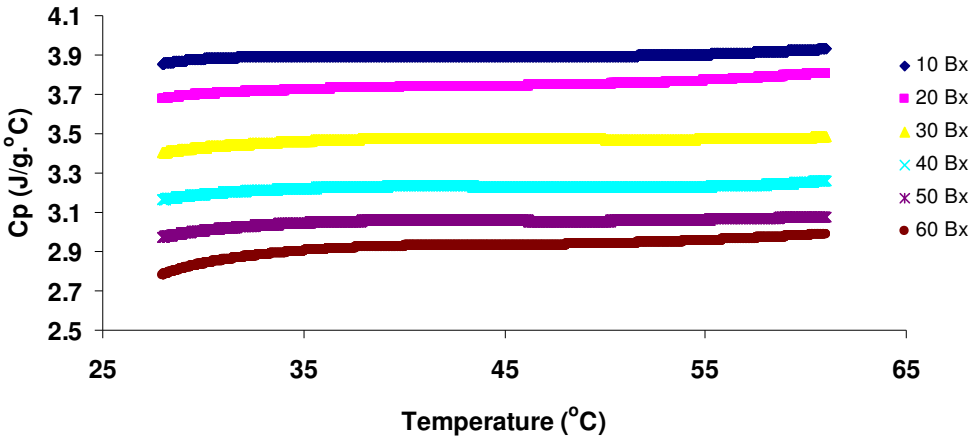

Figure 30 Heat Capacity change of apple juice w.r.t. sugar concentration (Brix) at constant temperature (^{o}C)

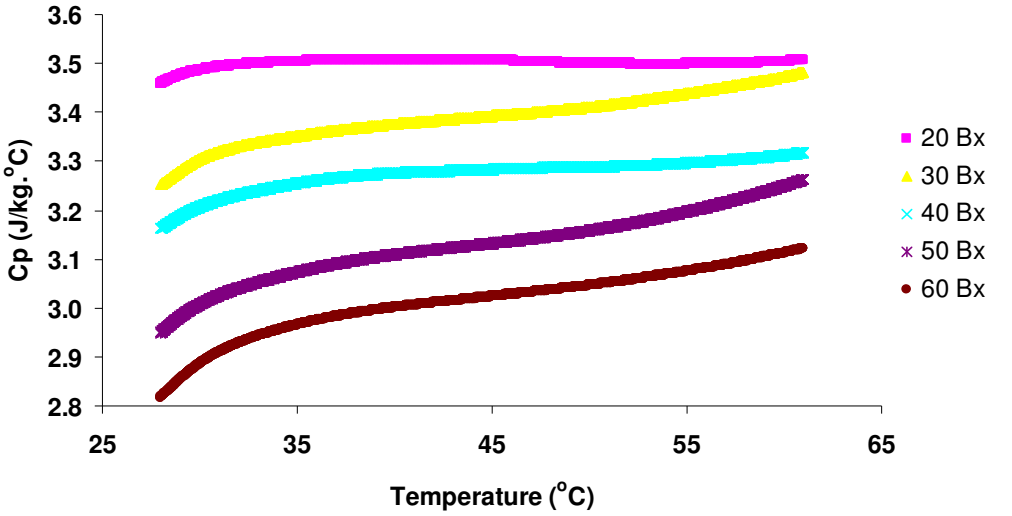

Figure 31 Heat Capacity change of grape juice w.r.t. sugar concentration (Brix) at constant temperature (°C)

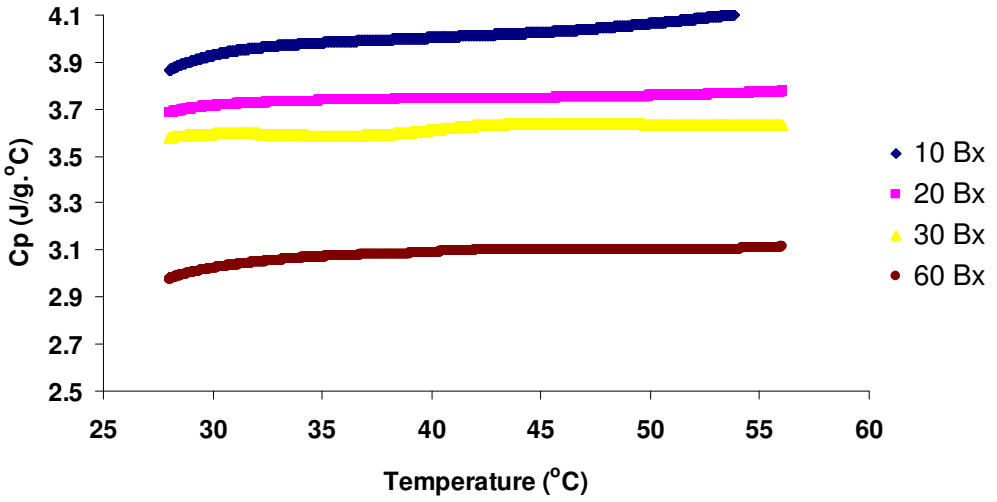

Figure 32 Heat Capacity change of sourcherry juice w.r.t. sugar concentration (Brix) at constant temperature (°C)

The influence of soluble solid content can be attributed to the decrease in water content of fruit juices. The heat capacity of water is the highest value in the group of food constituents. Similar dependences on both variables have been shown in previous studies (Constenla et al., 1989, Telis-Romero et al., 1998, Zainal et al., 2000, and Shamsudin et al., 2005). The results of the heat capacities of three different clear fruit juices indicate that they had a linear correlation with temperature and also their soluble solid concentrations. The multiple regressions were applied to these data to fit them with models. The models were given below. The linear models' parameters were temperature (T) and sugar concentrations (B). They showed high fitting to experimental results if their R^2 values were considered. The following models were for apple juice, grape juice and sourcherry juice respectively (Eqs.39-41).

For apple juice,

$$Cp = 4.01258 - 0.0204898 \cdot B + 0.00199994 \cdot T \qquad (\boldsymbol{R^2 = 0.985}) \qquad \textbf{(39)}$$

For grape juice,

$$Cp = 3.55664 - 0.0123801 \cdot B + 0.00447969 \cdot T \qquad (\boldsymbol{R^2 = 0.975}) \qquad \textbf{(40)}$$

For sourcherry juice,

$$Cp = 4.00607 - 0.0180636 \cdot B + 0.00356652 \cdot T \qquad (\boldsymbol{R^2 = 0.987}) \qquad \textbf{(41)}$$

The achieved model that fitted best the experimental heat capacity values for clarified fruit juices were far away from expectation of one model being valid for clarified fruit juices. Linear model (Eq.42) obtained by least square method has been proposed; however, the results were fitted to models with regression coefficient less than 0.90 which is not sufficient for representation. The model describing the relationship between heat capacities of the clarified fruit juices and both variables, temperature and soluble solid content can be given as:

$$Cp = 3.9193 - 1.7039\times10^{-2}\cdot B + 3.2437\times10^{-3}T \qquad (\mathbf{R^2 = 0.747}) \qquad \mathbf{(42)}$$

As can be seen from Equation (42) and **Figure 30-Figure 32,** the magnitude of the effect of soluble solid content was higher than that originated by temperature. The resulted model with relatively low level of regression coefficient is associated with the effects of compositional differences of the clarified fruit juices, despite those effects were neglected for other physical properties.

3.2 Optimization of Solid-Liquid Extraction of Resveratrol and Other Phenolic Compounds from Milled Grape Canes

Target phenolic compounds (*trans*-resveratrol, *trans*-ε-viniferin and ferulic acid) contributed more than 70% of total phenolic content of grape cane extracts. **Figure 33** shows the comparison of HPLC chromatograms (280 nm) of extracts obtained by a reference method (Perry, Greene, Maloney, 1997) and by the solid-liquid extraction method carried out using 58% ethanol/water (v/v) solution (70 mL/g) at 50°C. *trans*-Resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics of grape cane extracts produced by the solid-liquid method varied from 1.3 to 4.1 mg/g dw, from 0.8 to 2 mg/g dw, from 0.2 to 1 mg/g dw, and from 3.2 to 8.9 mg/g dw, respectively (**Table 3**).

trans-Resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics levels from the analysis of the extract obtained by the reference method were 3.85 ± 0.02 mg/g dw, 1.25 ± 0.03 mg/g dw, 0.16 ± 0.001 mg/g dw, and 6.54 ± 0.09 mg/g dw, respectively. The yield of stilbene compounds from grape cane achieved during optimization trials ranged from 34% to 106% for resveratrol, and from 64% to 160% for viniferin. Recently, Püssa, Floren, Kuldkepp, and Raal (2006) reported that stems of three frost-hardy grapevine varieties [Hasaine (Hasansky) sladki, Zilga, and Yubilei Novgoroda] from Estonia contain 1.1-3.2 mg/g dw of *trans*-resveratrol, and 0.3-1.7

mg/g dw of ε-viniferin. The *trans*-resveratrol and *trans*-ε-viniferin concentrations in the investigated grape cane samples compare favourably with this available dataset.

In red wines, the concentration of *trans*-resveratrol has been found to be in the range of 0.2-7.7 mg/L (Adrian, Jeandet, Breuil, Levite, Debord, Bessis, 2000, Pezet, Gindro, Viret, Spring, 2004, Abril, Negueruela, Pérez Juan Estopañán, 2005, Lamuela-Raventós, Waterhouse, 1993, Dong, 2003), and grape skins contain 0.01-0.2 mg/g resveratrol (Romero-Pérez, Lamuela-Raventós, Andrés-Lacueva, Carmen de la Torre-Boronat, 2001, Dong, 2003). Romero-Pérez, Lamuela-Raventós, Andrés-Lacueva, Carmen de la Torre-Boronat (2001) reported *trans*-resveratrol content of skins in the range of 11.0 to 47.6 μg/g dw for white grapes (Parellada, Macabeo, Chardonnay, and Xarelâlo) and 17.3 μg/g dw to 39.4 μg/g dw for red grapes (Carinñena, Cabernet Sauvignon, and Merlot). *trans*-Resveratrol yields determined in the solid-liquid extraction of grape cane samples are 10 to 400 times higher compared to published values for grape skins. Cultivar, climatic differences, and/or disease pressure are the effective factors on the synthesis of stilbenes. Resveratrol yields obtained from different parts of grape vines may be attributed to the sensitivity of the different parts of grape vine to these factors. Li, Wu, Wang, Li (2006) have reported the difference in the response of resveratrol synthesis in grape skin and grape seeds being associated with the variable sensitivity of different parts of grape vine to climate change.

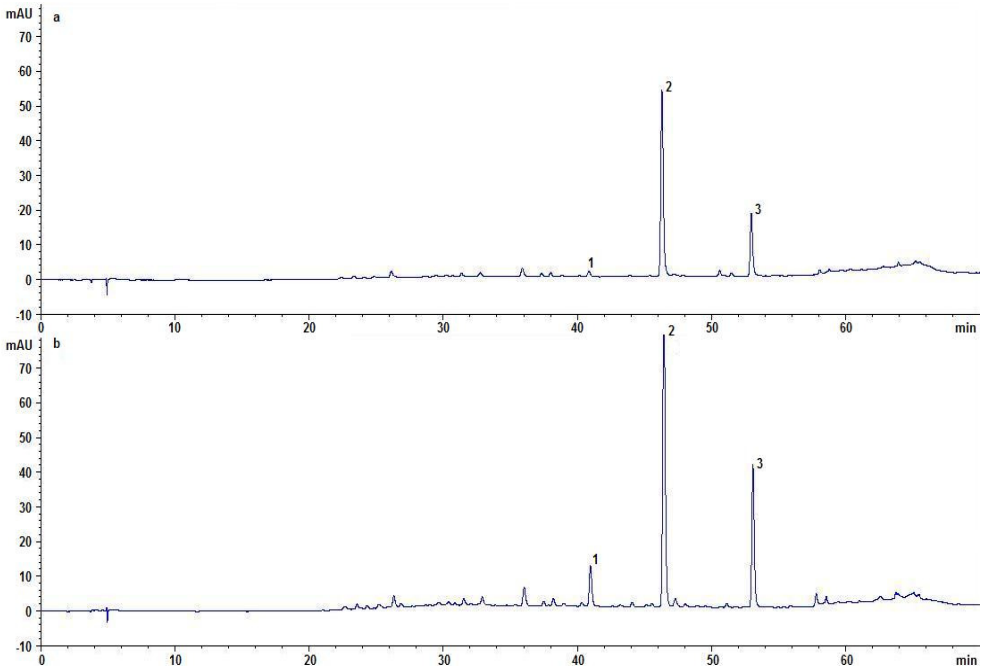

Figure 33 HPLC chromatograms (280 nm) of grape cane extracts

obtained by a reference method (Romero-Pérez et al., 2001) (a) and by the solid-liquid extraction method carried out using 58% ethanol/water (v/v) solution (70 mL/g) at 50°C (b).

Phenolic compounds;

1 ferulic acid

2 *trans*-resveratrol

3 *trans*-ε-viniferin

Table 3 Experimental data of the investigated responses of grape cane extracts under different extraction conditions shown in **Table 1** and independent effects of factors

standard order	*trans*-resveratrol yield[a]	*trans*-ε-viniferin yield[a]	ferulic acid yield[a]	total phenolics yield[b]	*trans*-resveratrol diffusivity[c], $D_{eff}*10^{13}$
1	1.59	0.88	0.95	3.80	6.33
2	3.66	1.73	0.19	7.57	15.79
3	1.71	0.93	0.95	3.98	5.24
4	3.78	1.76	0.21	7.97	16.22
5	3.19	1.15	0.56	6.35	5.79
6	3.93	2.00	0.20	8.02	17.97
7	3.22	1.18	0.43	6.16	6.23
8	3.91	1.78	0.16	7.46	17.66
9	2.41	1.02	0.90	5.12	3.12
10	4.06	1.87	0.17	8.91	26.61
11	3.14	1.90	0.73	6.93	15.52
12	3.02	1.68	0.82	7.16	13.97
13	1.29	0.83	0.64	3.19	13.76
14	3.74	1.64	0.15	6.78	5.77
15	3.17	1.70	0.73	7.22	14.67
16	3.12	1.72	0.77	7.24	13.98
17	3.22	1.67	0.70	7.29	13.53
18	3.08	1.66	0.87	7.06	13.53
main effects					
temperature	**	**	**	**	**
solvent to solid ratio	ns	ns	ns	ns	ns
ethanol concentration	**	*	**	**	ns

[a], In mg/g dw (dried weight). [b], Total phenolics yield in mg resveratrol equivalent/g dw of grape cane expressed as equivalent of resveratrol. [c], In m^2/s. [ns], Not significant; [*], significant at $p \leq 0.05$; [**], significant at $p \leq 0.01$.

The effective diffusivity of resveratrol in the solid phase, D_{eff} was also calculated by fitting the equilibrium concentrations of resveratrol in to the governing equation derived from Ficks' second law for diffusion. Results ranged from 3.1×10^{-13} to 26.6×10^{-13} m^2/s, and are represented in **Table 3**.

The surface response analysis for *trans*-resveratrol, *trans*-ε-viniferin, ferulic acid, and total phenolics indicated that temperature was the most effective independent variable ($p \leq 0.01$) (**Table 3**). Ethanol concentration was also a significant factor for yield of all phenolic compounds assayed and for total phenolics ($p \leq 0.05$, or $p \leq 0.01$). However, for the effective diffusivity of resveratrol, surface response analysis showed that ethanol concentration was not statistically significant (**Table 3**). Solvent to solid ratio was found not to be a significant factor for all responses ($p > 0.05$) (**Table 3**).

Models developed by surface response analysis for *trans*-resveratrol, *trans*-ε-viniferin, ferulic acid, total phenolics yields, and effective diffusivity of resveratrol were significant at the level of 0.001% of probabilities, and variability could be explained by the models (**Table 4**). Regression coefficient and analysis of variance of the adjusted polynomial second-order models for resveratrol, viniferin, ferulic acid, total phenolics, and effective diffusivity of resveratrol are summarized in **Table 4**. An ANOVA of the regression parameters of the surface response analysis of models for resveratrol, viniferin, ferulic acid, total phenolics yields, and effective diffusivity of resveratrol in the solid phase indicated the existence of the significant effects of linear and quadratic terms ($p \leq 0.001$ or $p \leq 0.01$) except linear terms of the yield of ferulic acid ($p > 0.05$). Interaction terms were only significant in the models for resveratrol, ferulic acid, and total phenolics yields ($p \leq 0.001$, and $p \leq 0.01$) (**Table 4**). Control of other model parameters, R^2 and R^2_{Adj}, indicated that model adequacies were good (**Table 4**).

Table 4 Regression coefficients of predicted models for the investigated responses of grape cane extracts

coefficient[a]	*trans*-resveratrol yield	*trans*-ε-viniferin yield	ferulic acid yield	total phenolics yield	effective diffusivity of *trans*-resveratrol ($*10^{13}$)
	calculated values of coefficients of fitted models for each responses				
β_0	-3.3968*** [c]	-1.4052***	0.7664*	-7.2617***	-11.4029*
β_1	0.0753***	0.0422***	-0.0015^{ns}	0.1416***	0.3060***
β_2	0.0001^{ns} [b]		-0.00002^{ns}	0.0008^{ns}	-0.0114^{ns}
β_3	0.1113***	0.0496***	0.0173*	0.2826***	0.4042**
β_{11}		-0.0003*	-0.0003***		
β_{22}					
β_{33}	-0.0004**	-0.0004***	-0.0003***	-0.0015***	-0.0038**
β_{12}					
β_{13}	-0.0008***		0.0002**	-0.0014***	
β_{23}					
model	***	***	***	***	***
linear	***	***	ns	***	***
quadratic	**	***	***	***	**
cross-product	***		**	***	
R^2	0.94	0.92	0.95	0.97	0.92
R^2_{Adj}	0.92	0.90	0.93	0.95	0.90

[a] Polynomial model $Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$ adjusted by backward elimination at the level of 0.1% with the lack-of-fit test, where β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient. Subscript 1, 2 and 3 located in the model coefficients indicates temperature, solvent to solid ratio, and ethanol concentration, respectively. [b] ns, not significant ($p > 0.05$); [c] *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$.

Solvent to solid ratio in the studied range of 36.8 mL/g to 103.6 mL/g was not significant for resveratrol, viniferin, ferulic acid, and total phenolics yields. Cacace and Mazza (2003a) reported that the solubility and equilibrium constant are modified by the main effect of solvent to solid ratio varying from 6 mL/g to 74 mL/g and extraction results in a higher yield being maximum at the highest solvent to solid ratio. However, no effect of solvent to solid ratio was observed. This may be attributed to the relatively high value of the lowest solvent to solid ratio selected, and/or the significant difference in the physico-chemical structure of the used milled grape cane versus berries.

3.2.1 Effect of Temperature

Total phenolics yield increased with increasing temperature (**Figure 34** and **Figure 35**). The increase in yield of total phenolics with temperature was practically linear at the lower and moderate ethanol concentrations, but the temperature effect almost disappeared with further increase in ethanol concentration (**Figure 35**). **Figure 35** also shows an interaction of temperature and ethanol concentration, particularly at lower temperatures and dilute ethanol solutions.

The combined effect of temperature and ethanol concentration on the yield of *trans*-resveratrol and *trans*-ε-viniferin is clearly shown in **Figure 36A** and **Figure 36B**. At lower ethanol concentration, a linear temperature effect on resveratrol yield was observed, however, when approaching the higher concentration level, change due to temperature nearly disappeared (**Figure 36A**). The yield of viniferin was mainly influenced by temperature. Curve shape temperature effect was observed in the whole range of ethanol concentration but it was more apparent at lower concentration levels (**Figure 36B** and **Table 3**).

Unlike resveratrol and viniferin, ferulic acid yield increased with the decrease of temperature (**Table 3**). Change due to temperature was strong and almost linear at

lower ethanol concentration levels, whereas a weak curve effect was seen when approaching higher ethanol concentrations (**Figure 36C**).

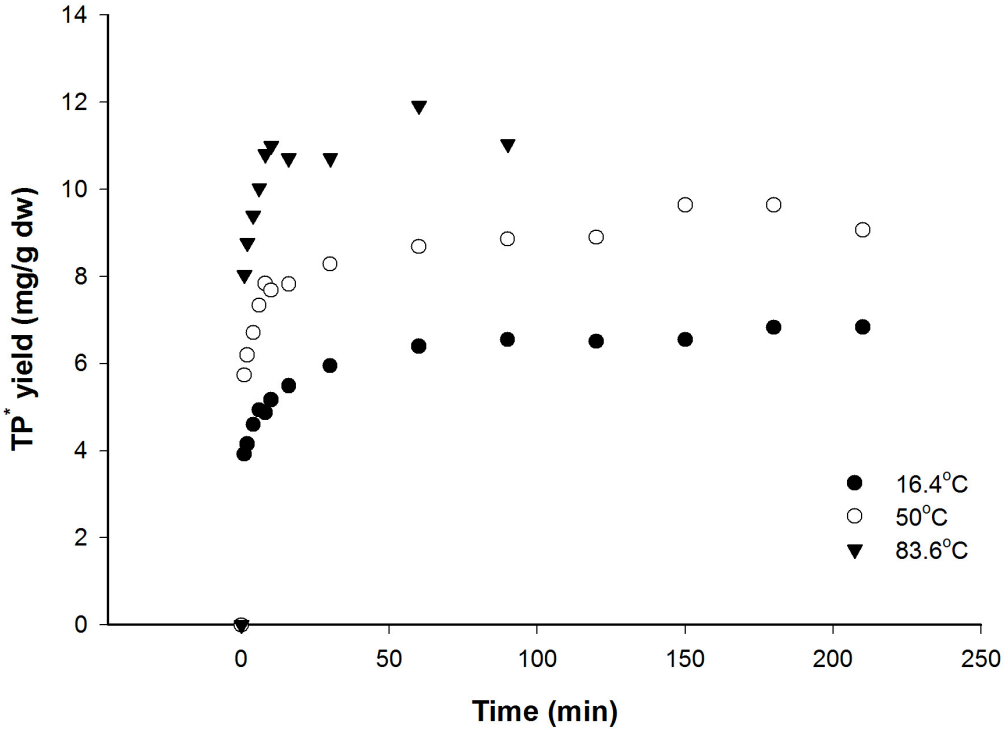

Figure 34 Effect of temperature on the extraction of total phenolics from milled grape canes

TP*, Total phenolics as equivalent of resveratrol

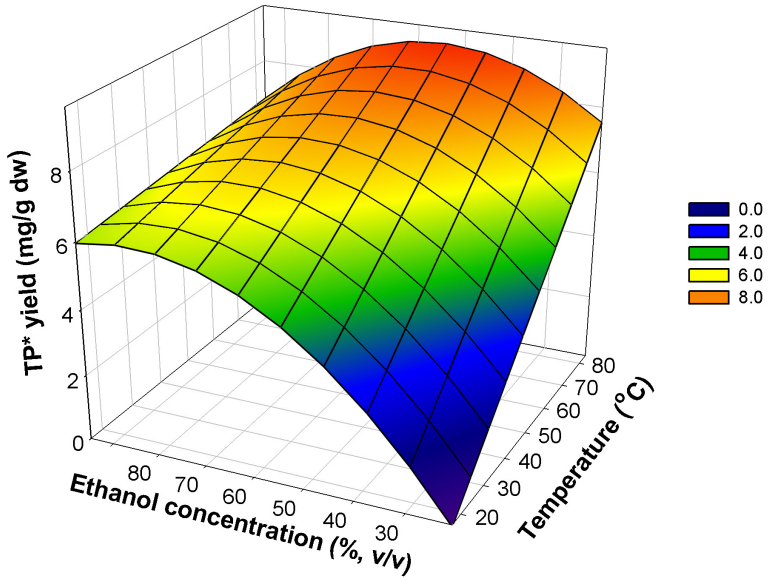

Figure 35 Response surfaces for the effects of temperature and ethanol concentration on the yield of total phenolics of grape cane extract at a constant solvent to solid ratio of 103.6 mL/g.
TP*, Total phenolics as equivalent of resveratrol

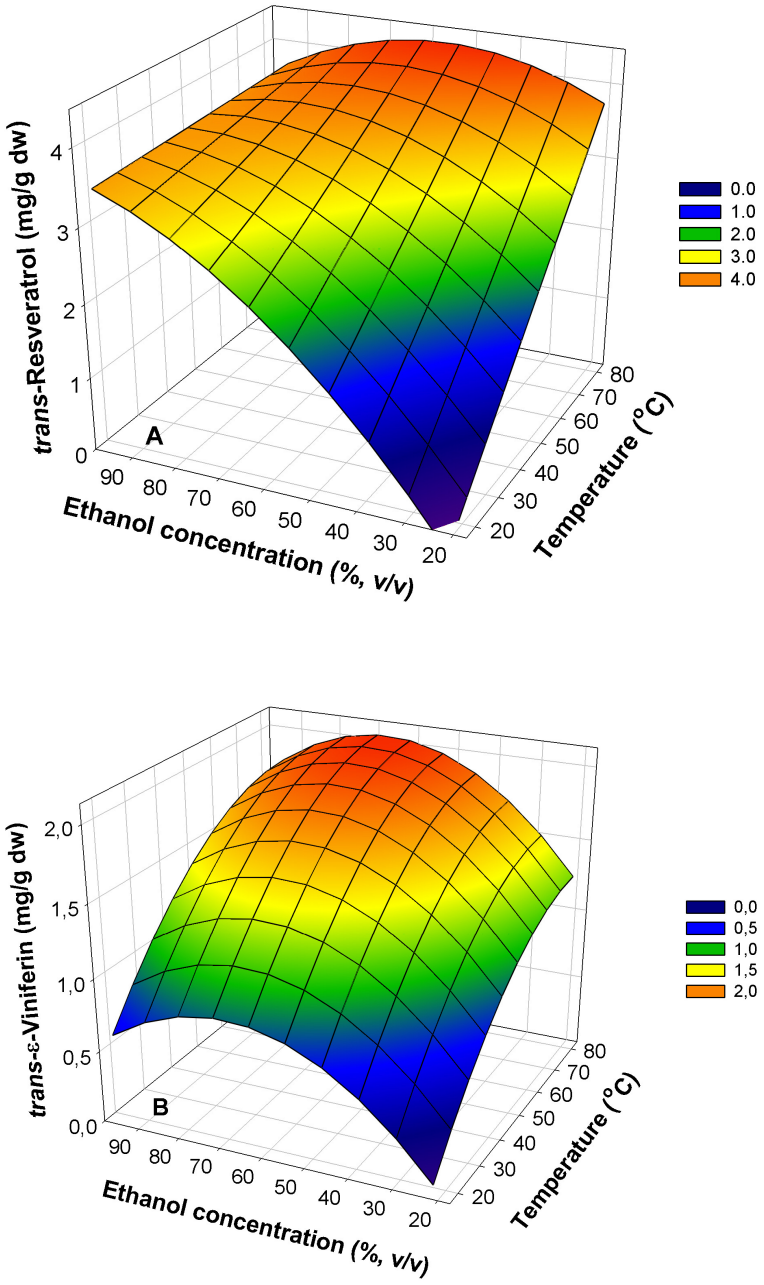

Figure 36 continued

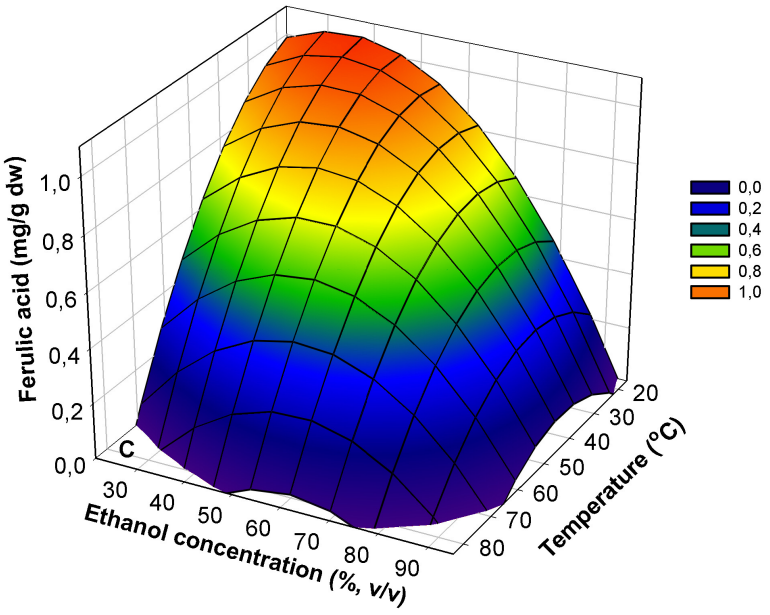

Figure 36 Response surfaces for the effects of temperature and ethanol concentration on phenolic compounds

A. *trans*-resveratrol yield with solvent to solid ratio levels of 103.6 mL/g,
B. *trans*-ε-viniferin yield with solvent to solid ratio levels of 70 mL/g,
C. ferulic acid at constant critical values of solvent to solid ratio levels of 36.8 mL/g from grape cane.

Increase of temperature favors extraction by enhancing solubility of compounds extracted. The solubility is associated with the property of the mixture (the activity coefficient) and the properties of the solute (entropy of fusion and melting point). Solubility increases with high temperature and low melting point. The solubility is called ideal, when the activity coefficient is equal to 1. The temperature dependence of solid solubility is determined not only by the ideal solubility, but also by changes in the activity coefficient with temperature (Frank, Downey, Gupta, 1999). By contrast, the decrease in the yield of ferulic acid with increasing temperature was observed during the solid-liquid extraction of bioactive compounds from grape cane samples. A high temperature may also degrade temperature-sensitive molecules and

accelerate oxidation. Thus, the lower yields for ferulic acid may be due to oxidation and degradation reactions favored by the higher extraction temperature (Mazzoleni, Caldentey, Silva, 1998, Maga, 1987).

3.2.2 Effect of Ethanol Concentration

There was almost a linear increase in yield of resveratrol with increasing ethanol concentration at low temperature levels, whereas the response surface for yield of resveratrol displayed the insignificant curve effect of ethanol concentration at higher temperature levels (**Figure 36A**). The highest yield of *trans*-resveratrol of 4.25 mg/g dw was predicted using the ethanol solution of 58% at the highest temperature of 83.6^{o}C being independent of solvent to solid ratio. Noticeable curve effect of ethanol concentration, being optimum in the range of 65% - 70%, on yield of viniferin was observed in the whole range of temperature (**Figure 36B**). The highest yield was achieved at the highest temperature. An ethanol solution of 68% was found to produce the maximum predicted yield of *trans*-ε-viniferin of 2.03 mg/g dw irrespective of solvent to solid ratio. Ethanol concentration displayed curve influence on ferulic acid yield in the range of temperature studied (**Figure 36C**). Using aqueous ethanol (35%), a maximum yield of 1.05 mg/g dw was calculated at 16.4^{o}C using fitted model for ferulic acid (**Table 4**).

Modification in extraction solvent by mixing different solvents over a limited compositional range can enhance the solubility of solute. The change in the physical properties of the solvents such as density, dynamic viscosity, and dielectric constant is attributed to ethanol concentration. Solubility of compounds, thus, can be modified by change in ethanol concentration, and this can affect the extractions of given compounds.

Lower solubility of compound in water is associated with the energy required to overcome the attraction between the water molecules. This energy due to the attraction between the partial charges of the water dipoles is important when a much

weaker interaction with covalent molecules is considered. The energy required to break the configuration of water molecules becomes dominant for non polar covalent molecules, and this could affect the extraction of given compound having low solubility in water (Cacace, Mazza, 2002, and 2003b, Frank, Downey, Gupta, 1999, Mackay, Mackay, 1981).

The yields of resveratrol and viniferin, having lower polarity are compatible with the above mentioned phenomenon by showing higher solubility in less polar solvents (moderate and concentrated aqueous ethanol solution) compared to dilute ethanol solutions. Ferulic acid, however, exhibited higher solubility in slightly moderate ethanol concentration (30% to 40%) which can be attributed to being a more polar phenolic compound than the stilbene compounds. Because phenolic acids with a carboxylic group and a hydrophobic glycosilated benzene ring may be considered as covalent polar molecules (Frank, Downey, Gupta, 1999).

The effect of ethanol concentration on the yield of total phenolics (**Figure 35**) was very similar to that for resveratrol (**Figure 36A**). This similarity can be attributed to resveratrol, having the highest contribution to the total phenolics yield. The yield of total phenolics increased with increase in ethanol content up to moderate concentration levels (50% - 60%) and decreased with further increase irrespective of the solvent to solid ratio and temperature studied (**Figure 35**). This trend is clear at higher temperature levels; however, the change in yield with ethanol concentration from dilute to moderate levels is stronger than the change observed between moderate and higher concentrations at lower temperature (**Figure 35**).

The maximum yield was predicted from combination of an ethanol concentration of 55%, solvent to solid ratio of 103.6 mL/g, and the highest temperature of 83.6^{o}C. The reason for the optimum ethanol concentration in the range of 50% to 70% for the extraction of both stilbene compounds, resveratrol and viniferin, can be attributed to the fact that these compounds have similar polarities, and thus show closer solubility in the same ethanol concentration. Total phenolics yield results indicated optimum ethanol concentration nearly in the same range as resveratrol and viniferin, and this

indicates that the stilbene compounds are the major phenolic group contributing to the total phenolics content of grape cane (**Table 3**).

3.2.3 Effective Diffusivity

Effective diffusivity of *trans*-resveratrol in the solid phase of grape cane extraction varied from 3.1×10^{-13} to 26.6×10^{-13} m^2/s. Surface response of effective diffusivity of resveratrol showed a saddle shape (**Figure 37**).

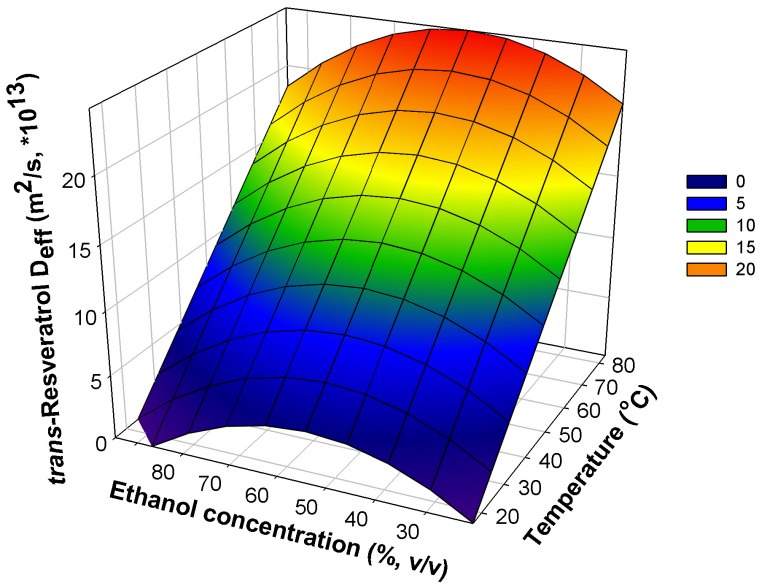

Figure 37 Response surfaces for the effects of temperature and ethanol concentration on the effective diffusivity of *trans*-resveratrol at a constant solvent to solid ratio of 36.8 mL/g.

Increase in the effective diffusivity was observed from 21% ethanol water mixture up to the range of 55% - 60%, thereafter diffusivity of *trans*-resveratrol decreased with

further increase in ethanol concentration as can be seen from **Figure 37** and from the slope of the models predicted by linear regression of τ (Dimensionless time value) versus time in **Figure 38**.

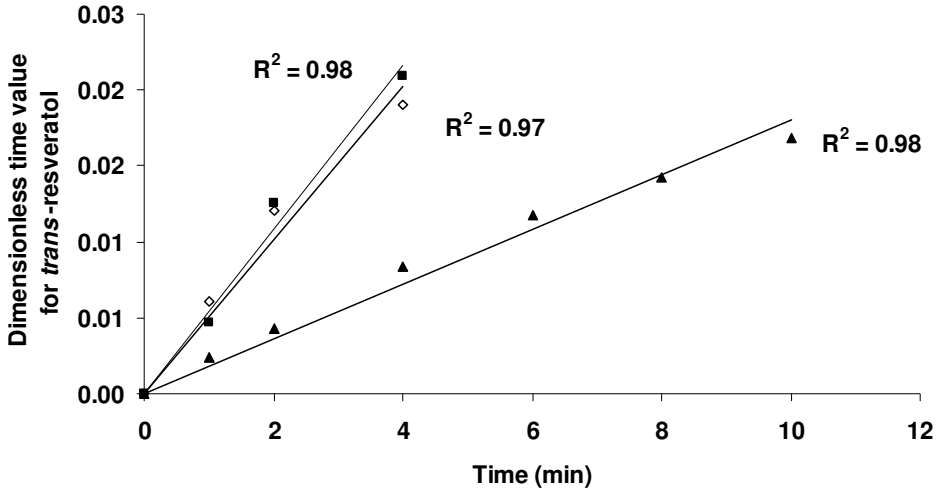

Figure 38 The change of dimensionless time values (τ) for *trans*-resveratrol at different ethanol concentrations
21% (◊), 58% (■), and 95% (▲) at 50°C and at the solvent solid ratio of 70 mL/g.

Predicted maximum effective diffusivity of resveratrol in solid phase (24.6×10^{-13} m^2/s) was achieved using ethanol concentration of 54% at the highest temperature (83.6°C) and the lowest solvent to solid ratio (36.8 mL/g) studied. Change in ethanol concentration enhances the diffusion of given compounds in the solid phase by altering the solvent properties such as density, dynamic viscosity, surface tension, and dielectric constant (Gertenbach, 2002, Frank, Downey, Gupta, 1999). **Figure 38** also shows that the change in effective diffusivity achieved with varying ethanol concentration from 58% to 95% was higher compared to the change achieved when using ethanol concentration in the range between 21% and 58%. This difference may

be associated with the alteration in the solid matrix. Solvent polarity increases with water content of ethanol solution. Extraction with dilute ethanol solution may cause the dissolution of other compound such as structural polysaccharides resulting in pore size enlargement. These changes in the solid matrix may have enhanced the penetration of solvent into the solid matrix and resulted in higher extraction rate. Increase of temperature resulted in increase in effective diffusivity (**Figure 37** and **Figure 39**).

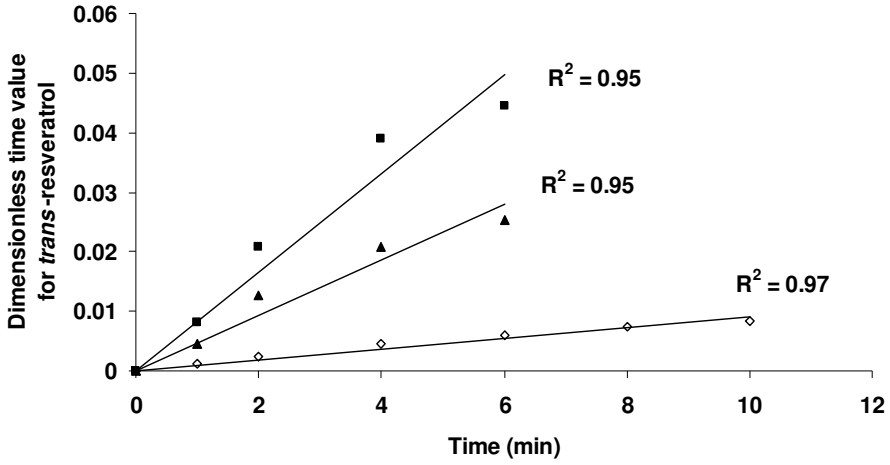

Figure 39 The change of dimensionless time values (τ) for *trans*-resveratrol at different temperatures
16.4°C (◊), 50°C (▲), and 83.6°C (■) with 58% ethanol solution at the solvent solid ratio of 70 mL/g.

The linear temperature effect on effective diffusivity was seen in the whole range of ethanol concentration and reflects the effect of temperature on resveratrol yield (**Figure 36A**). Temperature influence can be attributed to improvement of mass transfer, and solute-solid interactions. Increase in temperature produces efficient penetration of solvent in the solid phase reducing viscosities of solvents and enhances the diffusivity of solute in the solid and the liquid phases (Gertenbach,

2002). From another point of view, temperature effect on diffusivity can be associated with an increase in the internal energy of the molecules and thus their mobility, and a reduction of the dynamic viscosity coefficient. Thus, higher extraction rate and hence shorter time is achieved (Cacace, Mazza, 2003a).

Sineiro, Dominguez, Nuñez, Lema (1996) have reported the effective diffusivity values for polyphenols on sunflower press cake extraction varying from 2.2×10^{-13} to 6.1×10^{-13} m^2/s. These results were comparable to those obtained in the solid-liquid extraction of bioactive compounds of grape cane samples. Antioxidants extraction from grape byproducts (skins, seeds, and stems) using ethanol as a solvent also displayed similar effective diffusivity values ranged from 1.3×10^{-13} to 10.6×10^{-13} m^2/s (Pinelo, Sineiro, Nuñez, 2006). The effective diffusivity values for anthocynanins and total phenolics from milled berries being between 1.2×10^{-11} m^2/s and 25×10^{-11} m^2/s for aqueous ethanol extraction (Cacace, Mazza, 2003a) were higher than the calculated effective diffusivity results of the extractions of resveratrol and viniferin by PLPW. This difference may be attributed to the differences in the structure of samples studied. The structure of grape canes is obviously much more rigid than that of berries.

As a conclusion, the investigation and optimization of the effects of temperature, solvent to solid ratio, and ethanol concentration on the extraction of resveratrol, viniferin, ferulic acid, and total phenolics from milled grape canes indicated that the combination of a moderate ethanol concentration (50% - 70% ethanol/water mixture) and the highest temperature (83.6^oC) was the optimum for the extraction of phenolic compounds *trans*-resveratrol, *trans*-ε-viniferin, and total phenolics, except the yield of ferulic acid for which dilute ethanol concentration and the lowest temperature became the optimum conditions. Increase in temperature causes increase in the effective diffusivity of resveratrol, being maximum at the highest temperature using moderate ethanol/water solution.

3.3 Optimization of Antioxidant Activity of Extracts from Milled Pinot Noir Grape Canes

Antioxidant activity of grape cane extracts determined by the TEAC method and by the ORAC method varied from 85.6 to 238.6 μmol Trolox equivalent/g dry sample and from 308.4 to 1302.7 μmol Trolox equivalent/g dry sample, respectively (**Table 5**). The surface response analysis for antioxidant activity measured using the TEAC and ORAC methods showed that the main effects of ethanol concentration and temperature are statistically significant ($p \leq 0.01$, or $p \leq 0.001$), whereas solvent to solid ratio was found not to be significant ($p > 0.05$) (**Table 5**). The significant contribution of ethanol concentration and/or temperature to the total antioxidant activities of wheat and wheat bran has been reported by Liyana-Pathirana, and Shahidi (2005).

Table 5 Experimental data of the investigated responses of grape cane extracts under different extraction conditions shown in **Table 1** and independent effects of factors

standard order[a]	response-I	response-II	response-III
	TEAC[c]	ORAC[b]	total phenolic content of extracts (mg resveratrol equivalent/g dw)[c]
1	89.6	407.4	3.80
2	190.3	1048.6	7.57
3	91.1	457.8	3.98
4	217.7	999.0	7.97
5	131.5	1074.0	6.35
6	171.4	1302.7	8.02
7	142.5	963.0	6.16
8	184.0	1064.8	7.46
9	97.5	733.5	5.12
10	238.6	1259.6	8.91
11	139.0	836.0	6.93
12	147.4	817.8	7.16
13	85.6	308.4	3.19
14	121.3	645.5	6.78
15	158.6	994.8	7.22
16	160.1	1054.4	7.24
17	153.1	906.9	7.29
18	146.8	837.6	7.06
		main effects	
temperature	**	*	
solvent to solid ratio	ns	ns	
ethanol concentration	*	*	

[a] Not randomized. [b] Antioxidant activity expressed as equivalent of μmol Trolox/g dry sample. [c] Total phenolic content of grape cane extract as an equivalent of resveratrol (mg resveratrol equivalent/g dw). *, significant at $p \leq 0.01$; **, significant at $p \leq 0.001$; ns, not significant ($p > 0.05$).

Table 6 Regression coefficients of predicted models for the investigated responses of grape cane extracts.

coefficient[a]	TEAC	ORAC
	calculated values of coefficients of fitted models for each responses	
β_0	-154.731^{**b}	-1180.31*
β_1	2.36*	9.47^{ns}
β_2	0.245^{ns,c}	
β_3	5.927***	50.23***
β_{11}	0.02*	0.13^{ns}
β_{22}		
β_{33}	-0.03***	-0.27**
β_{12}		
β_{13}	-0.041***	-0.24*
β_{23}		
model	***	***
linear	***	***
quadratic	***	**
cross-product	***	*
R^2	0.96	0.87
R^2_{Adj}	0.94	0.81

[a] Polynomial model $Z = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$ adjusted by backward elimination at the level of 0.1% with the lack-of-fit test, where β_0 is the constant coefficient, β_i is the linear coefficient (main effect), β_{ii} is the quadratic coefficient, and β_{ij} is the two factors interaction coefficient. Subscript 1, 2 and 3 located in the model coefficients indicates temperature, solvent to solid ratio, and ethanol concentration, respectively. [b] *, significant at $p \leq 0.05$; **, significant at $p \leq 0.01$; ***, significant at $p \leq 0.001$. [c] ns, not significant ($p > 0.05$).

Surface response analysis for antioxidant activity of extracts determined by both methods indicated significant adequacies of the derived models at the 0.001% level of probability, and variability could be explained by the models (**Table 6**). Regression coefficient and analysis of variance of the adjusted polynomial second-order models for antioxidant activity of grape cane extracts are summarized in **Table 6**. As can be seen from an ANOVA of the regression parameters of the surface response analysis of the models, the linear, quadratic, and interaction terms have significant effects ($p \leq 0.001$, $p \leq 0.01$, or $p \leq 0.05$). Control of model parameters, R^2, R^2_{Adj}, confirmed the model adequacies (**Table 6**). The lack of fit test at the level of 0.05 indicates no evidence for lack of fit for both of the antioxidant activity models.

The linear increase in antioxidant activity (TEAC method) with increasing temperature was observed at low and moderate ethanol concentration levels, although, the effect of temperature almost disappeared at relatively higher solvent concentrations (**Figure 40**). The response surface displayed the curve effect of solvent concentration on the antioxidant activity (TEAC method). Trend of solvent concentration effect displayed change when moving from 16.4 to 83.6^{o}C. The increase in antioxidant activity (TEAC) was observed with increase in ethanol concentration (up to 95%) at lower temperature values. The decrease in ethanol content (up to 40%) resulted in an increase in the activity at high temperature (**Figure 40**). The highest predicted antioxidant activity of 260.8 μmol Trolox equivalent/g dry sample (TEAC) was achieved with 40.4% of ethanol concentration at 83.6^{o}C, when solvent to solid ratio was 103.5 mL/g. Similar ethanol concentration and antioxidant activity values were obtained with leaves and buds of black currant (Tabart, Kevers, Sipel, Pincemail, Defraigne, & Dommes, 2007).

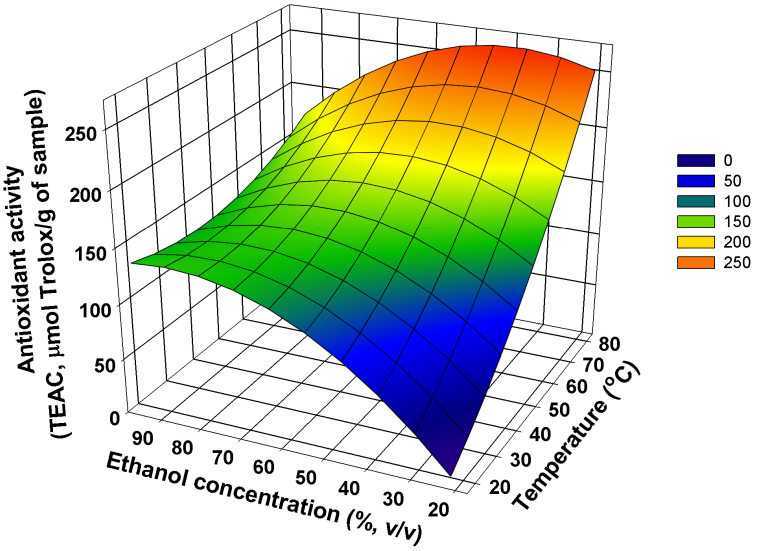

Figure 40 Response surfaces for the effects of temperature and ethanol concentration on the antioxidant activity (TEAC) of grape cane extracts
at a constant solvent to solid ratio of 103.5 mL/g.

Antioxidant activity (ORAC method) also increased linearly with increasing temperature level at moderate and lower solvent concentration levels (**Figure 41**). This strong effect of temperature decreased with increase in ethanol concentration up to 70%, and it disappeared with further increase in solvent concentration (**Figure 41**).

Ethanol concentration displayed curve influence on the antioxidant activity (ORAC method) of grape cane extract (**Figure 41**). The antioxidant activity (ORAC) increased with the increase of ethanol content up to moderate concentration levels (50% - 60%) and decreased with further increase at higher temperature levels (>60^{o}C). The trend of the solvent concentration effect displayed change, when moving from higher temperature levels to lower ones and the optimum point to obtain higher antioxidant activity (ORAC) shifted from moderate ethanol content

(50% - 60%) to its higher levels (95%) (**Figure 41**). The extraction with ethanol concentration of 55.4% at 83.6°C resulted in the highest predicted antioxidant activity (1378.7 µmol Trolox equivalent/g dry sample by ORAC) of grape cane irrespective of solvent to solid ratio.

These results (**Figure 40** and **Figure 41**) are found to be parallel to the results on the effect of ethanol concentration and temperature on the antioxidant activities of wheat and wheat bran extracts reported by Liyana-Pathirana & Shahidi, (2005). The results indicated that increase in temperature lead to increase in antioxidant activity (TEAC and ORAC methods), and this effect reflects the temperature effect on total phenolic content of the extracts (**Table 5**).

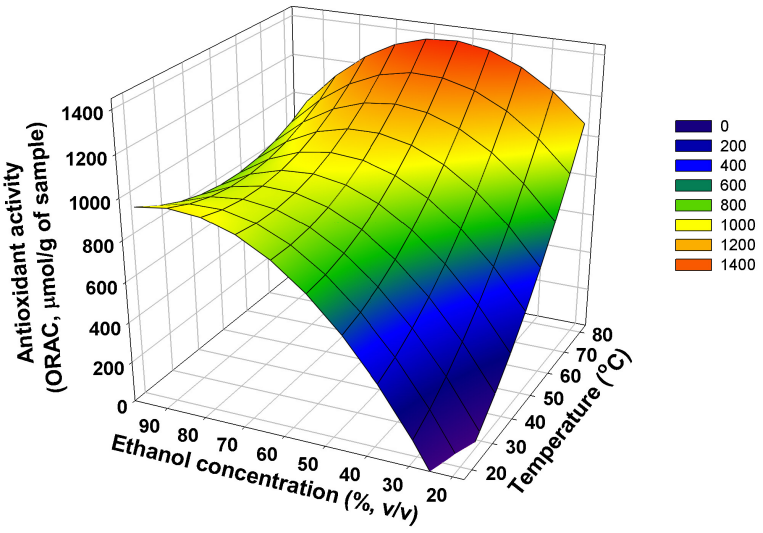

Figure 41 Response surfaces for the effects of temperature and ethanol concentration on the oxygen radical absorbing capacity (ORAC) of grape cane extracts.

Solvent concentration was found to be the most significant extraction factor affecting the antioxidant activity of grape cane extract determined by TEAC method and ORAC method. This effect may be attributed to the change of solvent polarity with

the modification in ethanol concentration. However, the surface response analysis for antioxidant activities measured by both methods, TEAC and ORAC, indicated that the interaction effect between solvent concentration and temperature was also significant (**Table 6**). The results indicated that the optimum solvent concentration levels were altered depending on the temperature of the extraction process. At low temperatures the solvent concentration of 95% (ethanol:water, v/v) was found to be the optimum to predict the highest antioxidant activities for both methods, whereas it shifted to 40% and 55% of ethanol content (v/v) for TEAC and ORAC, respectively (**Figure 40** and **Figure 41**). This may be due to the difference in the antioxidant activity methods used and/or the composition of extract which is affected by solvents and/or process temperature. It has been reported that polarity of solvent used in extraction directly affects not only the quantity of total phenolics, but also the composition/potency of phenolics (Yu, Ahmedna, & Goktepe, 2005). The structure of polyphenol is important factor affecting its antioxidant capacity. The polarity of phenolic compound extracted is consistent with the polarity of solvent used and the more polar phenolic compounds have more hydroxyl groups on the ring of polyphenols. The number and location of these groups generally determine the antioxidant capacity of that polyphenol (Cao, Sofic, & Prior, 1997, Lien, Ren, Bui, & Wang, 1999, and Fukumoto, & Mazza, 2000).

Antioxidant activity obtained by the ORAC method was significantly ($p \leq 0.001$) correlated with antioxidant activity determined by the TEAC method ($R^2 = 0.80$). Tabart et al (2007) has observed the relation between two different antioxidant measurement methods, the DPPH and ABTS, which gave consistent results with similar variations. In present study, the relations between the results of two different antioxidant measurement methods was expected since the antioxidant activity values measured by two methods, TEAC and ORAC were positively correlated with total phenolics ($p \leq 0.001$, $R = 0.95$ and $p \leq 0.001$, $R = 0.89$, respectively). Yu et al (2005) has presented similar correlation between total antioxidant activity and total phenolic content of peanut skin extracts obtained with water and ethanol. The high correlation between antioxidant activity and total phenolic content of bud and leaf extracts of black currant was also reported by Tabart et al (2007).

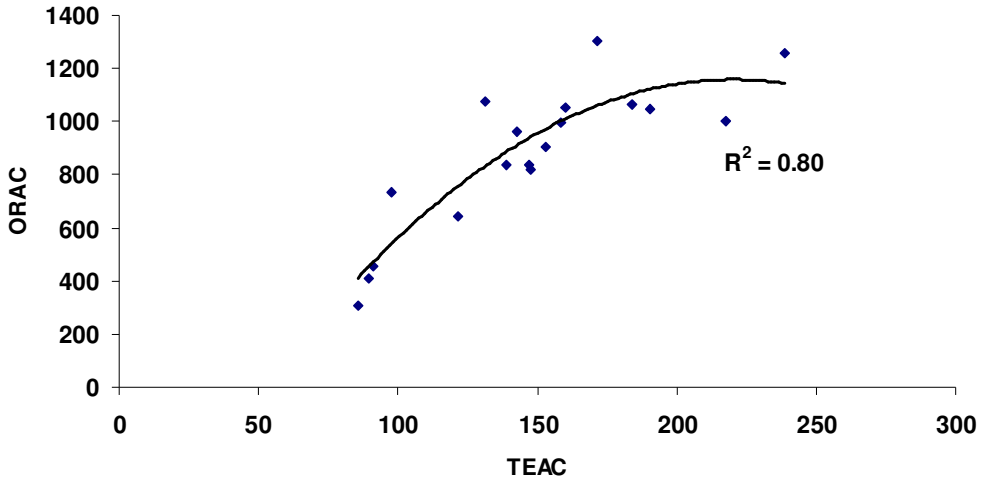

Figure 42 Correlation between antioxidant activities of extracts measured by TEAC and ORAC methods

3.4 Extraction of Bioactive Compounds from Milled Grape Canes (Vitis Vinifera) Using Pressurized Low Polarity Water Method

Total phenolic content of grape cane extracts produced by pressurized low polarity water extraction (PLPW) varied in the range of 4.97 mg/g dw to 7.78 mg/g dw depending on the studied extraction conditions (**Table 7**). The phenolic composition analysis of grape cane extracts indicated mainly the presence of two stilbene compounds, *trans*-resveratrol and *trans*-ε-viniferin varying from 1.95 mg/g dw to 3.65 mg/g dw and from 0.28 mg/g dw to 1.82 mg/g dw, respectively (**Table 7**). Besides the abundant two stilbene compounds, HPLC chromatograms showed the existence of ferulic acid in the water and aqueous ethanol extracts of grape cane samples in the range of 0.20 mg/g dw to 0.50 mg/g dw (**Table 7** and **Figure 43, Figure 44**). The contribution of three main phenolic compounds to the total phenolic

content of extracts was found to be in the range of 38% - 78% altering with extraction conditions employed in the present study.

In the solid-liquid extraction of bioactive compounds from grape cane samples, target compounds has been reported as the yields of *trans*-resveratrol (3.85 ± 0.02 mg/g dw), *trans*-ε-viniferin (1.25 ± 0.03 mg/g dw), ferulic acid (0.16 ± 0.001 mg/g dw), and total phenolics (6.54 ± 0.09 mg/g dw). Pressurized low polarity water extraction (PLPW) method displayed the improvement in the yields of *trans*-ε-viniferin being 1.5 times higher, and ferulic acid being 3 times higher compared to the yields of those bioactive compounds reported in the previous part of the present study, whereas the slight decrease in the yield of *trans*-resveratrol was observed.

Table 7 Extraction of bioactive compounds from milled grape canes by PLPW at 85 to 160°C using water and ethanol/water mixture (max. 25%, v/v), and 0.5 to 5.0 mL/min flow rate

temperature (°C)	ethanol concentration (%, v/v)	flow rate (mL/min)	total phenolics (mg/g dw)	*trans*-resveratrol (mg/g dw)	*trans*-ε-viniferin (mg/g dw)	ferulic acid (mg/g dw)	antioxidant activity*
Effect of temperature							
85	7.4	1	5.27 ± 0.78^{a}	3.16 ± 0.18^{a}	0.50 ± 0.05^{a}	0.20 ± 0.09^{a}	150 ± 22.1^{a}
95	7.4	1	6.15 ± 0.42^{a}	3.28 ± 0.04^{a}	0.74 ± 0.09^{a,b}	0.33 ± 0.10^{a}	189 ± 6.3^{a}
105	7.4	1	6.29 ± 0.28^{a}	2.98 ± 0.18^{a,b}	0.83 ± 0.14^{b}	0.24 ± 0.24^{a}	198 ± 14.2^{a}
120	7.4	1	6.13 ± 1.21^{a}	2.84 ± 0.16^{a,b}	0.94 ± 0.01^{b}	0.29 ± 0.09^{a}	194 ± 18.3^{a}
140	7.4	1	6.68 ± 0.37^{a}	2.51 ± 0.01^{b}	0.66 ± 0.09^{a,b}	0.32 ± 0.03^{a}	186 ± 25.9^{a}
160	7.4	1	7.12 ± 1.13^{a}	1.95 ± 0.07^{c}	0.49 ± 0.02^{a}	0.27 ± 0.03^{a}	172 ± 6.6^{a}
Effect of modifier concentration							
105	0	1	4.97 ± 0.74^{a}	2.76 ± 0.12^{a}	0.28 ± 0.05^{a}	0.32 ± 0.07^{a}	153 ± 23.6^{a}
105	7.4	1	6.29 ± 0.28^{a,b}	2.98 ± 0.18^{a}	0.83 ± 0.14^{b}	0.24 ± 0.24^{a}	198 ± 14.2^{a,b}
105	15	1	6.80 ± 0.56^{a,b}	3.23 ± 0.18^{a}	1.40 ± 0.00^{c}	0.32 ± 0.04^{a}	220 ± 13.6^{a,b}
105	25	1	7.18 ± 0.35^{b}	3.40 ± 0.18^{a}	1.65 ± 0.04^{c}	0.39 ± 0.10^{a}	249 ± 15.1^{b}
Effect of flow rate							
105	15	0.5	6.44 ± 0.42^{a}	2.98 ± 0.16^{a}	1.22 ± 0.01^{a}	0.31 ± 0.11^{a}	189 ± 6.0^{a}
105	15	1	6.80 ± 0.56^{a}	3.23 ± 0.18^{a}	1.40 ± 0.00^{b}	0.32 ± 0.04^{a}	220 ± 13.6^{a}
105	15	2	7.25 ± 0.32^{a}	3.37 ± 0.09^{a}	1.46 ± 0.02^{c}	0.33 ± 0.14^{a}	231 ± 27.7^{a}
105	15	3	7.08 ± 0.70^{a}	3.35 ± 0.23^{a}	1.52 ± 0.02^{d}	0.42 ± 0.01^{a}	225 ± 27.2^{a}
105	15	5	7.12 ± 0.11^{a}	3.40 ± 0.09^{a}	1.59 ± 0.01^{e}	0.40 ± 0.04^{a}	234 ± 38.2^{a}

*, the antioxidant activity, expressed as equivalent of μmol Trolox/g dry sample, was determined using the Trolox equivalent antioxidant capacity assay, (TEAC). Same letters mean that the differences between values having same letter in the same column and under same extraction variable topic is not significant *($p > 0.05$)*.

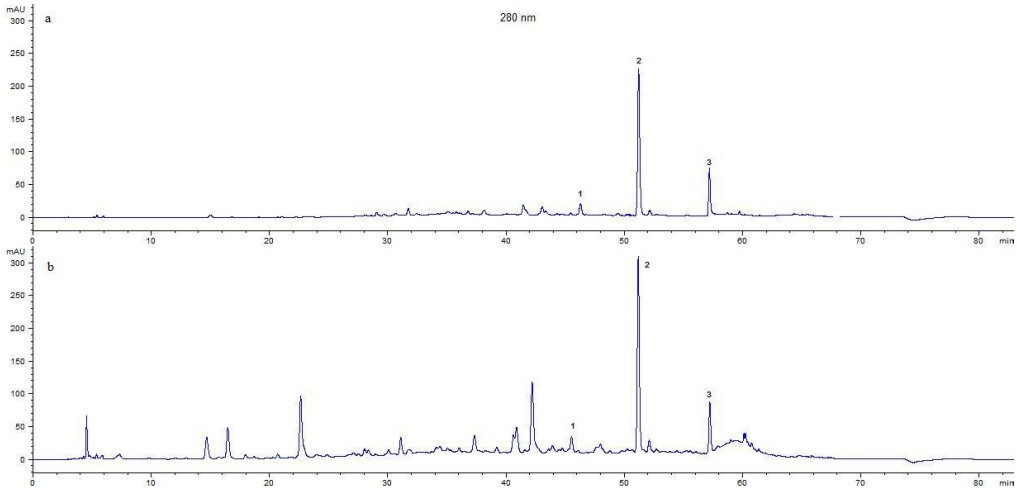

Figure 43 HPLC chromatograms (280 nm) of grape cane extracts obtained by pressurized low polarity water extraction at 105°C (extract volume of 300 mL) (a) and at 160°C (extract volume of 300 mL) (b). Phenolic compounds;

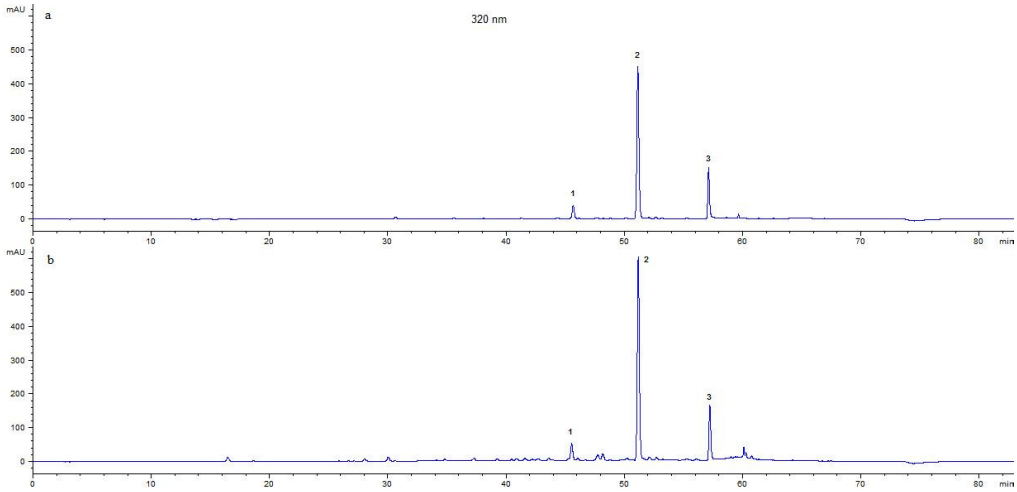

Figure 44 HPLC chromatograms (320 nm) of grape cane extracts obtained by pressurized low polarity water extraction at 105°C (extract volume of 300 mL) (a) and at 160°C (extract volume of 300 mL) (b). Phenolic compounds;

1 ferulic acid

2 *trans*-resveratrol

3 *trans*-ε-viniferin

3.4.1 Effect of Solvent Composition

Pressurized low polarity water extraction method using water without a modifier as a solvent was found not to be an efficient system for extraction of grape cane bioactives. As can be seen from **Table 7**, the extraction efficiency of pressurized water extraction resulted in the lowest yields of total phenolic and target bioactive compounds except ferulic acid yield. It was decided to employ ethanol as a modifier in the extraction of target phenolic compounds from grape canes due to the low extraction efficiency of water. Ong, Cheong, and Goh (2006) have also concluded that the presence of ethanol in the water enhanced the capacity of the solvent to solubilize the naturally occurring compounds in plant materials.

Table 7 indicated the favorable effect of ethanol varying in the range of 7.4 to 25% (v/v) on the extraction efficiencies of *trans*-resveratrol, and *trans*-ε-viniferin. Decrease in the yield of ferulic acid was observed when ethanol was at concentration of 7.4%, further increase of ethanol concentration caused the favorable effect on the ferulic acid content of extracts (**Table 7**). The increase in ethanol concentration from zero to 25% resulted in 1.5 fold increase in total phenolic content of grape cane extract. The highest improvement (above 5 fold) was observed in the yields of *trans*-ε-viniferin (**Table 7**). The enhancement in the viniferin yield with ethanol concentration was found to be statistically significant ($p \leq 0.05$). The slight rise (statistically insignificant, $p > 0.05$) was seen in the yield of *trans*-resveratrol (**Table 7**). The significant change in the yields of ferulic acid was not observed with the compositional change of employed solvent in extractions ($p > 0.05$) (**Table 7**).

The positive potent of the modifier may be attributed to the alteration of solvent polarity with changing of ethanol concentration. At elevated temperatures and moderate pressures the polarity of water can be reduced considerably, but the applicability is limited due to the possible thermal degradation of target compounds at elevated temperature levels. The modifier usage in low proportions, thus, became

necessary to adjust the polarity of solvent employed in PLPW system (Carabias-Martínez et al., 2005). Alonso-Salces, Korta, Barranco, Berrueta, Gallo, and Vicente (2001) have reported that the extraction yield of phenolic compounds from apple was favored by altering methanol concentration in the extraction solvent. Gonzalez-Rodriguez, Perez-Juan, and Luque de Castro (2003) has also investigated the effect of the ethanol concentration on the extraction of bioactive compounds from solid residues of winemaking processes and reported the value of 40% ethanol-water as the optimum concentration for the extraction efficiency of all the compounds of interest. Howard and Pandjaitan (2008) have compared the extraction of the flavonoids from spinach via pressurized liquid extraction with water and aqueous ethanol and it has been found that at elevated temperature level (higher than 110^{o}C), ethanol-water mixture was more efficient than water extraction. The literature results are compatible with the presented results of the favorable ethanol effect.

3.4.2 Effect of Temperature

As can be seen from **Table 7**, statistically significant change of the total phenolic content of grape cane extract with increasing temperature was not observed in the whole range of temperature ($p > 0.05$). The insignificant increase in the yield of *trans*-resveratrol ($p > 0.05$) was seen with increase in the narrow temperature range of 85 to 95^{o}C, further rise up to the highest studied level of 160^{o}C resulted in severe decrease in *trans*-resveratrol content ($p \leq 0.05$) (**Table 7**). **Table 7** indicated a favorable change in *trans*-ε-viniferin content of extract followed by sharp reduction with increasing temperature in the studied range. This observed temperature influence on the *trans*-ε-viniferin yield was found to be statistically significant ($p \leq 0.05$) (**Table 7**). The difference responses of *trans*-resveratrol and *trans*-ε-viniferin as a results of temperature change may be attributed to their possible different thermal stabilities. However any clear extraction trend was observed throughout the PLPW extraction of ferulic acid from grape cane samples. Temperature was found not to be significant extraction condition influencing the yield of ferulic acid ($p > 0.05$) (**Table 7**).

Figure 43 and **Figure 44** displayed the compositional change of phenolic content of extracts at temperature levels of 105°C and 160°C. The portion of *trans*-resveratrol and *trans*-ε-viniferin in the total phenolic yield of grape cane extract lowered from 70% to 38% when the highest temperature level of 160°C was employed. This may be attributed to the thermal degradation of target compounds and/or the increase in the solubility of other compounds with increasing temperature, especially after 120°C.

The performance of PLPW extraction process due in part to elevated temperature can be attributed to two main reasons: solubility and mass transfer effect, and disruption of surface equilibria (Richter et al., 1996). The capacity of solvent to solubilize the analyte is increased by using elevated temperature. Using higher temperature also results in faster diffusion rate. The strong solute-matrix interactions caused by van der Waals forces, hydrogen bonding, and dipole attractions of the solute molecules and active sites on the matrix can be disrupted and hydrogen bonding is weakened with increased temperature (Richter et al., 1996; Lou, Janssen, Cramers, 1997; Kawamura, Kikuchi, Ohira, Yatagai, 1999). Other properties such as viscosity, surface tension and density are also modified. Decreases in viscosity of solvent and surface tension of the solvent due to the increase in temperature level facilitate better contact of the analytes with solvent and enhance extraction (Richter et al., 1996; Cacace, Mazza, 2006).

The appreciable effect of thermal degradation of catechins at temperature values higher than 130°C for 10 min extraction cycles have been proven to be effective on the extract yield by Piñeiro, Palma, and Barroso (2004). These literature values and put forth results indicates that the thermal degradation of phenolic compounds should be considered when the extraction method required to employ elevated temperature levels like PLPW system. Piñeiro, Palma, and Barroso (2006) have reported the stability of *trans*-resveratrol during the methanolic extraction at even 150°C, but it may be attributed to short duration time in the extraction.

3.4.3 Effect of Solvent Flow

Although the significant change in the *trans*-ε-viniferin yield with increasing flow rate was observed ($p \leq 0.05$), the statistical analysis indicated the insignificant solvent flow rate dependencies of *trans*-resveratrol, ferulic acid, and total phenolic yields ($p > 0.05$) (**Table 7**). The increase in the extraction efficiency of *trans*-ε-viniferin can be associated with the effect of flow rate on the extraction rate through its impact on the local driving force (concentration gradient). The solvent flow rate favorably influenced the extraction efficiency of *trans*-ε-viniferin in the whole range of flow rate of 0.5 to 5 mL/min (**Table 7**).

Influence of flow rate may be attributed the internal diffusion of target compound in milled grape cane particles, controlling mass transfer (Ho, Cacace, Mazza, 2008). In the solid-liquid extraction study, the internal diffusion of resveratrol in the milled grape cane particle has been determined to control the mass transfer during solid-liquid extraction process. Insignificant effect of solvent flow rate on the yields of total phenolic and target compounds except *trans*-ε-viniferin may be attributed to diffusion of these compounds in the solid matrix being the controlling step of mass transfer taking place in the extraction process. Besides to the favorable effect of solvent flow rate, it should be noted that employing higher solvent flow rate resulted in more dilute extract in terms of target compounds and consumption of more solvent which were not warranted.

3.4.4 Antioxidant Activity

Antioxidant potentials of grape cane extracts were evaluated. The antioxidant activity of extracts measured by TEAC method displayed a correlation with their total phenolics contents, ($p < 0.0001$, $R = 0.82$) (**Figure 45a**). The relation between total phenolics content of grape cane extracts obtained by solid-liquid extraction and their

antioxidant activities measured by TEAC and ORAC methods have been reported. The similar correlations introduced by solid-liquid extraction and PLPW extraction were coincidence to figure out the relation between total phenolics and antioxidant activity. Yu, Ahmedna, and Goktepe (2005) and Tabart, Kevers, Sipel, Pincemail, Defraigne, and Dommes (2007) have presented the similar relation between total antioxidant activities and total phenolic contents of peanut skin extracts and bud and leaf extracts of black currant, respectively. Temperature influence on antioxidant activity of extracts was investigated. An increase in temperature was found not to have statistically significant effect on antioxidant activity ($p > 0.05$).

The contributions of the target bioactive compounds to the antioxidant activity of extracts were also investigated. *trans*-resveratrol and *trans*-ε-viniferin were positively correlated with antioxidant activities of extracts ($p < 0.005$, $R = 0.70$, and $p < 0.0001$, $R = 0.95$, respectively) (**Figure 45b** and **Figure 45c**). These results showed that the antioxidant activity of extract depended not only on the total phenolic content of extracts, but also on the distribution of phenolic compounds of extracts. In other words, the major contribution to the antioxidant capacity of extract is due to bioactive compounds, *trans*-resveratrol, and *trans*-ε-viniferin.

As can be seen from **Table 7**, the antioxidant activities were enhanced by modifier concentration in the solvent varying from 0 to 25% of ethanol in water (v/v), parallel to the change of total phenolic and target phenolic contents of grape cane extracts. The statistically significant difference was achieved in the antioxidant activity of extracts when 25% ethanol/water mixture was used compared to pure water as a solvent in PLPW system ($p \leq 0.05$) (**Table 7**).

The antioxidant capacity of polyphenol is generally determined with the number and location of hydroxyl groups on the ring of that polyphenol (Cao, Sofic & Prior, 1997, Lien, Ren, Bui & Wang, 1999, and Fukumoto & Mazza, 2000). The change in ethanol concentration resulted in an increase in the yields of target phenolic compounds which influences the antioxidant capacity of extracts due to their hydroxyl groups. This result also indicates the strong relation between antioxidant

activity and phenolic content and phenolic composition of extracts. The change in the antioxidant activities of extract due to the effect of flow rate was found not to be significant ($p > 0.05$) (**Table 7**).

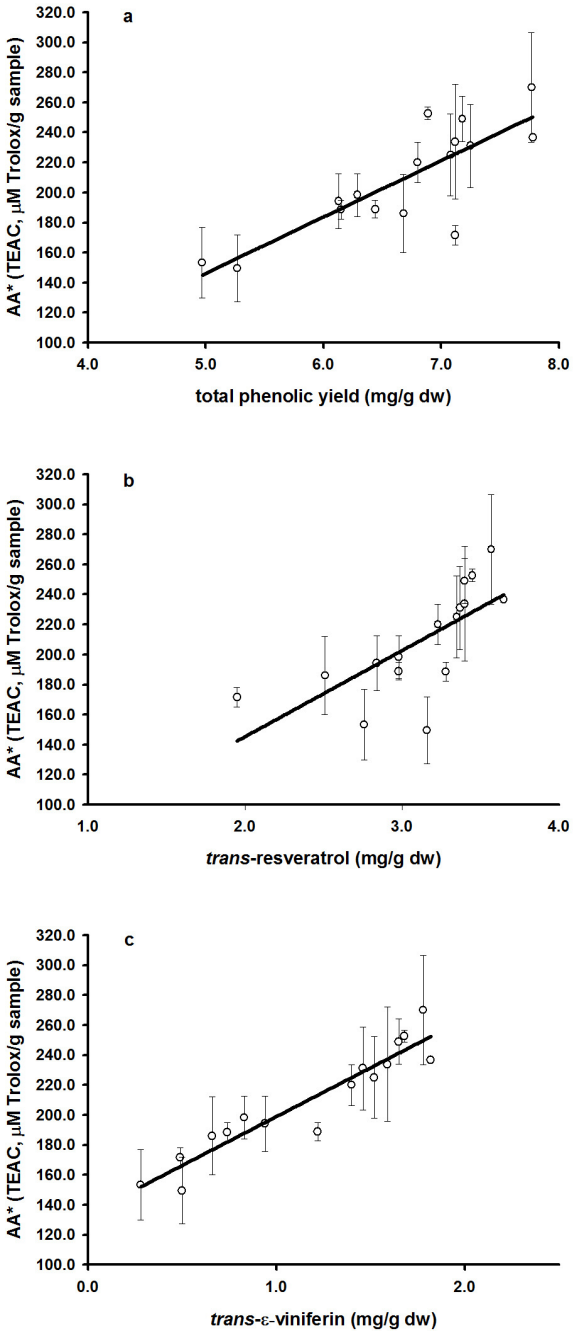

Figure 45 Change in antioxidant activities with total phenolic (a), *trans*-resveratrol (b), and *trans*-ε-viniferin (c) contents of grape cane extracts.

*, antioxidant activity, expressed as equivalent of μmol Trolox/g dry sample, and measured by TEAC method.

3.4.5 Effective Diffusivity

Effects of temperature and ethanol concentration on effective diffusivities of stilbene compounds were investigated. Effective diffusivities of *trans*-resveratrol and *trans*-ε-viniferin were favored from 2.9×10^{-11} to 10.4×10^{-11} m^2/s and from 0.9×10^{-11} to 7.7×10^{-11} m^2/s, respectively, with ascending temperature in the whole range of 95 to 160°C (**Table 8**). Increase in the temperature, although, accelerated the extraction of *trans*-resveratrol and *trans*-ε-viniferin about 3 times and almost 8 times, respectively, possible unwanted side reactions and thermal degradation limited using elevated temperature.

The results indicated that, as the ethanol concentration increased from 0 to 15% (v/v), the D_{eff} values of *trans*-resveratrol increased, whereas the favorable influence of increasing ethanol concentration on the effective diffusivities of *trans*-ε-viniferin was observed when rising concentration from 7.4% to 15% (**Figure 46**). Slight increase in the D_{eff} value at 7.4% ethanol/water mixture (v/v) was seen compared to result achieved water used alone as a solvent (**Figure 46**). Kim and Mazza (2007) have reported the effective diffusivity values for phenolic compounds of flax shives extraction by PLPW system varying from 9.1×10^{-11} to 15×10^{-11} m^2/s. These results are comparable to effective diffusivity values calculated for mass transfer of target compounds by PLPW extraction. However the effective diffusivity values of *trans*-resveratrol during the solid-liquid extraction of grape cane samples varied from 3.1×10^{-13} to 26.6×10^{-13} m^2/s were smaller than above results determined for the PLPW extraction of bioactive compounds from grape cane samples. Difference indicated the effectiveness of the PLPW system in the extraction of stilbene compounds from grape cane samples in terms of mass transfer phenomena compared to conventional solid-liquid extraction. Change in ethanol concentration enhances the diffusion of given compounds in the solid phase. The influence of ethanol concentration can be attributed to alteration in the solvent properties such as density,

dynamic viscosity, surface tension, and dielectric constant due to change of concentration (Frank, Downey, & Gupta, 1999, Gertenbach, 2002).

Temperature dependencies of the effective diffusivities of studied stilbene compounds can be attributed to improvement of mass transfer, and solute-solid interactions. (Gertenbach, 2002). From another point of view, temperature effect on diffusivity can be associated with an increase in the internal energy of the molecules and thus their mobility, and a reduction of the dynamic viscosity coefficient. Thus, higher extraction rate and hence shorter time is achieved (Cacace, Mazza, 2003b). The effective diffusivities corresponding 1/T values was assumed to obey the Arrhenius type equation;

$$D_{eff} = D_o \exp\left(-\frac{E_a}{RT}\right) \qquad (\mathbf{43})$$

Where D_{eff} and D_o are the effective diffusivity and initial diffusivity, respectively, E_a is the activation energy (kj.mol^{-1}) for diffusion, R is the universal gas constant (kj.mol^{-1}.K^{-1}) and T is the absolute temperature (K). As can be seen from **Figure 47** that the slope of plots of ln(D_{eff}) vs 1/T for resveratrol and viniferin were used to calculate the activation energies of diffusion of studied stilbene compounds. Activation energies of the diffusion of *trans*-resveratrol and *trans*-ε-viniferin were 28 and 44 kj.mol^{-1}, respectively. Spiro and Selwood (1984) and Spiro and Siddique (1981) have reported the comparable activation energy values of 32 and 23 kj.mol^{-1} for caffeine diffusion through coffee beans and tea leaf, respectively.

Table 8 Effective diffusivity determined using the linear (graphical) solution for PLPW extraction of *trans*-resveratrol and *trans*-ε-viniferin from grape cane samples at different temperature values

	effective diffusivity, $D_{eff} \times 10^{11}$ (m^2/s)*	
temperature (^oC)	trans-resveratrol	trans-ε-viniferin
95	2.9	0.9
105	3.3	1.7
120	5.4	2.8
140	8.6	6.5
160	10.4	7.8

*, PLPW extraction of *trans*-resveratrol and *trans*-ε-viniferin from grape cane samples were performed using 7.4% ethanol/water mixture (v/v), an extraction pressure of 5.2 MPa, and a 100 mm long × 19.3 mm ID extraction cell at the flow rate of 1 mL/s.

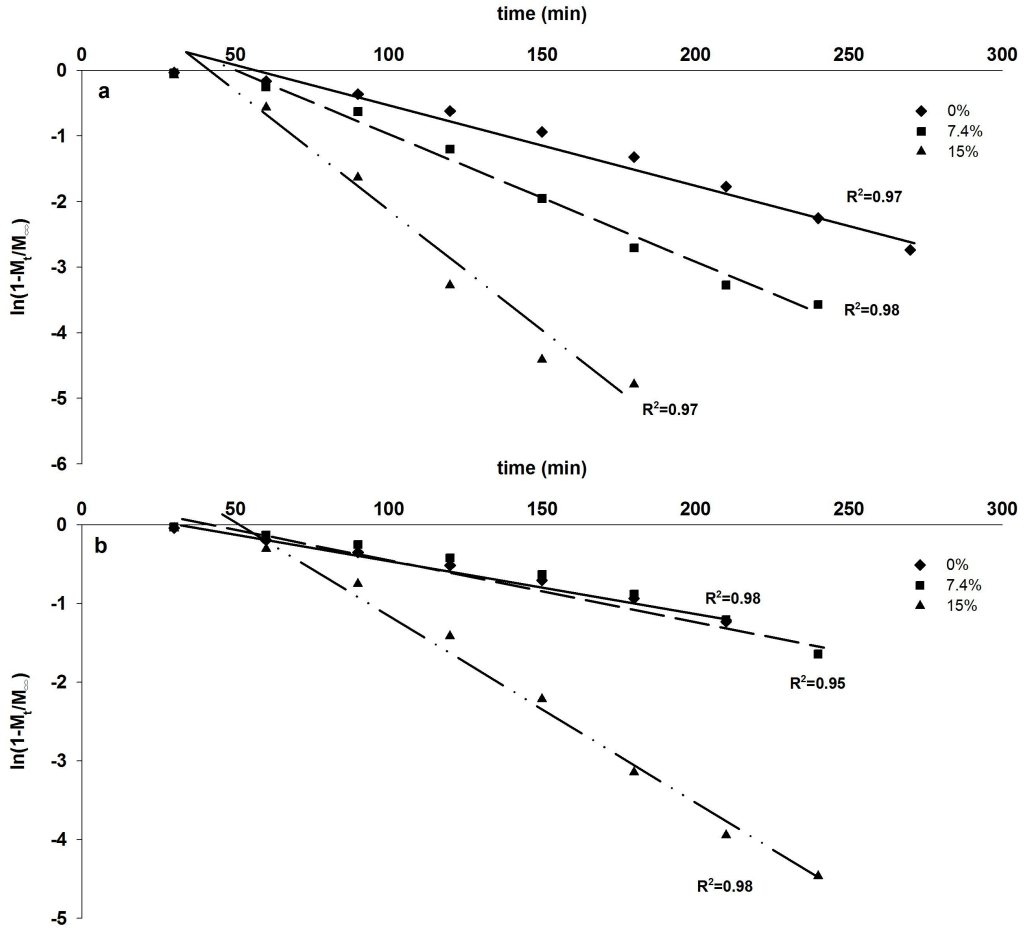

Figure 46 Plot of linear (graphical) solution using Fick's second law for the calculation of effective diffusivities of *trans*-resveratrol (a) and *trans*-ε-viniferin (b) by PLPW extraction.

Extraction conditions: PLPW extraction of *trans*-resveratrol and *trans*-ε-viniferin from grape cane samples were performed using an extraction pressure of 5.2 MPa and a 100 mm long × 19.3 mm ID extraction cell at a flow rate of 1 mL/s and temperature of 105^{o}C.

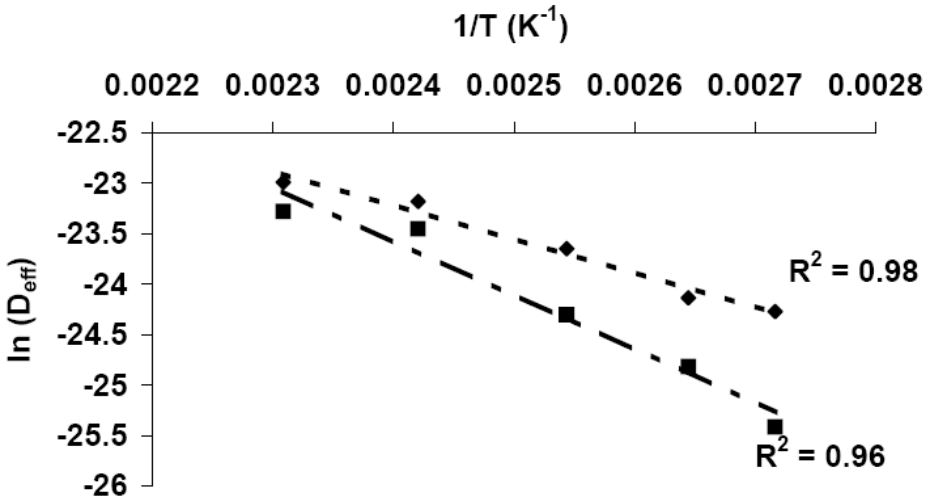

Figure 47 Arrhenius type relation between effective diffusivities of *trans*-resveratrol and *trans*-ε-viniferin and temperature using 7.4% ethanol/water mixture (v/v) at a flow rate of 1 mL/s.

3.4.6 Kinetic Model

Modified Gompertz Equation has been proposed to explain the extraction kinetic of *trans*-resveratrol and *trans*-ε-viniferin from grape cane samples by PLPW system. Temperature and ethanol concentration were two evaluated process parameters affecting the extraction yields of target compounds. **Figure 48a-b**, and **Figure 49a-b** show the change of yields of *trans*-resveratrol and *trans*-ε-viniferin under different temperature and ethanol concentration values. The fitted Modified Gompertz model lines for trans-resveratrol and trans-ε-viniferin at studied temperature levels and ethanol concentrations can be observed in **Figure 48a-b** and **Figure 49a-b**.

The goodness of the fit of proposed model was evaluated by computing the regression coefficient, (R^2) and mean square error (*MSE*) values (**Table 9**). Modified Gompertz Equation is found to be a satisfactory model to represent the extraction

kinetics of studied stilbene compounds from grape cane samples according to their computed R^2 and *MSE* values (**Table 9**).

Calculated maximum extraction yields of *trans*-resveratrol and *trans*-ε-viniferin are compatible with the yield values achieved experimentally (**Figure 48a-b**, **Table 7** and **Table 9**). Rising temperature favored the calculated maximum extraction yield of *trans*-resveratrol and *trans*-ε-viniferin up to 95°C and 120°C, respectively, further increase resulted in reduction in these values (**Figure 48a-b**, **Table 9**). Same trend can be followed for experimental yield results of studied stilbene compounds (**Table 7**). As can be seen from **Figure 49a-b**, and **Table 9**, higher ethanol concentration enhanced the calculated maximum extraction yields of *trans*-resveratrol and *trans*-ε-viniferin in the studied concentration range of 0% to 15%. Experimental ethanol concentration results from **Table 7** also supported the findings about maximum extraction yield results of stilbene compounds.

Parallel to the effective diffusivity results, rise in the fasted extraction rate values as a parameter of modified Gompertz Equation was observed with increase in the whole range of temperature (**Table 8** and **Table 9**). **Figure 48a-b** also indicated the favorable effect of temperature on the fastest extraction rate of *trans*-resveratrol and *trans*-ε-viniferin. The fastest extraction rate was found to be enhanced by increase in the ethanol concentration. As can be seen from **Figure 46a-b**, the favorable influence of modifier concentration on the effective diffusivities being the strong indicator of the extraction rate also supports the statement of the effect of ethanol concentration. Positive influence of ethanol concentration can be attributed to the alteration in the solvent polarity, density, and viscosity.

Third parameter of the proposed kinetic model is the time required to reach the beginning of the fastest extraction phase. Increase in ethanol concentration was found to shorten the time values for extraction of *trans*-resveratrol, whereas time value for *trans*-ε-viniferin extended and then shortened (**Figure 49a-b**, and **Table 9**). Acceleration in the extraction can be associated with the improvement in the dissolution of target compounds in the solvent with change in the concentration.

Decrease in the time values with rising temperature were observed after temperature values of 95^{o}C and 105^{o}C for *trans*-resveratrol and *trans*-ε-viniferin, respectively (**Table 9**).

Table 9 The non-linear regression values of fitted parameters of the Modified Gompertz Equations at studied extraction conditions

trans-resveratrol extraction								
temperature (°C)	Adj. Rsq	MSE	y_0 (mg/g dw)	st error	m (mg/g.min)	st error	λ (min)	st error
85	0.998053	0.0029	3.1316*	0.0561	0.0197*	0.0006	31.1858*	2.2901
95	0.999479	0.0011	3.3799*	0.0235	0.0248*	0.0005	37.6391*	1.2968
105	0.999611	0.0006	2.9411*	0.0204	0.0269*	0.0005	37.556*	1.0264
120	0.999727	0.0004	2.7554*	0.0118	0.0361*	0.0007	32.0052*	0.7287
140	0.999216	0.0011	2.517*	0.0157	0.0465*	0.0015	26.8204*	0.8943
160	0.999979	0	1.8934*	0.0022	0.0489*	0.0003	20.374*	0.0829
ethanol concentration (%, v/v)								
0	0.998986	0.0012	2.8677*	0.0459	0.0167*	0.0004	40.8752*	1.8692
7.4	0.999581	0.0007	2.9413*	0.0214	0.0269*	0.0006	37.5389*	1.0751
15	0.999582	0.0009	3.1143*	0.0125	0.0495*	0.0013	31.8222*	0.8738
trans-ε-viniferin extraction								
temperature (°C)								
85	0.973651	0.0008	0.47*	0.0338	0.0029*	0.0003	12.8762*	6.6187
95	0.993551	0.0004	0.7766*	0.0432	0.0031*	0.0001	38.705*	4.7033
105	0.99812	0.0002	0.9066*	0.0291	0.0042*	0.0001	46.433*	2.494
120	0.999281	0.0001	0.986*	0.0126	0.0069*	0.0002	37.9376*	1.4258
140	0.998942	0.0001	0.7317*	0.006	0.0099*	0.0004	30.0556*	1.3438
160	0.999947	0	0.4791*	0.0009	0.0102*	0.0001	21.6787*	0.1566
ethanol concentration (%, v/v)								
0	0.990202	0.0001	0.2686*	0.0179	0.0012*	0.0001	33.6151*	5.4895
7.4	0.9977	0.0002	0.9432*	0.0481	0.0042*	0.0001	46.7602*	2.57
15	0.999719	0.0001	1.4117*	0.0056	0.0141*	0.0002	35.1953*	0.8572

*, Significant at $p \leq 0.0001$.

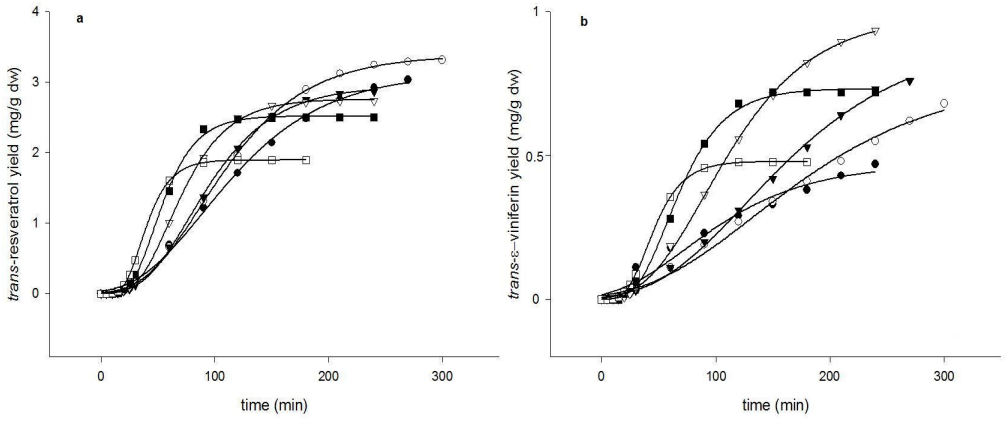

Figure 48 Temperature effect on the extraction yield of trans-resveratrol (a) and trans-ε-viniferin (b) by PLPW system

with 7.4% ethanol/water mixture at the flow rate of 1 mL/s: 85°C (●), 95°C (○), 105°C (▼), 120°C (▽), 140°C (■), and 160°C (□). The fitted Modified Gompertz Model (—).

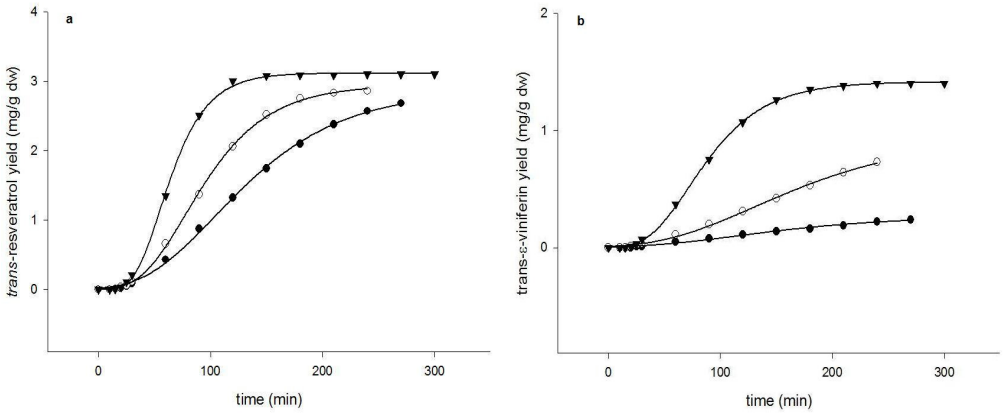

Figure 49 Ethanol concentration effect on the extraction yield of *trans*-resveratrol (a) and *trans*-ε-viniferin (b) by PLPW system

at 105 °C and the flow rate of 1 mL/s: ethanol concentration of 0% (●), 7.4% (○), and 15% (▼). The fitted Modified Gompertz Model (—).

4 CONCLUSIONS

The results of the filtration of grape juce indicated that variables in filtration process play important roles in the filtration efficiency and product quality. Usage of precoating and filter aid materials (diatomaceous earth) displayed positive effect on the filter life. Trials for the effect of pressure showed that the cake formed during filtration process was incompressible. Temperature favored flow rate by lowering the viscosity of grape juice. The influences of pretreatments, depectinization and clarification, were examined and their positive effects on filtration efficiency and product quality were determined. As a conclusion pretreatments were required for improvement in filtration process.

Exponential model was proposed to explain the filtration of grape juice, but it was found to be sufficient for the filtration of nonpectinized and nonclarified grape juice. Results for other filtration parameters indicated presence of insufficient scale of filter unit for the mathematical explanation of filtration of grape juice which was depectinized and clarified.

Single models were proposed to predict the density, viscosity and heat capacity of studied clarified fruit juices in the studied ranges of temperature and soluble solid content. The statistical analysises showed that the models for density and viscosity were valid for all studied fruit juices. In other words model for density can be used to predict this physical property for sourcherry, apple and grape juices. Same circumstance was also applicable for viscosity with high regression coefficient. However, the achieved model for heat capacity was not sufficient to represent the influences of temperature and soluble solid content. Higher soluble solid content resulted in higher density and viscosity but lower heat capacity of fruit juices. Temperature adversily affected the density and viscosity, meanwhile favorable influence of temperature was observed for heat capacity.

The optimization of the effects of extraction variables, being temperature, solvent to solid ratio, and ethanol concentration, on the yield of resveratrol, viniferin, ferulic acid, and total phenolics from milled grape canes has been studied. The results indicated that the extraction at the highest temperature (83.6 ^{o}C) with a moderate ethanol concentration (50% - 70% ethanol/water mixture) was the optimum for the extraction of phenolic compounds *trans*-resveratrol, *tran*s-ε-viniferin, and total phenolics. The yield of ferulic acid was optimized by the extraction of grape cane samples with a dilute ethanol solution (ethanol/water mixture) at the studied lowest temperature level.

Antioxidant capacities of extracts obtained from grape cane samples were also optimized. The maximum predicted activity levels were found to be 260.8 and 1378.7 μmol Trolox equivalent/g dry sample measured by the TEAC and ORAC methods, respectively. Ethanol/water mixture of 40.4% was optimum for maximum activity measured by TEAC method, whereas 55.4% of ethanol concentrations resulted in the highest activity measured by ORAC method, when the highest temperature (83.6^{o}C) was employed.

The extraction of bioactive compounds from milled grape canes employing pressurized low polarity water extraction (PLPW) was investigated. Temperature, modifier concentration (ethanol), and solvent flow rate were the extraction parameters. Temperature favored the extraction efficiencies of *trans*-resveratrol and *trans*-ε-viniferin, whereas the modifier concentration was found to be significant for total phenolic content and *trans*-ε-viniferin. Another parameter solvent flow rate significantly increased the yield of *trans*-ε-viniferin. Ferulic acid content displayed insignificant changes with investigated parameters. Antioxidant capacity of extracts positively correlated with total phenolic content and with stilbene compounds, *trans*-resveratrol and *trans*-ε-viniferin. The influence of modifier concentration was found to be statistically significant when the effects of extraction parameters on the antioxidant activity of extracts were investigated.

Increase in temperature causes increase in the effective diffusivity of resveratrol, being maximum at the highest temperature using moderate ethanol/water solution in the solid-liquid extraction. Temperature and ethanol concentration influences on the effective diffusivities of *trans*-resveratrol and *trans*-ε-viniferin were also investigated. Calculated effective diffusivity values indicated that PLPW system for extraction of stilbene compounds from grape cane samples was faster than the effective diffusivities of resveratrol calculated in the solid-liquid extraction method.

Kinetic analysis of the extraction of *trans*-resveratrol and *trans*-ε-viniferin by PLPW was performed using modified Gompertz equation. Regression parameters indicated the high goodness of fits for modified Gompertz equation.

5 RECOMMENDATIONS

In addition to density, viscosity and heat capacity of sourcherry, apple and grape juices, other physical properties like thermal conductivity, thermal diffusivity can be modeled. Other clarified fruit juices can be investigated in this respect. Rheological analysis of clarified fruit juices may be helpful to understand the processes and products better. The effect of compositional differences between studied fruit juices on heat capacity values can be studied.

Grape canes were displayed as a high potential natural source of phytochemicals, but environmental factors and seasonal effects could be investigated.

Pressurized low polarity water extraction displayed possible high potential for the extraction of bioactives from natural sources, but it requires more and detailed studies for its application to different materials and target compounds. It also requires additional studies for industrial applications.

REFERENCES

Abril, M., Negueruela, A.I., Pérez C., Juan T., & Estopañán G., (2005). Preliminary study of resveratrol content in Aragón red and rosé wines. *Food Chemistry*, 92, 729–736.

Adrian, M., Jeandet, P., Breuil, A.C., Levite, D., Debord, S., & Bessis, R., (2000). Assay of resveratrol and derivative stilbenes in wines by direct injection high performance liquid chromatography. *American Journal of Enology and Viticulture,* 51 (1), 37-41.

Aggarwal, B. B., Bhardway, A., Aggarwal, R. S., Seeram, N. P., Shishodia, S., & Takada, Y., (2004). Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res.,* 24, 2783-2840.

Aguado, M. A., & Ibarz, A., (1988). Variación de la densidad de un zumo de manzana con la temperatura y concentración. *Alimentación Equipos y Tecnología*, pp. 209–216.

Ali, S. D., Ramaswamy, H. S., & Awuah, G.B., (2002). Thermo-physical properties of selected vegetables as influenced by temperature and moisture content. *Journal of Food Process Engineering*, 25, 417-433.

Alonso-Salces, R.M.; Korta, E.; Barranco, A.; Berrueta, L.A.; Gallo, B.; & Vicente, F., (2001). Pressurized liquid extraction for the determination of polyphenols in apple. *J. of Chromatography A*, 933, 37-43.

Alvarado, J.D., & Romero, C. H., (1989). Physical properties of fruits I. density and viscosity of juices as functions of soluble solids content and temperature. *Latin American Applied Research*, 19: 15-21.

Aruoma, O. I., Murcia, A., Butler, J., & Halliwell, B., (1993). Evaluation of the antioxidant and prooxidant actions of gallic acid and its derivatives. *Journal of Agricultural and Food Chemistry*, *41*, 1880-1885.

Azoubel, P.M., Cipriani, D.C., El-Aouar, A.A., Antonio, G.C. & Murr, F.E.X., (2005). Effect of concentration on the physical properties of cashew juice. *Journal of Food Engineering*, 66: 413–417.

Bailey, J. A., (1982). *Phytoalexins*; Blackie: Glasgow and London, Chapter 9.

Bayındırlı, L., (1989). Mathematical analysis of apple juice filtration and apple juice concentrate production by freeze concentration, *PhD Thesis*.

Bayındırlı, L., (1992). Mathematical Analysis of Variation of Density and Viscosity of Apple Juice With Temperature and Concentration. *J. of Food Proc. and Pres.*, 16: 23-28.

Bayındırlı, L., (1993). Density and viscosity of grape juice as a function of concentration and temperature. *Journal of Food Processing Preservation*, 17:147-151.

Bayındırlı, L., & Erşan, C., (1998). Mathematical Modeling of Variation of Density and Viscosity of Clear Fruit Juices With Temperature and Concentration, *Gıda Teknolojisi*, 4, 66-69.

Bayındırlı, L., Özilgen, M. & Ungan, S., (1989). Modeling of Apple Juice Filtrations, *J. of Food Sci.*, 54,4, 1003-1006.

Belguendouz, L., Fremont, L., & Linard, A. (1997). Resveratrol inhibits metal ion-dependent and independent peroxidation of porcine lowdensity lipoproteins. *Biochemical Pharmacology*, *53*, 1347–1355.

Bhat, K.P.L.; Kosmeder, J.W.; & Pezzuto, J.M., (2001). Biological effects of resveratrol. *Antioxid. Redox Signaling*, 3, 1041–1064.

Bourne, M. C., (1982). Principles of objective texture measurement. In: Stewart, G. F., Schweigert, B. S., Hawthorn, J. (eds) *Food texture and viscosity: concept and measurement*. Academic, New York, pp 45–117.

Brennan, J.G., Butters, J.R., Cowell, N.D. & Lilley, A.E.V., (1990). *Food engineering operations*. (third ed.), Elsevier Applied Science, London

Burda, S., & Oleszek, W., (2001). Antioxidant and antiradical activities of flavonoids. *Journal of Agricultural and Food Chemistry*, *49*, 2774-2779.

Cacace, J.E., & Mazza, G., (2002). Extraction of anthocyanins and other phenolics from black currants with sulfured water. *Journal of Agricultural and Food Chemistry,* 50, 5939-5946.

Cacace, J.E., & Mazza, G., (2003a). Optimization of extraction of anthocyanins from black currants with aqueous ethanol. *Journal of Food Science,* 68, 240-248.

Cacace, J. E., & Mazza, G., (2003b). Mass transfer process during extraction of phenolic compounds from milled berries. *J. Food Eng.*, *59*, 379-389.

Cacace, J. E., & Mazza, G., (2006). Pressurized low polarity water extraction of lignans from whole flaxseed. *J. Food Eng.*, *77*, 1087-1095.

Cao, G., Sofic, E., & Prior, R. L., (1997). Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationship. *Free Radical Biology and Medicine*, 22, 749–760.

Carabias-Martínez, R., Rodríguez-Gonzalo, E., Revilla-Ruiz, P., & Hernández-Méndez, J., (2005). Pressurized liquid extraction in the analysis of food and biological samples. *J. of Chromatography A*, 1089, 1-17.

Caruso, F., Tanski, J., Villegas-Estrada, A., & Rossi, M., (2004). Structural Basis for Antioxidant Activity of trans-Resveratrol: Ab Initio Calculations and Crystal and Molecular Structure. *Journal of Agricultural and Food Chemistry,* 52, 7279-7285

Castelluccio, C., Paganga, G., Melikian, N., Bolwell, G. P., Pridham, J., Sampson, J., & Rice-Evans, C., (1995). Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Letters*, *368*, 188-192.

Cepeda, E. & Villarán, M.C., (1999). Density and viscosity of Malus floribunda juice as a function of concentration and temperature. *Journal of Food Engineering*, 41: 103-107.

Cepeda, E., Villaran, M. C., & Ibarz, A., (1998). Rheological properties of cloudy and clarified juice of Malus floribunda as a function of concentration and temperature, *Journal of Texture Studies*, Submitted.

Choi, M. P. K., Chan, K. K. C., Leung, H. W., & Huie, C. W., (2003). Pressurized liquid extraction of active ingredients (ginsenosides) from medicinal plants using non-ionic surfactant solutions. *Journal of Chromatography A*, 983, 153–162.

Constenla, D.T., Lozano, J.E. & Crapiste, G.H., (1989). Thermophysical properties of clarified apple juice as a function of concentration and temperature. *Journal of Food Science*, 4(3):663-668.

Counet, C., Callemien, D., & Collin, S., (2006). Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. *Food Chemistry*, 98, 649-657.

Darcy, H. P. G., (1856). Les Fontaines Publiques de la Ville de Dijon, Dalamont.

De La Garza, F., & Boulton, R., (1984). The modeling of Wine Filtrations. *Am. J. Enol. Vitic*. 35, 189-195.

de Moura, S.C.S.R., Germer, S.P.M., Jardim, D.C.P. & Sadahira, M.S., (1998). Thermophysical properties of tropical fruit juices. *Brazilian Journal of Food Technology*, 1(1–2): 70–76.

Dong, Z., (2003). Molecular mechanism of the chemopreventive effect of resveratrol. *Mutat. Res., 523/524*, 145-150.

Fernandez DP, Goodwin ARH, Lemmon EW, Levelt-Sengers JMH, & Williams RC., (1997). A formulation for the static permittivity of water and steam at temperatures from 238 K to 873 K at pressures up to 1200 MPa, including derivatives and Debye-Hückel coefficients. *J. Phys. Chem Ref Data*, 26, 1125–66.

Franco, D., Sineiro, J., Pinelo, M., & Nuñez M.J., (2007). Ethanolic extraction of *Rosa rubiginosa* soluble substances: Oil solubility equilibra and kinetic studies. *Journal of Food Engineering*, 79, 150-157.

Frank, T.C., Downey, J.R., & Gupta, S.K., (1999). Quickly screen solvent for organic solids. *Chemical Engineering Progress,* (Dec), 41-46.

Frémont, L., (2000). Biological effects of resveratrol. *Life Sciences,* 66 (8), 663-673.

Fukumoto, L. R., & Mazza, G., (2000). Assessing antioxidant and prooxidant activity of phenolic compounds. *Journal of Agricultural and Food Chemistry*, *48*, 3597-3604.

Gertenbach, D.D., (2002). Solid-liquid extraction technologies for manufacturing nutraceuticals. In *Functional foods: biochemical and processing aspects*; Shi, J., Mazza, G., Le Maguer, M., Eds.; Crc Press: New York, Vol. 2, 331-366.

Gonzalez-Rodriguez, J., Perez-Juan, P., & Luque de Castro, M. D., (2003). Superheated liquids for extraction of solid residues from winemaking processes. *Analytical and Bioanalytical Chemistry,* 377, 1190–1195.

Gratao, A. C. A., Junior, V. S., Polizelli, M.A., & Telis-Romero, J., (2005). Thermal properties of passion fruit juice as affected by temperature and water content. *Journal of Food Process Engineering*, 27, 413-431.

Halliwell, B., & Gutteridge, J. M. C., (2000). *Free Radicals in Biology and Medicine*. Oxford: Oxford University Press.

Herrero, M., Alvarez, P. J., Senorans, F. J., Cifuentes, A., & Ibanez, E., (2005). Optimization of accelerated solvent extraction of antoxidants from *Spirulina platensis microalga. Food Chem.*, *93*, 417-423.

Herrero, M., Cifuentes, A., & Ibanez, E., (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae a review. *Food Chemistry,* 98, 136-148.

Ho, C. H. L., Cacace, J. E., & Mazza, G., (2008). Mass transfer during pressurized low polarity water extraction of lignans from flaxseed meal. *Journal of Food Engineering*, 89, 64-71.

Holdsworth, S.D., (1993). Rheological models used for the prediction of the flow properties of food products: a literature review. Transactions of Institute of Chemical Engineering, Part C, *Food and Bioproducts Processing*, 71c: 139–179.

Howard, L., & Pandjaitan, N., (2008). Pressurized liquid extraction of flavonoids from spinach. *Journal of Food Science*, 73, 151-157.

Hwang, M. P. & Hayakawa, K. I., (1979). A specific heat calorimeter for foods. *J. Food Sci*., 44, 435-438, 448.

Jean-Denis, J.B., Pezet, R., & Tabacchi, R., (2006). Rapid analysis of stilbenes and derivatives from downy mildew-infected grapevine leaves by liquid chromatography-atmospheric pressure photoionisation mass spectrometry. *Journal of Chromatography A*, 1112, 263-268.

Jeandet, P., Céline Breuil, A., Adrian, M., Weston, L. A., Debord, S., Meunier, P., Maume, G., & Bessis, R., (1997). HPLC analysis of grapevine phytoalexins coupling photodiode array detection and fluorometry. *Analytical Chemistry*, 69 (24), 5172-5177.

Jerkovic, V. & Collin, S., (2007). Occurrence of resveratrol and piceid in American and Europe hop cones. *J.Agri. Food Chem.*, 55, 8754-8758.

Jowitt, R. J., Escher, F., Hallsrom, B., Meffert, H., Spiess, W. & Vos, G. (1983). *Physical Properties of Foods.* Applied Science Publishers, London.

Ju, Z.Y., & Howard, L.R., (2003). Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. *J. Agric. Food Chem.,* 51*,* 5207-5213.

Ju, Z.Y., & Howard, L.R., (2005). Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin. *J. Food Science,* 70, 270-276.

Kähkönen, M. P., Hopia, A. I., & Heinonen, M., (2001). Berry phenolics and their antioxidant activity. *Journal of Agricultural and Food Chemistry*, *49*, 4076-4082.

Kawamura, F., Kikuchi, Y., Ohira, T., & Yatagai, M., (1999). Accelerated solvent extraction of paclitaxel and related compounds from the bark of *Taxus cuspidata*. *Journal of Nattural Products*, *62*, 244-247.

Ketsawatsakul, U., Whiteman, M., & Halliwell, B., (2000). A reevaluation of the peroxynitrite scavenging activity of some dietary phenolics. *Biochem. Biophys. Res. Commun.,* 279, 692–699.

Kim, J.W., & Mazza, G., (2006). Optimization of extraction of phenolic compounds from flax shives by pressurized low-polarity water. *Journal of Agricultural and Food Chemistry,* 54, 7575-7584.

Kim, J.W., & Mazza, G., (2007). Mass transfer during pressurized low-polarity water extraction of phenolics and carbohydrates from flax shives. *Ind. Eng. Chem. Res.,* 46, 7221-7230.

King, R. E., Bomser, J. A. & Min, D. B., (2006). Bioactivity of resveratrol. *Comprehensive Food Science and Food Safety,* 5, 65-70.

Kinsella, J. E., Frankel, E., German, B., & Kanner, J., (1993). Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Food Technol.*, 47, 85-89.

Krokida, M.K., Maroulis, Z.B., & Saravacos, G.D., (2001) Rheological properties of fluid fruit and vegetable puree products: compilation of literature data. *International Journal of Food Properties*, 4(2): 179–200.

Lamb, J., (1976). Influence of water on the thermal properties of foods, *Chem. Ind.*, 24, 1046-1048.

Lamuela-Raventós, R. M., & Waterhouse, A.L., (1993). Occurrence of resveratrol in selected California wines by a new HPLC method. *Journal of Agricultural and Food Chemistry,* 41 (4), 521–3.

Langcake, P., (1981). Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, ε-viniferin, α-viniferin and pterostilbene. *Physiol Plant Pathol*, 18, 213-226.

Lau, A.K., (1991). Evaporators, in *Encyclopedia of foodscience and technology*, Hui, Y.H. (ed). (Wiley and Sons Press, New York, USA), pp 751–765.

Lewis, M. J., (1987). *Physical Properties of Foods and Food Processing Materials*. Ellis Horwood, London.

Lewis, M. J., (1996). *Physical Properties of Foods and Food Processing Materials*. Woodhead Publishing Limited, Cambridge.

Li, X., Wu, B., Wang, L., & Li, S., (2006). Extractable amounts of *trans*-resveratrol in seed and berry skin in *vitis* evaluated at the germplasm level. *Journal of Agricultural and Food Chemistry*, 54, 8804-8811.

Lien, E. J., Ren, S., Bui, H., & Wang, R., (1999). Quantitative structure–activity relationship analysis of phenolic antioxidants. *Free Radical Biology and Medicine*, 26, 285–294.

Liyana-Pathirana C., & Shahidi, F., (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry*, 93, 47-56.

Lo, H. H., Chung, J. G., (1999). The effects of plant phenolics, caffeic acid, chlorogenic acid and ferulic acid on arylamine*N*-acetyltransferase activities in human gastrointestinal microflora. *Anticancer Res.,* 19, 133–139.

Lou, X., Janssen, H., & Cramers, C. A., (1997). Parameters affecting the accelerated solvent extraction of polymeric samples. *Analytical Chemistry*, *69*, 1598-1603.

Lucas-Abellán, C., Mercaderros, M. T., Zafrilla, M. P., Fortea, M. I., Gabaldón, J. A., & Núñez-Delicado, E., (2008). Orac-Fluorescein assay to determine

the oxygen radical absorbance capacity of resveratrol complexed in cyclodextrins. *Journal of Agricultural and Food Chemistry, 56,* 2254–2259

Lyons, M. M., Yu, C., Toma, R. B., Cho, S. Y., Reiboldt, W., Lee, J., & van Bremen, R. B., (2003). Resveratrol in raw and baked blueberries and bilberries. *J. Agric. Food Chem*., *51*, 5867- 5870.

Mackay, K.M., & Mackay, R.A., (1981). Solution chemistry. In *Introduction to modern inorganic chemistry*, Third edition; International textbook: London, U.K., 86-103.

Maga, J.A., (1987). The flavor chemistry of wood smoke. *Food Rev. Internat.*, 3, 139-183.

Magerramov, M.A., Abdulagatov, A.I., Azizov, N.D. & Abdulagatov, I.M., (2007). Effect of temperature, concentration, and pressure on the viscosity of pomegranate and pear juice concentrates. *Journal of Food Engineering*, 80: 476–489.

Makris, D.P., Boskou, G., Nikolaos K., & Andrikopoulos, N.K., (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. *Journal of Food Composition and Analysis,* 20, 125–132.

Mazzoleni, V., Caldentey, P., & Silva, A., (1998). Phenolic compounds in cork used for production of wine stoppers as affected by storage and boiling of cork slabs. *American Journal of Enology and Viticulture,* 49, 6-10.

Merken, H.M., & Beecher, G.R. (2000). Measurement of food flavonoids by high-performance liquid chromatography: a review, *J. Agric. Food Chem.,* 48, 577-599.

Miles, C. A., Van Beek, G. & Veerkamp, C. H., (1983). Calculation of thermophysical properties of foods. In: R. Jowitt et al. (Eds.), *Physical Properties of Foods*. Applied Science Publishers, London, pp. 269-312.

Mooney, M., (1951). The Viscosity of Concentrated Suspensions of Spherical Particles, *J. Colloid. Sci*., 6, 162.

Morales-Muñoz, S., Luque-García, J.L., & Luque de Castro, M.D., (2006). Pure and modified water assisted by auxiliary energies: An environmental friendly extractant for sample preparation. *Analytica Chimica Acta*, 557, 278–286.

Mori, H., Kawabata, K., Yoshimi, N., Tanaka, T., Murakami, T., Okada, T., & Murai, H., (1999). Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. *Anticancer Res.*, 19, 3775–3778.

Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus*, and *Ribes*. *Journal of Agricultural and Food Chemistry*, *50*, 519-525.

Muramatsu, Y., Tagawa, A., & Kasai, T., (2005). Thermal conductivity of several liquid foods. *Food Sci. Technol. Res.,* 11, 3, 288-294.

Myers, R. H., & Montgomery, D. C., (2002). *Response surface methodology: Process and product optimization using designed experiments*. New York: Wiley.

Naczk, M., & Shahidi, F., (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, 41, 1523–1542.

Nagy, S., Chen, C. S., & Shaw, P. E., (1993). *Fruit Juice Processing Technology*. Agscience, Auburndale, Florida.

Nindo, C.I., Tang, J., Powers, J.R. & Bolland, K., (2004). Energy consumption during Refractance Window evaporation of selected berry juices. *International Journal of Energy Research*, 28(12): 1089–1100.

Nindo, C.I., Tang, J., Powers, J.R. & Singh, P., (2005). Viscosity of blueberry and raspberry juices for processing applications. *Journal of Food Engineering,* 69: 343–350.

Ong, E. S., Cheong, J. S. H., & Goh, D., (2006). Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials. *Journal of Chromatography A*, 1112, 92–102.

Orr, C., (1977). *Filtration, Principles and Practices, Part 1*, Marcel Dekker, New York.

Ou, B., Hampsch-Woodill, M., & Prior, R. L., (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry,* 49, 4619-4626.

Ou, S., & Kwok, K., (2004). Ferulic acid: pharmaceutical functions, preparation and applications in foods. *Journal of the Science of Food and Agriculture*, 84, 1261-1269.

Özilgen, M., (1998). *Food Process Modeling and Control*, Gordon and Breach Science Publishers, Amsterdam.

Peleg, Y., & Brown, R.C., (1976). Methods of Evaluating the Filterability of Wine and Similar Fluids, *J. Food Sci.*, 41, 805-808.

Pellegrini, N., Re, R., Yang, M., & Rice-Evans, C., (1999). Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. *Methods in Enzymology,* 299, 379-389.

Perry, R.H., Greene, D.W., & Maloney, J.O., (1997). Section 18. In *Perry's Chemical Engineers' Handbook*; McGraw Hill: New York, 5-17.

Pezet, R., Gindro, K., Viret, O., & Spring, J. L., (2004). Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. *Physiol. Mol. Plant Pathol., 65*, 297-303.

Piñeiro, Z., Palma, M., & Barroso, C.G., (2004). Determination of catechins by means of extraction with pressurized liquids. *Journal of Chromatography A*, 1026, 19-23.

Piñeiro, Z., Palma, M., & Barroso, C.G., (2006). Determination of trans-resveratrol in grapes by pressurised liquid extraction and fast high-performance liquid chromatography. *Journal of Chromatography A*, 1110, 61–65.

Pinelo, M., Sineiro, J., & Núñez, M.J., (2006). Mass transfer during continuous solid–liquid extraction of antioxidants from grape byproducts. *Journal of Food Engineering,* 77, 57-63.

Piver, B., Berthou, F., Dreano, Y., & Lucas, D., (2003). Differential inhibition of human cytochrome P450 enzymes by ε-viniferin, the dimer of resveratrol: comparison with resveratrol and polyphenols from alcoholized beverages. *Life Sciences.,* 73, 1199–1213.

Plato, F., (1900). *Kaiserliche normaleichungs konmission wissenschafiliche abhandlungen*, *2*, 153.

Poyrazoğlu, E., Gökmen, V. & Artık, N., (2002). Organic acids and phenolic compounds in pomegranates. *J. of Food Composition and Analysis*, 15, 567-575.

Privat, C., Telo, J. P., Bernardes-Genisson, V., Vieira, A., Souchard, J. P., & Nepveu, F., (2002). Antioxidant properties of *ε-viniferin* as compared to stilbene derivatives in aqueous and nonaqueous media. *Journal of Agricultural and Food Chemistry,* 50, 1213–1217.

Püssa, T., Floren, J., Kuldkepp, P., & Raal, A., (2006). Survey of grapevine vitis vinifera stem polyphenols by liquid chromatography-diode array detection-tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 54, 7488-7494.

Ramos, A. M. & Ibarz, A., (1998). Density of juice and fruit puree as a function of soluble solids content and temperature. *Journal of Food Engineering*, 35: 57-63.

Rao, M. A., (1999). Rheological behavior of processed fluid and semisolid foods, in *Rheology of fluid and semisolid foods: principles and applications*, Rao, M. A. (ed) (Aspen Pub. Inc. Press, Gaithersburg, Maryland, USA), pp 219–255.

Rayne, S., Karacabey, E., & Mazza, G., (2008). Grape cane waste as a source of *trans*-resveratrol and *trans*-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications. *Industrial Crops and Products*, 27, 335-340.

Rice-Evans, C. A., Miller, N. J., & Paganga, G., (1996). Structure-antioxidant activity relationships of flavonoids and phenolics acids. *Free Radical Biology and Medicine*, *20*, 933-956.

Rice-Evans, C. A., Miller, N. J., & Paganga, G., (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science,* 2, 152-159.

Richter, B. E., Jones, B. A., Ezzell, J. L., & Porter, N. L., (1996). Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, 68, 1033-1039.

Riedel, L., (1951). The refrigerating effect required to freeze fruits and vegetables, *Refrig. Eng*., 59, 670-673.

Roemer, K., & Mahyar-Roemer, M., (2002). The basis for the chemopreventive action of resveratrol. *Drugs Today,* 38, 571–580.

Romero-Pérez, A.I., Lamuela-Raventós, R.M., Andrés-Lacueva, C. & Carmen de la Torre-Boronat, M., (2001). Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food Chemistry*, 49, 210-215.

Sakai, S., Kawamata, H., Kogure, T., Mantani, N., Terasawa, K., Umatake M., & Ochiai, H., (1999). Inhibitory effect of ferulic acid and isoferulic acid on the production of macrophage inflammatory protein-2 in response to respiratory syncytial virus infection in RAW264.7 cells. *Mediators Inflammation,* 8, 173–175.

Saravacos, G. D. & Kostaropoulos, A. E., (1995). Transport properties in processing of fruits and vegetables, *Food Technol.,* 49, 99-109.

Schwartzberg, H. G., (1975). Mathematical analysis of solubilization. Kinetics and diffusion in foods. *Journal of Food Science*, 40, 211–213.

Schwartzberg, H.G., & Chao, R.Y., (1982). Solute diffusivities in leaching processes. *Food Technology*, 36, 73-86.

Shahidi, F., & Wanasundara, P. K. J. P. D., (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, *32*, 67-103.

Shamsudin, R., Mohamed, I.O. & Yaman N.K.M., (2005). Thermophysical properties of Thai seedless guava juice as affected by temperature and concentration. Journal of Food Engineering 66: 395–399.

Sineiro, J., Dominguez, H., Nuñez M.J., & Lema, J.M., (1996). Ethanol extraction of polyphenols in an immersion extractor. Effect of pulsing flow. *Journal of American Oil Chemistry Society,* 73, 1121-1125.

Sperry, D. R., (1917). The principles of Filtration, *Chem. Met. Eng*., 17: 161.

Spiro, M., & Selwood, R. M., (1984). The kinetics and mechanicsm of caffeine infusion from coffee: the effect of the particle size. *Journal of the Science of Food and Agriculture*, 35, 915–924.

Spiro, M., & Siddique, S., (1981). Kinetics and equilibria of tea infusion. Analysis and partition constants of theaflavins, thearubigins, and caffeine in Koonsong Broken Pekoe. *J. of the Science of Food and Agriculture*, 32, 1027–1032.

Sweat, V.E., (1995). Thermal properties of foods, in *Engineering properties of foods* (2nd edn), Rao, M.A. and Rizvi, S.S.H. (eds) (Marcel Dekker, Inc., New York, USA), pp 99-138.

Şahin, S., (1992). Mathematical analysis of sour cherry juice filtration, *Master Thesis.*

Şahin, S. & Bayındırlı, L., (1993). The Effect of Depectinization and Clarification on the Filtration of Sour Cherry Juice, *J. of Food Eng*., 19, 237-245.

Tabart, J., Kevers, C., Sipel, A., Pincemail, J., Defraigne, J. O., & Dommes, J., (2007). Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. *Food Chemistry,* 105, 1268-1275.

Telis-Romero, J., Telis, V. R. N., Gabas, A. L. & Yamashitah, F., (1998). Thermophysical Properties of Brazilian Orange Juice as Affected by Temperature and Water Content, *Journal of Food Engineering,* 38, 27-40.

Velioglu, Y.S., Mazza, G., Gao, L., & Oomah, B.D., (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.,* 46, 4113-4117.

Wolter, F., & Stein, J., (2002). Biological activities of resveratrol and its analogs. *Drugs of the Future*, 27, 949-959.

Yang, Y., Belghazi, M., Lagadec, A., Miller, D. J., & Hawthorne, S. B., (1998). Elution of organic solutes from different polarity solvents using subcritical water. *J. Chromatography A*, *810*, 149-159.

Yu, J., Ahmedna, M., & Goktepe, I., (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chemistry*, 90, 199–206.

Zainal, B.S., Abdul Rahman, R., Ariff, A.B., Saari, B.N. & Asbi, B.A., (2000). Effects of temperature on the physical properties of pink guava juice at two different concentrations. *Journal of Food Engineering*, 43:55-59.

Zuritz, C.A., Muñoz Puntes, E., Mathey, H.H., Pérez, E.H., Gascón, A., Rubio, L.A., Carullo, C.A., Chernikoff, R.E. & Cabeza, M.S., (2005). Density, viscosity and coefficient of thermal expansion of clarified grape juice at different soluble solid concentrations and temperatures. *Journal of Food Engineering*, 71: 143–149.

APPENDIX A

Filtration Conditions

Table A.1 Experimental conditions of clarified grape juice filtration and calculated resistance parameters

Experiment Conditions	
Pressure (atm.gauge) (Bar)	0.65
Filter aid material	-
Amount of precoating (g)	-
Amount of filter aid (g)	-
Temperature (^{o}C)	21
Brix of grape juice	28.6
pH of grape juice	3.7
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.002709
Pressure difference (Pa or N/m^2)	65000
Filtration area (m^2)	0.00332
Filter medium resistance, R_m, (1/m)	15504.08
Exponential fouling coefficient, β, (1/m^3)	-0.00238

CLF, clarified grape juice.

Filtration Process					
filtrate (mL)	time (s)	dt/dV	V (m^3)	R_{Total} (m^{-1})	ln(R_{Total})
0					
100	18	0.18	50	14340.46	9.57084
200	30	0.12	150	9560.308	9.165375
300	41	0.11	250	8763.616	9.078364
400	51	0.1	350	7966.923	8.983054
500	57	0.06	450	4780.154	8.472228

R_{Total}, total resistance.

Table A.2 Experimental conditions of non-clarified grape juice filtration and calculated resistance parameters

Experiment Conditions	
Pressure (atm. gauge) (Bar)	0.65
Filter aid material	-
Amount of precoating (g)	-
Amount of filter aid (g)	-
Temperature (^{o}C)	21
Brix of grape juice	27.2
pH of grape juice	3.7
Clarification condition	Non-CLF
Viscosity of grape juice (Pa.s)	0.002436
Pressure difference (Pa or N/m^2)	65000
Filtration area (m^2)	0.00332
Filter medium resistance, R_m, (1/m)	835443.4
Exponential fouling coefficient, β (1/m^3)	0.03151

Non-CLF, non-clarified grape juice.

Filtration Process					
filtrate (mL)	time (s)	dt/dV	V (m^3)	R_{Total} (m^{-1})	ln(R_{Total})
0	0				
31	494	15.93548	15.5	1411538	14.16019
36	600	21.2	33.5	1877860	14.44564
49	1200	46.15385	42.5	4088229	15.22362
60	1800	54.54545	54.5	4831544	15.39068
69	2400	66.66667	64.5	5905220	15.59135
81	3000	50	75	4428915	15.30367
90	3600	66.66667	85.5	5905220	15.59135

R_{Total}, total resistance.

Table A.3 Experimental conditions of grape juice filtration to evaluate addition of precoating material

Experiment Conditions	
Amount of precoating (g/cm^2 filter area)	0.05, 0.1,0.25
Pressure (atm. gauge) (Bar)	0.65
Filter aid and precoating material	D.E.
Amount of filter aid (g/mL fruit juice)	0.005
Temperature (^{o}C)	21
Brix of grape juice	27.6
pH of grape juice	3.9
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.00251
Pressure difference (Pa or N/m^2)	65000
filtration area (m^2)	0.00332

D.E., Diatomaceous earth. CLF, clarified grape juice.

Table A.4 Experimental conditions of grape juice filtration to evaluate addition of filter aid material

Experiment Conditions	
Amount of filter aid (g/mL fruit juice)	0.002, 0.005, 0.01
Pressure (atm. gauge) (Bar)	0.65
Filter aid and precoating material	D.E.
Amount of precoating (g/cm^2 filter area)	0.1
Temperature (^{o}C)	21
Brix of grape juice	27.0
pH of grape juice	3.8
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.002406
Pressure difference (Pa or N/m^2)	65000
Filtration area (m^2)	0.00332

D.E., Diatomaceous earth. CLF, clarified grape juice.

Table A.5 Experimental conditions of grape juice filtration to evaluate effect of temperature

Experiment Conditions	
Temperature (^{o}C)	8, 21, 34
Pressure (atm. gauge) (Bar)	0.65
Filter aid and precoating material	D.E.
Amount of precoating (g/cm^2 filter area)	0.1
Amount of filter aid (g/mL fruit juice)	0.005
Brix of grape juice	27.4
pH of grape juice	3.6
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.002596
Pressure difference (Pa or N/m^2)	65000
Filtration area (m^2)	0.00332

D.E., Diatomaceous earth. CLF, clarified grape juice.

Table A.6 Experimental conditions of grape juice filtration to evaluate effect of pressure

Experiment Conditions	
Pressure (atm. gauge) (Bar)	0.3, 0.65, 1, 1.5, 2
Filter aid and precoating material	D.E.
Amount of precoating (g/cm^2 filter area)	0.1
Amount of filter aid (g/mL fruit juice)	0.005
Temperature (^{o}C)	21
Brix of grape juice	26.8
pH of grape juice	3.7
Clarification condition	CLF
Viscosity of grape juice (Pa.s)	0.002388
Filtration area (m^2)	0.00332

D.E., Diatomaceous earth. CLF, clarified grape juice.

Table A.7 Experimental conditions of grape juice filtration to evaluate effect of soluble solid content

Experiment Conditions		
Brix of grape juice	28.6	16.0
Pressure (atm. gauge) (Bar)	0.65	0.65
Filter aid and precoating material	-	-
Amount of precoating (g/cm^2 filter area)	-	-
Amount of filter aid (g/mL fruit juice)	-	-
Temperature (oC)	21	21
pH of grape juice	3.66	3.6
clarification condition	CLF	CLF
Viscosity of grape juice (Pa.s)	0.002709	0.001297
pressure difference (Pa or N/m^2)	65000	65000
filtration area (m^2)	0.00332	0.00332

CLF, clarified grape juice.

APPENDIX B

ANOVA Tables of Optimization Studies

Table B1 Statistical Analysis of optimization of total phenolic extraction

The analysis was done using uncoded units
Estimated Regression Coefficients for Total Phenolic

Term	Coef	SE Coef	T	P
Constant	-7.2617	1.0597	-6.8520	0.0000
Temperature	0.1416	0.0161	8.7790	0.0000
Solvent:Solid	0.0008	0.0045	0.1850	0.8570
Ethanol Conc.	0.2826	0.0257	10.9980	0.0000
Ethanol Conc.*Ethanol Conc.	-0.0015	0.0002	-8.2940	0.0000
Temperature*Ethanol Conc.	-0.0014	0.0003	-5.0990	0.0000

$R^2 = 0.968$ $R^2_{adj} = 0.955$

Analysis of Variance for Total Phenolic

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	40.277	40.277	8.055	72.910	0.000
Linear	3	29.805	14.962	4.987	45.140	0.000
Square	1	7.600	7.600	7.600	68.780	0.000
Interaction	1	2.873	2.873	2.873	26.000	0.000
Residual Error	12	1.326	1.326	0.110		
Lack-of-Fit	9	1.295	1.295	0.144	14.020	0.026
Pure Error	3	0.031	0.031	0.010		
Total	17	41.603				

Table B2 Statistical Analysis of optimization of *trans*-resveratrol extraction

The analysis was done using uncoded units
Estimated Regression Coefficients for Resveratrol

Term	Coef	SE Coef	T	P
Constant	-3.3968	0.7336	-4.6300	0.0010
Temperature	0.0753	0.0112	6.7390	0.0000
Solvent:Solid	0.0001	0.0031	0.0460	0.9640
Ethanol Conc.	0.1113	0.0178	6.2550	0.0000
Ethanol Conc.*Ethanol Conc.	-0.0004	0.0001	-3.1770	0.0080
Temperature*Ethanol Conc.	-0.0008	0.0002	-4.1670	0.0010

R^2 = 0.944 R^2_{adj} = 0.921
Analysis of Variance for Resveratrol

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	10.790	10.790	2.158	40.750	0.000
Linear	3	9.336	2.995	0.998	18.850	0.000
Square	1	0.535	0.535	0.535	10.100	0.008
Interaction	1	0.919	0.919	0.919	17.360	0.001
Residual Error	12	0.635	0.635	0.053		
Lack-of-Fit	9	0.624	0.624	0.069	18.360	0.018
Pure Error	3	0.011	0.011	0.004		
Total	17	11.425				

Table B3 Statistical Analysis of optimization of *trans*-ε-viniferin extraction

The analysis was done using uncoded units
Estimated Regression Coefficients for Viniferin

Term	Coef	SE Coef	T	P
Constant	-1.4052	0.3313	-4.2410	0.0010
Temperature	0.0422	0.0087	4.8330	0.0000
Ethanol Conc.	0.0496	0.0083	5.9530	0.0000
Temperature*Temperature	-0.0003	0.0001	-2.9700	0.0110
Ethanol Conc.*Ethanol Conc.	-0.0004	0.0001	-5.1700	0.0000

R^2 = 0.922 R^2_{adj} = 0.897
Analysis of Variance for Viniferin

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	2.360	2.360	0.590	38.150	0.000
Linear	2	1.878	0.783	0.391	25.310	0.000
Square	2	0.482	0.482	0.241	15.590	0.000
Residual Error	13	0.201	0.201	0.015		
Lack-of-Fit	4	0.134	0.134	0.033	4.460	0.029
Pure Error	9	0.067	0.067	0.007		
Total	17	2.561				

Table B4 Statistical Analysis of optimization of ferulic acid extraction

The analysis was done using uncoded units
Estimated Regression Coefficients for Ferulic acid

Term	Coef	SE Coef	T	P
Constant	0.76642	0.30295	2.53000	0.028
Temperature	-0.00146	0.00697	-0.20900	0.838
Solvent:Solid	-0.00002	0.00112	-0.02100	0.983
Ethanol Conc.	0.01734	0.00646	2.68400	0.021
Temperature*Temperature	-0.00025	0.00006	-4.44000	0.001
Ethanol Conc.*Ethanol Conc.	-0.00031	0.00005	-6.55200	0.000
Temperature*Ethanol Conc.	0.00025	0.00007	3.73900	0.003

R^2 = 0.953 R^2_{adj} = 0.928
Analysis of Variance for Ferulic acid

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	6	1.527	1.527	0.255	37.24	0.000
Linear	3	1.061	0.063	0.021	3.07	0.073
Square	2	0.371	0.371	0.185	27.10	0.000
Interaction	1	0.096	0.096	0.096	13.98	0.003
Residual Error	11	0.075	0.075	0.007		
Lack-of-Fit	8	0.059	0.059	0.007	1.42	0.424
Pure Error	3	0.016	0.016	0.005		
Total	17	1.603				

Table B5 Statistical Analysis of optimization of effective diffusivity of *trans*-resveratrol extraction

The analysis was done using uncoded units
Estimated Regression Coefficients for Effective diffusivity of resveratrol

Term	Coef	SE Coef	T	P
Constant	-11.4029	4.1491	-2.7480	0.0170
Temperature	0.3060	0.0258	11.8570	0.0000
Solvent:Solid	-0.0114	0.0259	-0.4420	0.6660
Ethanol Conc.	0.4042	0.1260	3.2090	0.0070
Ethanol Conc*Ethanol Conc.	-0.0038	0.0011	-3.5180	0.0040

R^2 = 0.923 R^2_{adj} = 0.899
Analysis of Variance for Effective diffusivity of resveratrol

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	563.321	563.321	140.830	38.73	0.000
Linear	3	518.324	549.363	183.121	50.36	0.000
Square	1	44.997	44.997	44.997	12.38	0.004
Residual Error	13	47.269	47.269	3.636		
Lack-of-Fit	10	46.394	46.394	4.639	15.91	0.022
Pure Error	3	0.875	0.875	0.292		
Total	17	610.590				

Table B6 Statistical Analysis of optimization of TEAC

The analysis was done using uncoded units
Estimated Regression Coefficients for TEAC

Term	Coef	SE Coef	T	P
Constant	-154.731	38.2905	-4.041	0.002
Temperature	2.360	0.8803	2.680	0.021
Solvent:Solid	0.245	0.1419	1.729	0.112
Ethanol Conc.	5.927	0.8166	7.258	0.000
Temperature*Temperature	0.020	0.0072	2.843	0.016
Ethanol Conc.*Ethanol Conc.	-0.030	0.0059	-5.108	0.000
Temperature*Ethanol Conc.	-0.041	0.0084	-4.939	0.000

$R^2 = 0.962$ $R^2_{adj} = 0.941$

Analysis of Variance for TEAC

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	6	29981.8	29981.8	4996.97	45.76	0.000
Linear	3	22905.3	6089.1	2029.72	18.59	0.000
Square	2	4412.6	4412.6	2206.28	20.20	0.000
Interaction	1	2664.0	2664.0	2663.99	24.39	0.000
Residual Error	11	1201.3	1201.3	109.21		
Lack-of-Fit	8	1092.7	1092.7	136.59	3.77	0.151
Pure Error	3	108.6	108.6	36.20		
Total	17	31183.1				

Table B7 Statistical Analysis of optimization of ORAC

The analysis was done using uncoded units
Estimated Regression Coefficients for ORAC

Term	Coef	SE Coef	T	P
Constant	-1180.31	426.221	-2.769	0.017
Temperature	9.47	10.144	0.934	0.369
EthanolConc.	50.23	9.410	5.338	0.000
Temperature*Temperature	0.13	0.083	1.609	0.133
Ethanol Conc.*Ethanol Conc.	-0.27	0.068	-3.942	0.002
Temperature*Ethanol Conc.	-0.24	0.097	-2.501	0.028

$R^2 = 0.866$ $R^2_{adj} = 0.810$

Analysis of Variance for ORAC

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	5	1125752	1125752	225150	15.53	0.000
Linear	2	731280	438927	219463	15.13	0.001
Square	2	303763	303763	151881	10.47	0.002
Interaction	1	90709	90709	90709	6.26	0.028
Residual Error	12	174011	174011	14501		
Lack-of-Fit	3	89796	89796	29932	3.2	0.077
Pure Error	9	84215	84215	9357		
Total	17	1299763				

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name: Karacabey, Erkan

Nationality: Turkish (TC)

Date and Place of Birth: 24 August 1979, Ankara

Marital Status: Single

Phone: +90 312 210 57 93

Cell Phone: +90 533 604 5288

Fax: +90 312 210 2767

e-mail: erkaraca@metu.edu.tr, erkan.karacabey@gmail.com

EDUCATION

Degree	Institution	Year of Graduation
BS	Ankara University Food Engineering	2001
High School	Ankara Atatürk High School, Ankara	1996

WORK EXPERIENCE

Year	Place	Enrollment
2002-Present	METU Food Engineering	Teaching Assistant

FOREIGN LANGUAGES

Advanced English

PUBLICATIONS

A. Science Citation Index

1. E. Karacabey, G. Mazza, (2008). Optimization of solid-liquid extraction of resveratrol and other phenolics compounds from milled grape canes (Vitis vinifera). *Journal of Agricultural and Food Chemistry*, 56, 6318-6325.

2. E. Karacabey, G. Mazza, (2009). Optimisation of antioxidant activity of grape cane extracts using response surface methodology. *Food Chemistry*, (2009), doi:10.1016/j.foodchem.2009.06.029.

B. Science Citation Index-Expanded

1. S. Rayne, E. Karacabey, G. Mazza, (2008). Grape cane waste as a source of *trans*-resveratrol and *trans*-ε-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic applications. *Industrial. Crops and Products*, *27*, 335–340.

International Conference Paper

1. E. Karacabey, G. Mazza, (2008). Solid-Liquid Extraction Kinetics of *trans*-Resveratrol and *trans*-ε-Viniferin of Grape Cane Sample. 1st European Food Congress
2. E. Karacabey, L. Bayındırlı, G. Mazza, (2009). Extraction Kinetics of trans-Resveratrol and trans-ε-Viniferin of Grape Cane Sample by Pressurized Low Polarity Water. 2009 CIGR Section VI International Symposium

SCIENTIFIC REVIEWER

Plant Foods for Human Nutrition (Impact Factor: 0.885)

African Journal of Microbiology Research