Investigation Of Methods For The Extraction Of Polyphenols From Grape (*Vitis vinifera* L.) Pomace

by

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ABSTRACT

INVESTIGATION OF METHODS FOR THE EXTRACTION OF POLYPHENOLS FROM GRAPE (*VITIS VINIFERA* L.) POMACE

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Grapes (*Vitis vinifera* L.) are one of the largest food crops grown in the world, with global production of around 77.8 million tons. They are primarily used for wine production (85%) of which approximately 10 million tons give rise to solid waste "pomace". Pomace or Wine Marc consists of grape skin, seeds, and stalks and their proportion depends on the grape variety. Pomace is an excellent source of bioactive compounds such a polyphenols, flavonoids and dietary fibers, which could be further processed to yield nutraceuticals that can be used in the food, pharmaceutical and cosmetic industries. Conventional extraction methods such as heat-reflux, Soxhlet and solvent extraction are energy and time intensive and often generate lower yield. Advanced techniques including microwave-assisted extraction (MBE) and ultrasound can be used to overcome the constraints of conventional processes. The objective of this thesis is to intensify the extraction yield of bioactive components from grape pomace using advance extraction techniques including MBE and ultrasound by evaluating the effect of process parameters including type of solvent, solvent concentration, extraction time and temperature. Total polyphenol concentration was quantified using Folin-Ciocalteau method and antioxidant activity was quantified using free radical scavenging techniques including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Fe^{3+}-tripyridyltraizine (FRAP). It was observed that MBE process yielded high concentration of polyphenols in comparison to ultrasound and convention heat-reflux methods.

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CONTRIBUTIONS OF AUTHORS

The work reported in this thesis was performed by Gagan Sharma and supervised by Dr. Ashutosh Singh & Dr. Rafael Santos of the School of Engineering, University of Guelph, Guelph. Ontario. The entire research work was conducted in the Food Research Lab, School of Engineering, University of Guelph, Guelph. Ontario.

Co-authors, Prabhjot Kaur (Ph.D. Student, school of Engineering, University of Guelph), Dr. Rafael Santos (Assistant Professor, School of Engineering, University of Guelph) and Dr. Ashutosh Singh (Associate Professor, School of Engineering, University of Guelph), provided guidance and support in data analysis and in the development of the manuscripts.

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LIST OF ABBREVIATIONS

AE – ascorbic acid equivalent

DPPH – 2,2-diphenyl-1-picrylhydrazyl

EM- Electromagnetic waves

FRAP – ferric reducing antioxidant potential

GAE – gallic acid equivalent

GPE- grape pomace extract

HPLC – high performance liquid chromatography

HE – heat reflux extraction

MBE – microwave-based extraction

MAD – microwave-assisted digestion

PFE – pressurized fluid extraction

PI – Process Intensification

SFE – supercritical fluid extraction

SPM – secondary plant metabolites

TP- Total Phenols

TPC – total phenolic content

TPTZ – 2,4,6-Tri(2-pyridyl)-s-triazine

UAE – ultrasound-assisted extraction

CHAPTER 1

INTRODUCTION

Grapes (Vitis sp.) are grown in almost 74, 900 square kilometers of the world. With 10,000 different types of varieties, grapes are the sixth largest consumed fruit. Out of the total grape production of 77.8 million tons, about 85% is utilized for wine making (Hogervorst, Miljić, & Puškaš, 2017). They are also consumed as table fruit or as jams, jellies, and in the form of squashes and juices. Grapes are preferred for their high nutritional content. Besides vitamins and minerals, grapes have high amount of various triterpenoid (ursolic acid) and polyphenols including flavanols, flavonols, anthocyanins and stilbenes like resveratrol (Pintać et al., 2018a). Polyphenols are antioxidants and are known to protect body from cardiovascular diseases(Otero-Pareja, Casas, Fernández-Ponce, Mantell, & Ossa, 2015), inflammation (Manca et al., 2016), tumours (M. José Jara-Palacios et al., 2015), aging (Manca et al., 2016) and microbes (Oliveira et al., 2013). Typical polyphenol content in grape pomace ranges from

As stated earlier, grapes are consumed widely due to high value of polyphenols in it (Pintać et al., 2018a). In the process of wine making, after crushing the juice out of the grapes, 25% of the weight of grape is pomace. Pomace comprises of peels, seeds and stems of the grapes (Tournour et al., 2015). Wine Marc or grape pomace is the most prominent waste produced by the wine industry. Most of the pomace waste generated is either used in biogas plants or is subjected to landfill (Beres et al., 2017). This adds to the emission of greenhouse gases to the environment. Pomace is an excellent source of anthocyanins and flavanols (flavan-3-ol,catechins,epicatechins), thus can be used as a viable source of these bioactive compounds (Makris, Boskou, & Andrikopoulos, 2007). Hence, valorization or value addition of grape pomace waste into nutraceutical compounds using conventional and advanced extraction methods could convert this wine industry by-product into

economical and environment friendly product. Existing extraction techniques work on three steps: extraction, concentration, and separation. These techniques involve large amount of solvent, longer extraction time and high operating cost. This has resulted in shift from traditional extraction techniques to modern ones. Extractions based on microwave and ultrasonic waves though involve low solvent and time, appropriate optimization of the process parameters is needed to implement these techniques on commercial scale.

1.1 Hypothesis

The hypothesis of this research was that microwave-assisted extraction (MBE) can extract greater yield of antioxidants from grape pomace as compared to conventional extraction techniques.

1.2 Research Objective

1.2.1 Overall Objective

The overall objective of this research was to optimize the extraction of polyphenols and antioxidants from grape pomace.

1.2.2 Specific objectives

The specific objectives were:

1. Solvent selection for efficient extraction of polyphenols and antioxidants from grape pomace.
2. Process parameter optimization of advance microwave-assisted and ultrasound extraction techniques for extraction of polyphenols and antioxidants from grape pomace.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The food industries around the world focus on enhanced shelf life, nutritional and organoleptic quality of the processed food products. To achieve this, synthetic anti-oxidants such as Propyl gallate (PG) , Butylated hydroxytoluene (BHT) , Butylated hydroxyl anisole (BHA) and *tert*-Butyl hydroquinone (TBHQ) are widely used (Villamena, 2013). However, animal model studies on adverse health effects of synthetic antioxidants suggest that they increase mutagenic activities and formation of tumors (Teixeira et al., 2014). Because of these health concerns and consumer interests in food products with natural ingredients, food industries have focused their research and development in the area of finding natural substitutes to synthetic antioxidants.

There are naturally occurring antioxidants such as anthocyanins, carotenoids, flavonoids, iso-flavonoids, tannins, polyphenols and catechins (Al-juhaimi et al., 2018). Of all of these, polyphenols are the most abundant natural antioxidant present in plants and their by-products. Fresh fruits and vegetables are considered as one the most common source of these natural antioxidants with health benefits including reduced risk of chronic diseases such as cancers, heart disease and neurological disorders such as Parkinson's and Alzheimer's (Singh et al., 2011). Several polyphenols including ascorbic acid (Vitamin C), chlorogenic acid , neo-chlorogenic acid have been identified in various agro industrial by-products such as potato peels (Singh et al., 2011), peanut skin (Ballard et al., 2009)and grape seeds (Hayat et al., 2010).

Grapes (*Vitis vinifera* L.) are one of the largest food crops grown in the world, with total production of around 77.8 million tons. Of this 85% is used for wine making, which involves fermentation of grapes and conversion of sugars into ethanol. The major steps in wine making include:

De-stemming: Removal of stalks and stems, thus setting grapes free from bunches is a manual process. Partial or total removal of woody parts of grape bunches is called de-stemming (Unterkofler et al., 2020).

Crushing: Crushing helps in extracting pulp from grapes. It may be done manually or mechanically using roller presses (Zollinger et al., 2006). White wine requires grape pulp without peels whereas red wine is fermented with peels which helps in releasing the rose colour (Medina et al., 2005).

Alcoholic Fermentation: Fermentation results in conversion of grape sugars to alcohols (Ugliano et al., 2006).

Aeration and Stirring: Wine requires regular stirring because at times grape constituents being lighter form the scum and fermentation curd may settle down. Regular stir will help in making the fermentation consistent.

Pressing: In case of Red wines, pressing is done after fermentation. This helps in adding colour and flavour to the fermented mixture. Though, for white wines this step is skipped. The residue obtained from the pressing process is called as Grape Pomace (GPE) (Guerra-Rivas et al., 2017).

Grape Pomace (GPE) is the main by-product obtained during the wine making process. In certain countries, this pomace is used to make low quality wine, but in most of the cases the volume or alcoholic content of GPE determines its further processing. Constituents of GPE include:

Grape Seed: It is rich in phenolic content and comprises of extractable flavonoids, resveratrol, anthocyanins, lipids, and minerals. Essential fatty acid content of grape seed ranges between 13 to 19 %. Some nonphenolic antioxidants like beta carotene are also present (Lutterodt et al., 2011).

Peel and Stalks: Generally, stalks are present in red wine manufactured pomace as red grapes are subjected to fermentation without crushing. These stalks are rich in dietary fibers and antioxidants (M José Jara-Palacios et al., 2014).

Marc: It consists of small amount of dissolvable solute present in pomace which contains fermented buffer and acts as a starter culture for second grade pomace-based wine. It contains polyphenols and some toxic compounds (M José Jara-Palacios et al., 2014).

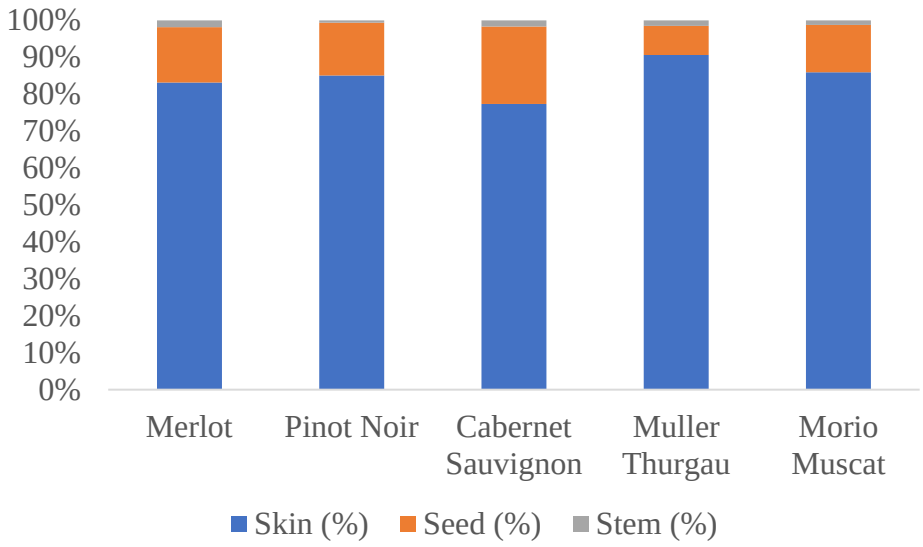

Figure 2.1 Composition of grape pomace for five grape varieties (Dwyer et al., 2014)

Currently utilization of grape pomace depends on its chemical composition, which varies with type of grapes used, wine extraction method and climatic conditions. Generally, grape pomace is utilized as animal feed, for biogas production or compost.

2.2 Characterization

Characterization of pomace waste into its various components can help in extracting and isolating important bioactive ingredients such as flavanols, catechins, epicatechin, pro-anthocyanins, malvidin acetaldehyde, quercetin gluconitrile, lupeol, oleanolic acid and β sitosterol etc. (Fontana et al., 2013). These products can contribute in production of functional derivatives, nutraceuticals, and pharma chemicals.

2.2.1 Phenolic Compounds

The classification of phenols is done on the basis of their molecular weight and chemical structure as : simple phenols (C6-C1 and C6-C3), polymeric compounds (comprises lignins, condensed and hydrolyzable tannins, lignin), flavonoids (C6-C3-C6 and oligomers), and miscellaneous phenol groups having quite distinct structures (stilbenes, betacyanines, and so on) (El Gharras, 2009).

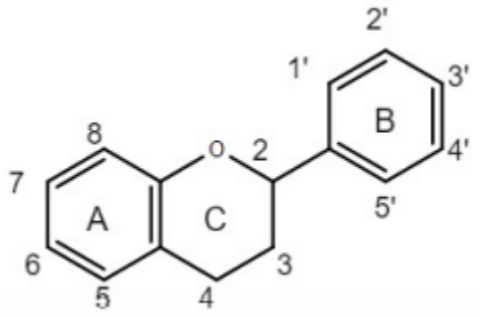

Figure 2.2 : Basic structure of flavonoid (Adopted from Routray & Orsat, 2012)

Red grape skin has more phenolic acids in comparison to the white grapes. The skin of grapes is rich in hydroxycinnamic acids (C6-C3) mainly tartarics esters such as coutaric acid and caftaric acid while seeds mostly contain protocatechic acid and gallic acid. (Kammerer et al., 2004; Teixeira et al., 2014).

Grape pomace contains flavanols and anthocyanins in abundance and all others are in minor amounts. As per the composition of anthocyanins in *Vitis vinifera* red varieties, is considered, malvidin-3-*O*-glucoside is found in abundant followed by peonidin and delphinidin-3-glucoside respectively based on the species and type of grape(Amico et al., 2008; Gonzalez-San Jose & Diez de Bethencourt, 1987; Kammerer et al., 2004; Pérez-Magariño & González-San José, 2004). The white grapes do not have anthocyanins so major phenol is flavanols in pomace of white wine. Seeds contain higher portion of flavonols about 56% to 65% in comparison to skin containing only 14% to 21%. Gallocatechins are the chief flavonols present in grape seeds (Czochanska et al., 1979; Montealegre et al., 2006), while the skin is rich in epigallocatechin (Escribano-Bailón et al., 1994; Montealegre et al., 2006).

Moreover, polymers and oligomers (from 2 to 5 units) of flavanols are in abundant but type-B proanthocyanidins are significantly higher than other counterparts. (Da Silva et al., 1991). Proanthocyanidins from grape seeds have a lower average degree of polymerization (10 to 20 units) than the skins (25 to 35 units) (Ky et al., 2014). The two flavanols as polymers and oligomers cannot be extracted in winemaking as they have low levels of solubility and remain in the grape pomace.

Phenolic compounds work as precursors to many types of chemicals. They lead to neuron protection from oxidative stress as well as exhibit anti-cancerous, vaso dilating properties (Stój et al., 2018). By products of wineries acts as natural source for phenols and have a lot of applications (José Jara-Palacios et al., 2017, 2014; José Jara-Palacios, 2019). Nowadays, as demand for natural compounds has increased, and both food and pharmaceutical industries are seeking new sources such as grape seeds, canola cake (obtained after pressing of oil from canola seeds) (Beres et al.,

2017). Phenolic compounds obtained from these sources can be used as ingredients for functional foods or can be directly used as nutraceuticals (García-Lomillo & González-SanJosé, 2017).

2.3 Valorization of Pomace

2.3.1 Current pomace valorization practices

Currently, pomace waste is directed as animal feed, for biogas production, compost or landfill. There are certain cases in which this waste is also incinerated (Cáceres, Cáceres, Hein, Molina, & Pia, 2012). Among these, conversion of pomace into cattle feed is the most commonly implemented and cost-effective strategy (Bhalla & Joshi, 1994). This conversion is limited as only unfermented pomace can be utilized. This is because the fermented pomace contain high amounts of phenolic compounds , which can cause gastric indigestion and sometime lead to allergic reactions in animals (Yan & Kim, 2011). Composting is another method that can be used as an environmentally friendly waste disposal alternative to landfilling. Composting may help in adding value to pomace waste in terms of reducing the amount of organic waste (Lei & VanderGheynst, 2000) but operational costs of such methods do not promote utilization on small scale.

Incineration is used globally since it reduces the waste load on landfills (Kumanda, Mlambo, & Mnisi, 2019). Most of pomace waste is disposed at landfill sites. Landfilling is costly and also contributes to large amount of greenhouse gases emission (Rada, Ragazzi, Fiori, & Antolini, 2009). Figure 1 presents the current waste management techniques being used for grape pomace disposal and recovery (Gassara et al., 2011).

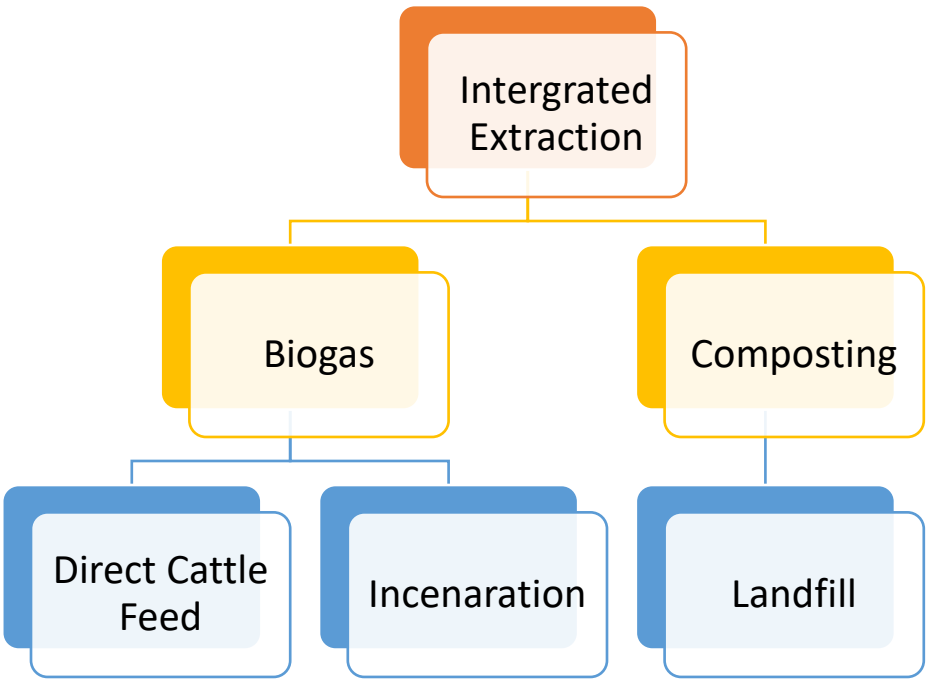

Figure 2.3: Pomace waste management starting from most to least favourable option

2.3.2 Conventional Techniques

Extraction of bioactive compounds from fruits and vegetable wastes consists of three to four steps including: pre-treatment, extraction with solvent, isolation/purification of compounds and if required encapsulation (Figure 2). Pre-treatment methods include milling which helps in increasing the surface area for solvent extraction (Leite, Salgado, Venâncio, Domínguez, & Belo, 2016). For example, grape pomace can be finely chopped or grinded to increase surface area, this in turn helps in increasing solute solvent interaction (Villano, Fernandez-Pachon, Moya, Troncoso, & Garcia-Parrilla, 2007)(Álvarez et al., 2017). Freeze Drying can also help in increasing the surface area, bioavailability of viable compounds but being high in cost, simple milling is preferred. In the case of grape pomace clarification is required to separate the pomace into components such as skin, seeds, and pulp (Larrauri, Rupérez, & Saura-Calixto, 1997). Table 2.1 reports the list of various polyphenols, their sources and the solvents used for their extraction.

Table 2.1: List of various phenols obtained from fruits and vegetables

Raw material *Solvent	Results	Reference
Grape skin (Vitis vinifera L.) Water	AA: 53.47 mg Trolox/100 g FW	(González-Centeno et al., 2014)
Berries Ethanol	Anthocyanins T: 22.73 mg cyanidin E/g DW.	(Celli et al., 2015)
Fruits of blackberry (Morus nigra) Enzyme (Pectinex UF)	Totals Flavonoids: 379.24 mg/100 mL	(Tchabo et al., 2015)
Rhizomes of Curcuma longa L. Bromine solution	Totals Curcuminoids: 5.72 g/100 g	(Xu et al., 2015)
Azufaifo (Ziziphus lotus). Ethanol.	TPC: 40.782 mg GAE/g DW. AA: 0.289 mg/mL.	(Hammi et al., 2015)
Blackberry leaves Methanol	TPC: 80.19 mg GAE/g DW.	(Aybastıer et al., 2013)
Phlomidoschema parviflorum Methanol 80%.	TPC: 15.4 mg GAE/g DB.	(Majd et al., 2014)
Black Aronia (Aronia melanocarpa) Ethanol 50%.	TPC: 15.41 mg GAE/mL	(Ramić et al., 2015)

Sugar beet molasses Ethanol.	TPC: 17.36 mg GAE/100 mL. AA: 16.66 mg/g	(Chen et al., 2015)
Rhizomes of S. (Stoloniferum Buch.-Ham) Ethanol	TPC: 881.12 µg/g DW.	(Wang et al., 2013)
Blueberry wine waste (Vaccinium ashei) Ethanol	TPC: 16.41 mg GAE/g DW.	(He et al., 2016)

GAE: Gallic Acid used as calibration standard and total phenols are reported as gallic acid equivalent (GAE).

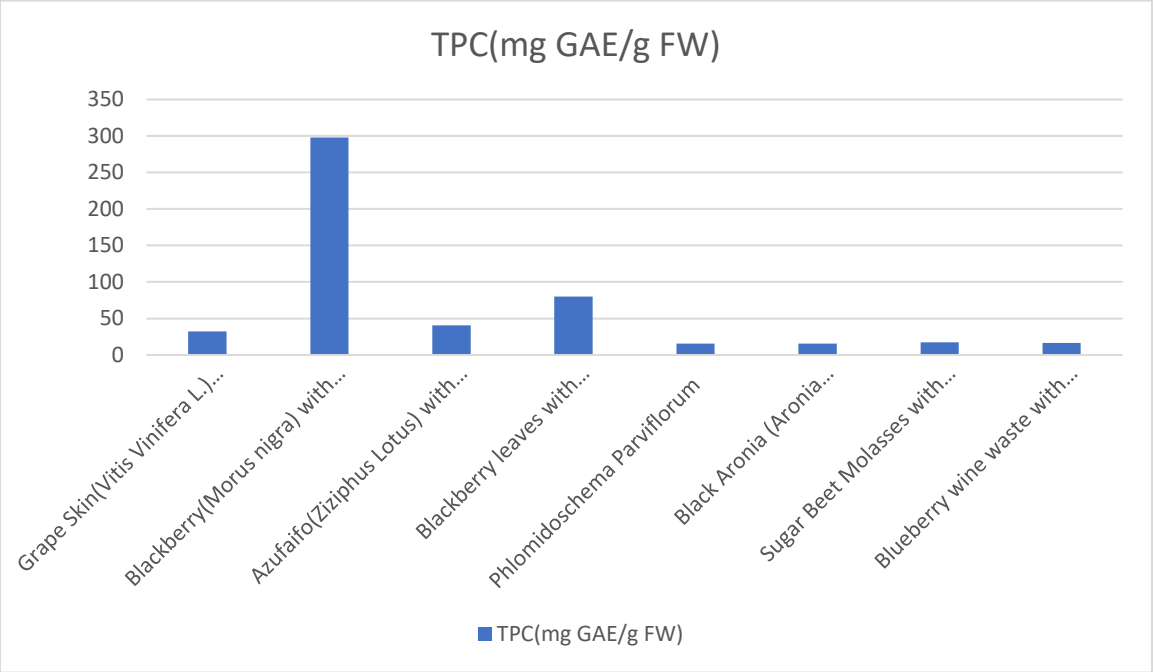

Figure 2.4: TPC of various fruits and vegetables

Bioactive compounds can be obtained by solvents such as non polar ethanol and methanol. Conventional Heat reflux method supplies heat and agitation to aid this extraction (Pan, Niu, &

Liu, 2002). This cost effective process depends on the type of bioactive compound to be extracted from the food matrix. (Tsali & Goula, 2018).

Soxhlet extraction: Similar to heat reflux , extraction of bioactive compounds is significantly low due to high exposure of heat and processing time (Luque de Castro & Priego-Capote, 2010). In the Soxhlet extraction process, chemical solvents including methanol, ethanol, hexane, acetone, etc. are boiled in a distillation column, the solvent vapor generated due to the heating process is then condensed and trickled onto the sample. Low volatility of solute than solvents , keeps the solute into the extraction vessel as the solvent vaporises. (Szolar, Rost, Braun, & Loibner, 2002). Many solvents including methanol, hexane and acetone used in the Soxhlet process are not considered safe for direct consumption by humans. These solvents could not be fully removed from the extracted compounds making it unsafe for nutraceuticals, or functional food processing.(Hawthorne, Grabanski, Martin, & Miller, 2000).

The drawbacks and constraints of the aforementioned conventional method has led to an increased research in development, optimization and implementation of advanced extraction techniques, such as, microwave-assisted extraction (MBE), supercritical fluid extraction (SFE) and ultrasonic extraction (USE), separation of valuable compounds from biological waste (Joana Gil-Chávez et al., 2013).

2.3.3 Ultrasonic-assisted extraction (UAE)

The principle involved in UAE is ultrasonic waves ranging in 20 kHz. Continuous vibrations caused due to ultrasound produces bubbles in the media. The cycle of formation and disruption results in breakdown of solid surface aids solute solvent interaction. This process is called cavitation. (Dolatowski et al., 2007; Vilkhu et al., 2008). Similar principle was applied by Kim et. al 2003, in plums for flavonoids and polyphenols extraction, longyan pulp (Yang, Zhao, Shi, Yang,

& Jiang, 2008), ginger (Balachandran, Kentish, Mawson, & Ashokkumar, 2006) and green walnut husk (Routray & Orsat, 2014). In the study conducted by Mungrat et. al in 2018 anthocyanins were extracted from purple corn cob variety and the solvent used was 50% ethanol-water solution with solid: liquid ratio of 1:20 (Muangrat, Pongsirikul, & Blanco, 2018).

Figure 2.4 represents the basic process involved in ultrasound assisted extraction (Shirsath et al., 2012).

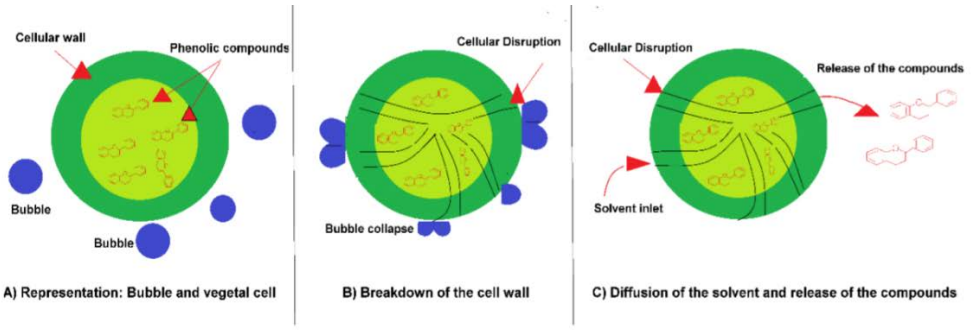

Figure 2.5: Heat transfer inside the cell structure (Shirsath et al., 2012).

Considering the versatility of power ultrasound it can used in several other processes like oil extraction (Sharma & Gupta, 2004) sterilization of the surface (Zhou et al., 2009)inactivation of microbes (Baumann et al., 2009) enzyme inactivation (Raviyan et al., 2005) protein-starch separation (Zhang et al., 2005). Other than this ultrasound can also increase surface area enhance hydration and increase pores of cell wall (Vinatoru, 2001). UAE has shown some promising results in phyto-pharmaceutical industry for extraction of phytonutrients from several herbal plants (Chemat et al., 2017).

Ultrasound assisted extraction is cheap, simple and efficient and results into better extraction yield but, few disadvantages like additional filtration is required after the whole process, limit its utilization in the industry (Garcia-Castello et al., 2015).

2.3.4 Supercritical fluid extraction (SFE)

The supercritical fluid extraction (SFE) process involves application of higher temperature and pressure combination to increase solvent transfer into the sample matrix (Reverchon, 1997). Major solvents used in this case is CO_2 which is nontoxic, inexpensive and non-flammable (Pourmortazavi & Hajimirsadeghi, 2007). SFE process has been widely used for extraction of oils from black pepper (Ferreira, Nikolov, Doraiswamy, Meireles, & Petenate, 1999), phenolic compounds from olive leaves (de Lucas, Martinez de la Ossa, Rincón, Blanco, & Gracia, 2002), and lycopene from tomato skin (Kassama, Shi, & Mittal, 2008). The choice of SFE as a novel extraction process is dependent on its advantages over the conventional heat reflux and Soxhlet processes. SFE has the extensive ability to solvate the sample and solvent by use of various temperature and pressure combinations, this leads to higher yield but SFE process is costly and its difficult to optimize as compared to other advance and novel extraction processes (Reverchon, 1997).

2.3.5 Pressurized fluid extraction (PFE)

PFE is relatively a new process and involves application of high temperature and pressure such as 40-200°C and 20 MPa respectively for short duration(Husni et al., 2009) thus increasing solute solvent interaction by reducing the molecular bond strength(Raynie, 2006). In recent time, it has been used widely for extraction of bioactive compounds from fruits and vegetables (Raynie, 2006).

2.3.6 Microwave-assisted extraction (MBE)

MBE is the advanced extraction technique which is extensively used for extraction of phenolic compounds from biological matrices. Microwave is an electromagnetic (EM) wave., which sits between infrared (higher wavelength) and radio waves (lower wavelength) in EM spectrum. The frequency range for microwave is 300 MHz-300 GHz, out of this range 915 MHz

is mostly used for industrial purposes due to its higher penetration depth (Gonzalez-San Jose & Diez De Bethencourt, 1987). Microwaves penetrate solute solvent medium to create vibrations due to dipole movement and ionic conduction which lead to increase in temperature of the medium. The amount of absorbed energy depends on the dielectric properties of the plant matrix and the solvent used. Equation 1 suggests the relationship of dielectric properties of the solvent or plant matrix:

$$\varepsilon^{*} = \varepsilon^{'} - j\varepsilon^{''} \tag{1}$$

Where, $j = \sqrt{-1}$, ε'=Dielectric constant, ε^{*} = is the relative permittivity to that of free space, and ε'' = Dielectric loss factor. Equation 2 is used to estimate the attenuation of microwave power.

$$\tan\delta = \frac{\varepsilon^{'}}{\varepsilon^{''}} \tag{2}$$

As stated earlier the microwave extraction process involves energy transfer by two phenomena: ionic conduction and rotation of dipoles in the solute and solvent (Kubrakova & Toropchenova, 2008).

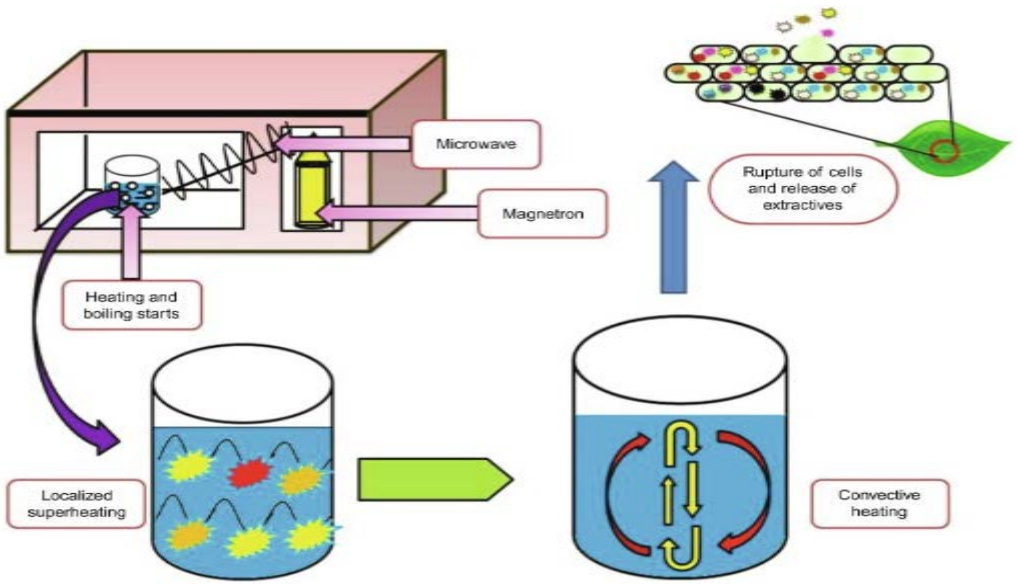

Figure 2.6: Extraction mechanism (adapted from (Kubrakova & Toropchenova, 2008)

Microwaves can either be used directly or as pre-treatment for extraction as pre-treatment to enhance the yield of final product. For instance, higher flavonoid content was achieved when microwave pre-treatment was given to apple mash before extracting juice (Roberts & Gerard, 2004).

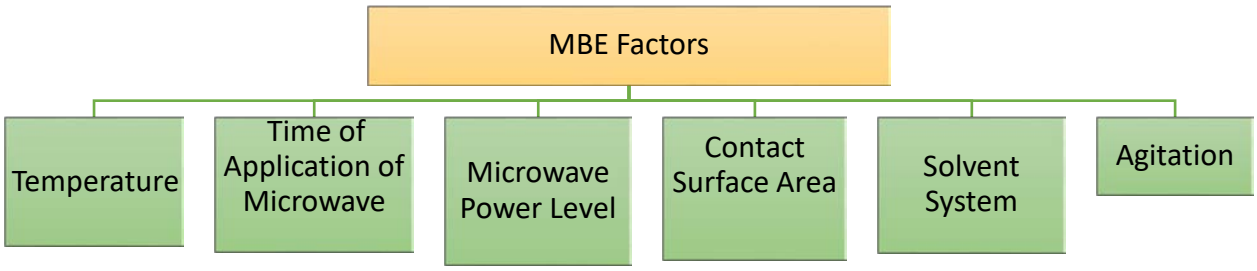

Figure 2.7: Factors affecting microwave extraction efficiency (Roberts & Gerard, 2004).

Conclusion

Fruits and vegetable wastes contain several beneficial phenolic compounds, which can be extracted to be used as part of functional food, nutraceutical or in the pharma industry. Several techniques exist for the extraction of these compounds from plant biomatrix, but their selection depends on their individual advantages and disadvantages. Conventional techniques including heat-reflux and Soxhlet are commonly used but due to the time and energy required for their operation they are not ideal for extraction of high value compounds. Advanced and novel techniques including microwave, ultrasound, high pressure and supercritical fluid are viable but their too have their own limitations.

CHAPTER 3
SOLVENT SELECTION FOR EFFICIENT EXTRACTION OF ANTIOXIDANTS AND TOTAL PHENOLICS FROM GRAPE POMACE

3.1 Introduction

Grape pomace or grape marc is the residual portion of grape or wine processing industry such as skin, stems and seeds that remain after the processing of grapes. These by-products are either discarded as waste or dumped into landfills or utilized for animal feed (Theagarajan, Malur Narayanaswamy, Dutta, Moses, & Chinnaswamy, 2019). Total grape production in the world is reported to be 77.8 million tonnes (Hogervorst, Miljić, & Puškaš, 2017) of which 85% is used for wine production. Grape is a rich source of bioactive components such as organic acid, vitamins, minerals, polyphenols, and antioxidants. Polyphenols such as flavonols, quercetin, myricetin, kaempferol, catechin, epicatechin, anthocyanin, and resveratrol are present in high quantity in grapes (Pintać et al., 2018b). Based on the phenolic content these polyphenols are strong antioxidants that provide various health benefits such as reduction of oxidative stress, free radical scavenging properties, lowers the risk of cancer, and cholesterol. Besides these polyphenolic compounds, the triterpenoids compound present in grape marc is considered as anti-inflammatory, antimicrobial, anticancer, and antiaging agents (Bonfigli, Godoy, Reinheimer, & Scenna, 2017). Hence, effective extraction, isolation, and further utilization of bioactive components of grape and its by-products have been attracting interest in the production of high-value pharmaceuticals and nutraceuticals in the past decade.

The recovery of high-value components from wine industry by-products or grape marc remained after processing of grapes is of great importance, not because of the aforementioned health benefits, but also because it could exploit a large amount of waste (5-9 million tons per year)

generated during processing (Louli, Ragoussis, & Magoulas, 2004). This processing consequently reduces the environmental impact caused due to disposal and landfilling.

The extraction of bioactive components is the major step to recover high-value components from grape by-products is an important way to increase the value of the waste. Phenolic compounds and antioxidants can be extracted from plant by-products with conventional extraction technologies such as maceration, solid-solvent extraction, and Soxhlet extraction as well as novel technologies. In conventional extraction such as Soxhlet and heat reflux, the bioactive component from the analytical compound can be extracted with non polar chemicals such as hexane, methanol, ethyl acetate, ethanol and sometimes water coupled with heat and frequent stirring (Singh et al., 2011). Heat reflux and Soxhlet are periodically used methods for deriving phenolics and antioxidants as it is a simple and cost-effective process (Routray & Orsat, 2013).

So far, the extraction of bioactive components such as phenolic compounds and antioxidants from plant by-products or cellular tissues of fruits has been achieved with extraction solvents such as ethanol, methanol, ethyl acetate, and water (Louli et al., 2004). The extraction of bioactive components from the cellular biomass not only depends on the type of solvent used but also depends on the quality, composition, and origin of the plant material as well as the pre-treatment given to the biomass. All these parameters are considered during the extraction to produce high quality with good antioxidant activity and other high-value components (Shi et al., 2005).

In particular, numerous studies have addressed the extraction of phenolic compounds and antioxidants from food industry by-products with different organic solvents. These organic solvents cause problems during their use in pharmaceutical products and require further downstream processing. Polyphenols are polar and are soluble in an aqueous mixture of polar solvents such as ethanol, methanol, or acetone and can be easily removed from the bio-active

components by evaporation. Hence their extraction in GRAS (Generally Recognized As Safe) solvents provides various benefits such as a cost-effective extraction process, a pure component in a polar solvent that doesn't affect their use in the production of high-value products (Casagrande et al., 2019).

The main objective of this study was to identify the most suitable solvent for the heat-reflux extraction of polyphenols and antioxidants from grape pomace. Grape pomace or marc obtained during the processing of grape into wine was chosen to be studied for the extraction of bioactive components. Three solvents methanol, ethanol, water, and a mixed ratio of ethanol and water were considered for the extraction of bioactive components from pomace. Further investigation of the extracted components such as antioxidant activity by DPPH and FRAP along with phenolic content by TPC was evaluated with the spectrophotometric assay.

3.2 Materials and Methods

3.2.1 Pomace and Chemicals

Grape Pomace (GPE) sample was procured from a winery named Andrew Peller Inc. located at 697 S Service Rd, Grimsby, ON in the Niagara region. GPE was purplish-brown the residue obtained during the processing of grapes into wine. Being fermented during the process of winemaking, its strong odor resembled that of the wine with pH 4.24. Four varieties of red grapes (Baco Noir, Pinot Noir, Merlot, and Cabernet Franc) were used to make wine and leftover pomace was packed in 400 gm sample bags. These were stored in a deep freezer at approximately -25°C.

Chemicals used: Regents such as Folin-Ciocalteu, sodium bicarbonate ($NaHCO_3$), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were procured from Sigma Aldrich. Sodium Acetate, Sodium hydroxide, ferric chloride was purchased from Thermo-Fisher

Scientific. HPLC grade methanol and ethanol solvents, Gallic Acid and Ascorbic Acid were supplied by Thermo-Fisher Scientific. Water as a solvent was utilized from the Milli-Q system.

3.2.2 Preparation of samples

For sample preparation, 100 gm of deep-frozen pomace was thawed at room temperature. Homogenization of the freshly thawed grape pomace was done by crushing it in pestle and mortar. For extraction of bio-active components, 2g of homogenized pomace sample was mixed with 20 ml of solvent (ethanol, water, and methanol) and kept at room temperature for 5 minutes to settle down. These samples were treated at different temperatures for constant period of time with different GRAS solvents to measure the total phenolic content, radical scavenging activity, and ferric reducing antioxidant potential.

Table 3.1 Temperature-Time combinations for major solvents used for extractions

Solvent	**Temperature**	**Time**
Methanol	30,50, and 70°C	90 minutes
Ethanol	30,50, and 70°C	90 minutes
Water	30,50, and 70°C	90 minutes
Ethanol: Water(1:1)	30,50, and 70°C	90 minutes

3.2.3 Extraction

Extraction of bioactive components from grape pomace was done at three different temperatures for constant period of time. Triplicates of all the prepared samples were kept at 30,50 and 70°C for 90 minutes respectively. Working with temperature as low as 30°C gives the advantage of restricting degradation due to heat sensitivity, though low temperatures may alter extraction rates

too. The higher temperature of the range 70°C helps in better extraction rates but there are chances of degradation of compounds. This wide range helped in analyzing the extraction-degradation relationship. After keeping the samples for 90 minutes in a hot water bath, they were kept at room temperature for a while. Then triplicates were centrifuged at 10,000 rpm using Servell Enclosed Superspeed Centrifuge (USA). The supernatant (Extract) was collected and kept at 4°C for further analysis.

3.3.1 Determination of Total Phenolic Content

Total Phenolic content of the grape pomace extract was determined by a modified protocol reported by (Singh et al., 2011). 1 mL extracts obtained after the heat reflux treatment, was mixed with 7.5 mL double distilled water and 0.5 mL of Follin Ciocalteau Reagent to make 9 mL of total volume. After 5 minutes of resting time, 1 mL of 5% of Sodium Bicarbonate Solution was added into the mixture and incubated at 23°C for 90 minutes. After incubation of 90 minutes, the absorbance of solution mixture was measured at 765 nm using UV-visible spectrophotometer (Thermo Scientific GENESYS 20, Canada).

Standard curves of pure Gallic Acid for the estimation of total phenolic content was measured at different concentrations (0.02,0.04.0.06,0.08 and 0.1 mg/mL) for all four solvents with their intercept at (0,0).

Table 3.2 Gallic Acid Standard Curve equations for different solvents used

Solvent	**Equation**	**R^2**
Ethanol	$y = 4.3791x$	R^2=0.9803

Ethanol & Water (1:1)	$y = 4.6729x + 0.0135$	R^2=0.9929
Water	$y = 4.17x + 0.0307$	R^2=0.9796
Methanol	$y = 6.1829x + 0.0185$	R^2=0.9909

Total Phenolic Content was expressed as Gallic Acid Equivalents(GAE) in mg per g of fresh weight. Here, x is the concentration of Total Phenols in terms of mg GAE per g of fresh weight.

3.3.2 Determination of Radical Scavenging activity

Free radical scavenging activity was calculated with a modified version of the method proposed by (Brar, Subramanian, Nair, Singh, & Engineering, 2020). Fifty µL of the extract was added to 1.5 mL of DPPH solution. The solution is kept for 20 minutes during which its color fades. The discoloration was observed due to a reduction in free electrons in DPPH. This reduction helps in determining the Radical scavenging activity of DPPH by measuring absorption at 517 nm by a spectrophotometer.

$$Scavenging\ \ activity(\%) = 100 \times \left(\frac{Abs_{control}^{517nm} - Abs_{sample}^{517nm}}{Abs_{control}^{517nm}} \right)$$

Here, $Abs_{control}$ is the absorption of blank and Abs_{sample} is the absorption of the extract at 517 nm.

3.3.3 Determination of ferric reduction antioxidant potential (FRAP)

The ferric reducing antioxidant potential (FRAP) method measures antioxidant capacity spectrophotometrically by Ferric Reducing Antioxidant Power Assay. The procedure being followed is taken from the modified method suggested by (Routray & Orsat, 2013). Due to low pH, colorless Ferric ion (Fe+3 of TPTZ radical) reduces to form a blue colored complex (Fe+2 - tripyridyltriazine). FRAP was prepared using fresh 10mM TPTZ, 2.5 ml of HCl (40 mM), 2.5 ml

of ferric chloride solution (20 mM) in 25 mL of sodium acetate buffer (300 mM). Twenty microliters of extract were mixed with three hundred microliters of DDW. To this, 2 ml of FRAP was added. This was further kept at 37°C for 30 minutes incubation time. The absorbance was recorded at 595nm.

Ascorbic Acid Equivalent (AE) was used as a standard for the solvent (0.02,0.04.0.06,0.08 and 0.1 mg/mL) and its absorbance by spectrophotometer calibrated at 595 nm. The following curves were used for the assessment of FRAP in terms of AE/gm fresh weight of the food sample.

Table 3.3 Ascorbic Acid Standard Curve equations for different solvents used

Solvent	**Equation**	**R^2**
Ethanol	$y = 3.4891\ x$	R^2=0.9978
Ethanol & Water (1:1)	$y = 3.28\ x$	R^2=0.9941
Water	$y = 2.9036\ x$	R^2=0.9901
Methanol	$y = 7.6175x$	R^2=0.9909

Here, y is the absorbance at 595 nm and x is the AE concentration of the sample.

3.4 Statistical Analysis

All the quantitative measurements were taken in triplicates and results were presented as mean ± standard deviation. ANOVA (Analysis of Variance) and Duncan's Tukey post hoc analysis were carried out using MINITAB software (version 17.5) and significant difference between different samples were measured at a 95% significance level ($p<0.05$).

3.5 Results and Discussions

For the recovery of antioxidant and total phenolic from the grape pomace and their utilization in the pharmaceutical, nutraceutical industry, and food processing industry the solvent selection is

the most important step. To this purpose, three solvents ethanol, methanol, water, and the mixed ratio of ethanol and water were examined for the extraction of bioactive components and optimized according to the maximum yield obtained.

3.5.1 Determination of Total phenolics

The total phenolic content of grape pomace extract is reported in Table 3.4. The total phenolic content of fresh grape pomace ranged from 21.5 to 37.08 mg GAE/ g FW. A study conducted by (Pintać et al., 2018b) reported almost similar content of phenolic content in grape pomace extract treated with a mixed ratio of acidified solutions of ethanol, and methanol. It is observed that after the processing of grape into grape juice or wine, the pomace obtained, contained almost all the skin and seeds which induce a great number of polyphenols and antioxidants (Aliakbarian, Fathi, Perego, & Dehghani, 2012). The results of total phenolic content revealed that aqueous methanol solution gave a higher yield of total phenols compared to other green solvents such as ethanol and water. It can be induced that aqueous methanol solution can increase the partial hydrolysis or cleave the non-polar compounds which released the reactivity of compounds in TPC assay, which further increased the phenolic content. On the other hand, ethanol also gave a comparable yield of total phenolic from the grape pomace extract. Water and an aqueous mixture of ethanol and water gave a lower yield of phenolics which are in correlation with the results obtained by (Goula, Thymiatis, & Kaderides, 2016). From the aforementioned observations, it was revealed that methanol solution gave maximum yield but the comparable yield of ethanol solvent also influenced the usage of GRAS (Generally Recognized As Safe) solvent ethanol for the extraction of bioactive components.

Table 3.4: Total Phenolic Content of grape pomace extract at various temperatures with different solvents based on heat reflux method (mg GAE/ g FW)

Solvent	Temperature (°C)	TPC (mg GAE/g fw)
Ethanol	30	21.69±0.41 [a]
	50	23.41±0.69 [b]
	70	26.79±0.84 [c]
Water	30	9.03±0.36 [a]
	50	14.30±0.32 [b]
	70	13.98±6.31 [b]
Methanol	30	13.17±0.76 [a]
	50	35.13±0.30 [ab]
	70	37.08±0.40 [ab]
Ethanol: Water (1:1)	30	15.67±0.39 [a]
	50	20.85±5.63 [ab]
	70	20.52±0.75 [ab]

Note: Values are presented as Mean± SD of three replicates. Different letters in the same column indicate statistically significant difference at ($p<0.05$).

Grape pomace was treated with different solvents using heat reflux at 30, 50, and 70°C for 90 minutes. With an increase in temperature, an increase in total phenolic content was also observed. For fresh grape pomace, the extraction yield of TPC increased from 30 to 70°C, thereafter, decreased with an increase in temperature due to degradation and oxidation of temperature-sensitive components. Hence, temperature also influenced the yield of phenolic content. ANOVA analysis of total phenolics for grape pomace at a 95% confidence level revealed that extraction temperature significantly affected the yield of polyphenols.

3.5.2 Determination of radical scavenging activity

Scavenging activity is the measure of the capacity of the antioxidants present in the extract to react and bind with the free radicals. In this assay, the hydrogen ions present in the active component change the purple color of DPPH into yellow which indicated the presence of antioxidants. A higher scavenging activity percentage or extinction of purple color represents the higher value of antioxidants. The scavenging activity of grape pomace extract varied from 13 to 27% for different solvents such as ethanol, methanol, and water. Ethanol and methanol solvents gave the maximum yield of scavenging activity of around 26-27% as compared to the water (Table 3.5). Similar results for the antioxidant activity of the grape pomace treated with alcoholic solvents by heat reflux were reported by (Y. Xu, Burton, Kim, & Sismour, 2016).

Table 3.5: Radical Scavenging Activity of DPPH free radical for grape pomace extract at different heat reflux extraction temperatures with different solvents

Solvent	**Temperature (°C)**	**DPPH (%)**
Ethanol	30	24.09±0.60 [a]
	50	27.73±0.59 [b]
	70	28.52+0.58 [b]
Water	30	13.96±0.97 [a]
	50	17.13±0.30 [b]
	70	15.21±0.32 [c]
Methanol	30	25.80±0.78 [a]
	50	27.57±0.53 [b]
	70	27.94±0.39 [b]

Ethanol: Water (1:1)	30	22.77±0.30 [a]
	50	20.66±0.81 [a]
	70	27.19±1.85 [b]

Note: Values are presented as Mean± SD of three replicates. Different letters in the same column indicate statistically significant difference at ($p<0.05$).

Extraction temperature significantly affects the antioxidant activity of the grape pomace sample during heat reflux extraction. The percentage of antioxidant activity of the grape pomace sample increased with increase in temperature for each solvent. This can be attributed to the fact that longer temperature treatment affects the oxidative ions and release the ions which reacts with free radicals and extinction of purple color which gave the indication of more antioxidant activity (Elbadrawy & Sello, 2016). For methanolic extract of grape pomace, a decrease in antioxidant activity was observed with increase in temperature beyond 60°C. This decrease in antioxidant content was observed because of the low boiling temperature of alcoholic solutions. Prolong this boiling temperature, an increase in boiling point will cause degradation of the bioactive components. Hence, for all the different solvents, there was a significant difference between the extraction yield was observed at 30, 50 and 70°C.

ANOVA analysis of the obtained data determined the signification of the treatment factors such solvent and temperature on the extraction yield of antioxidant activity by DPPH and FRAP. ANOVA analysis of the observed data generated a p-value <0.05 which revealed that both solvent type and extraction temperature were the significant factors with the interaction term (solvent*temperature) of $p<0.05$. From ANOVA analysis the determination coefficient observed was 0.003 which determines the significance of the treatment factors on the extraction yield. P-value less than 0.05 indicated the significance of the treatment factors (Table 3.5). Therefore, the

developed model gave a p-value of 0.003 which indicated that obtained results are significant and had different antioxidant activity for given different treatments.

3.5.3 Determination of Ferric reducing antioxidant potential (FRAP)

FRAP is another method to determine the antioxidant potential in the grape pomace extract. FRAP assay measures the capacity of antioxidant compounds to reduce the ferric complex (Fe3+) to the ferrous complex (Fe2+) with change in color and absorbance. FRAP of grape pomace was measured using a method proposed by (Routray & Orsat, 2014). FRAP for grape pomace ranged from 26.1 to 32.3 mg AE/g FW for aqueous mixture of alcoholic solvents (Table 3.7). (El Hassani et al., 2019) reported similar results for the ferric reduction potential for grape pomace and marc treated at 40-60°C. For all the solvents the maximum yield was obtained at 70°C which further decreased when temperature was increased to 80°C due to the near boiling point range of alcoholic solutions which further caused degradation of nutrients or bioactive components present in the grape pomace. A similar observation for increase in antioxidant content with increase in temperature was observed by (Routray & Orsat, 2014) where antioxidant activity by ferric reduction potential of blueberry leaves extract was observed to be increased from 45°C to 60°C, which further decreased when temperature was increased to 75°C. FRAP for water extract of grape pomace gave antioxidant yield of around 22-24.61mg AE/g FW at all temperatures (Table 3.6). Hence, a significant difference in antioxidant activity by FRAP was observed between the yield of water and alcoholic solution extract. As methanol solution gave better yield corresponding to other solvents. However, ethanol being considered as GRAS solvent also gave comparable yield with methanol.

Here, different temperature treatment at same interval exhibited significant effect on the extraction of antioxidants with ferric ions reduction potential. For water extract of grape pomace slight

increase in antioxidant content was observed with increase in temperature from 30-70°C. However, for methanol and ethanol grape pomace extract, a decrease in FRAP activity was observed after 50°C.

Table 3.7: Ferric complex (Fe3+) reduction antioxidant potential (FRAP) for grape pomace extract by MBE at different temperatures with different solvents

Solvent	Temperature (°C)	FRAP (mg AE/g)
Ethanol	30	26.32±0.22 [a]
	50	29.81±0.66 [ab]
	70	30.48±0.68 [ab]
Water	30	22.04±5.87 [a]
	50	24.85±0.26 [ab]
	70	24.61±0.72 [ab]
Methanol	30	31.94±0.37 [a]
	50	32.25±0.36 [ab]
	70	33.08±0.29 [ab]
Ethanol: Water (1:1)	30	23.57±1.37 [ab]
	50	27.54±0.83 [bc]
	70	29.67±0.98 [ab]

ANOVA analysis of FRAP results indicated that temperature and solvent were the significant factors ($p<0.05$) for the antioxidant activity. Whereas the interaction term such as temperature and solvent were not the significant factors for the yield of antioxidant activity ($p>0.05$).

Table 3.8: ANOVA for yield of TPC, DPPH, and FRAP

ANOVA	**Total Phenolics (mg GAE/g FW)**				**DPPH scavenging activity (%)**				**FRAP (mg AE/ g FW)**			
Source of Variation	***SS***	***df***	***P-value***	***F crit***	***SS***	***df***	***P-value***	***F crit***	***SS***	***df***	***P-value***	***F crit***
A: Solvent	2464.522	3	2.52E-37*	3.008787	875.9821	3	2.47E-21*	3.008787	280.9429	3	4.73E-08*	3.008787
B: Temperature	134.0011	2	7.95E-23*	3.402826	57.07643	2	7.61E-09*	3.402826	110.0354	2	2.95E-05*	3.402826
Interaction A*B	20.65682	6	1.37E-11*	2.508189	54.86029	6	6.44E-07*	2.508189	25.86726	6	0.293852	2.508189
Within	1.957867	24			15.22737	24			79.43456	24		
Total	2621.138	35			1003.146	35			496.2801	35		

Note: Values are presented as Mean± SD of three replicates. Different letters in the same column indicate statistically significant difference at ($p<0.05$).

3.6 Relationship between phenolic content and antioxidant activity

The phenolic content present in fruits and vegetables can be attributed to the presence of antioxidants which possess antioxidants properties by scavenging free radicals, metal chelation, and hydrogenation (DiNardo, Subramanian, & Singh, 2018). Hence, the determination of correlation between TPC and antioxidants in grape pomace is indicator of inhibiting oxidation. The association of antioxidants and TP for grape pomace extract is estimated in figure 1. From the figure, it can be deduced that antioxidant activity by DPPH and TPC was observed indicating correlation value of (R^2=0.88). On the other hand, antioxidant activity determined by FRAP also gave a positive correlation with total phenolics. These observations and correlation values indicated that the residue from grape processing industries were rich in phenolic components as well as contained high antioxidizing agents. Former related literature also supported the identical results which indicated that with increase in TPC, a consistent increase in antioxidant properties were also observed with correlation value of R^2=0.89 (Spigno, Tramelli, & De Faveri, 2007).

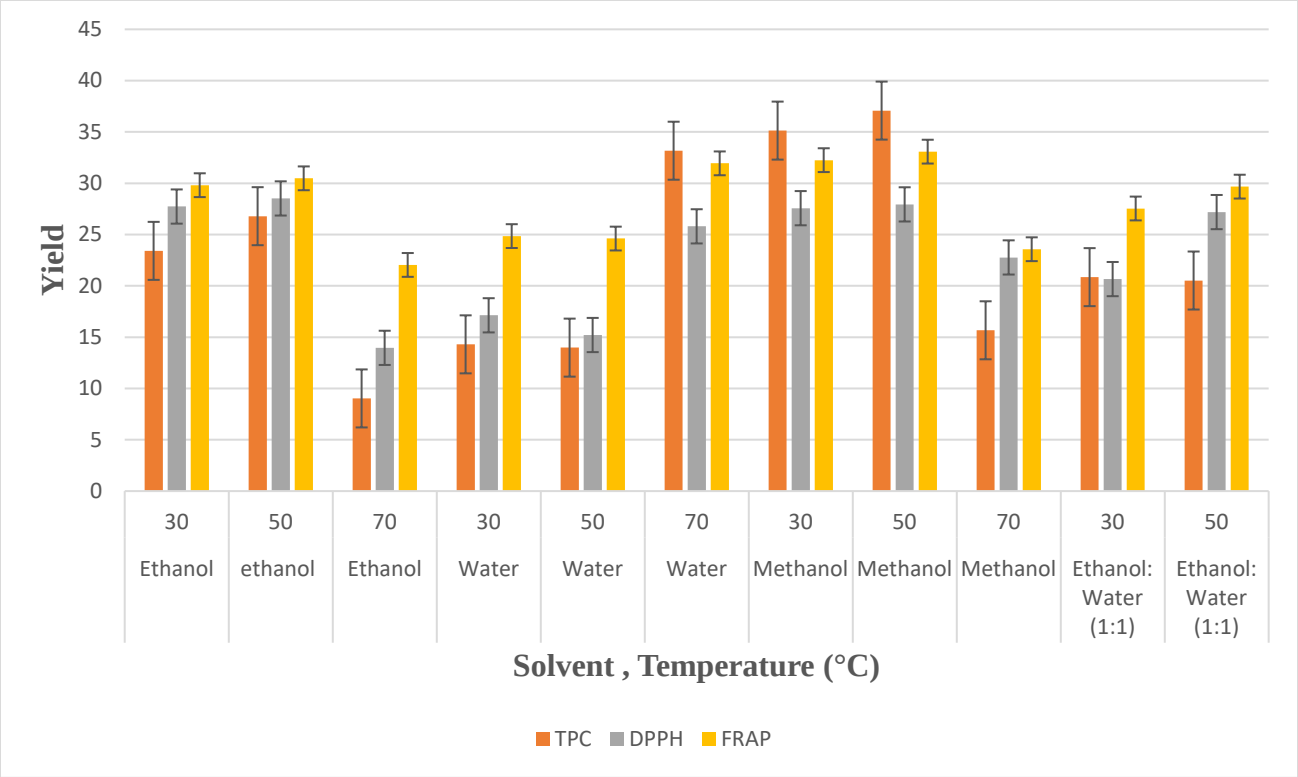

Figure 3.1: Correlation of extraction yield with different solvent and temperature

On the contrary, the correlation between TPC and antioxidant content for the extract with solvent was observed to have low correlation for antioxidant activity by both methods DPPH as well as FRAP (Figure 1). This low activity in antioxidants could be observed due to water which gave less extraction rate. This relationship could also contribute explanation to the other bioactive components present in GPE.

3.7 Conclusion

To choose the best suitable solvent for bioactive chemical extraction from grape pomace extract can be related to various factors such as the variety and material formation, type of component being extracted and solvent, extraction conditions being used for the extraction. Hence, in this chapter the selection of efficient, safe, cost-effective, and environmentally friendly solvent was

done to extract high-value bioactive components from the grape pomace. Aqueous solution of methanol and ethanol gave higher concentration of phenolic content and antioxidant activity. But from the industrial and environmental point of view ethanol is considered as GRAS (Generally Recognized As Safe) solvent. Therefore, aqueous solution of ethanol is the best solvent for the optimum yield of polyphenols as well as antioxidants. Further, this study also evaluated the usage of grape pomace obtained from the wine processing industry to produce bioactive components. Hence, results in the present research indicated that grape pomace or marc is a rich source of total phenols, and antioxidants which could be used to produce high-value pharmaceutical, cosmetic, and food products.

CHAPTER 4
INTENSIFICATION OF BIOACTIVE COMPONENTS FROM GRAPE POMACE BY NOVEL EXTRACTION TECHNOLOGIES (MICROWAVE AND ULTRASOUND ASSISTED EXTRACTION)

4.1 Introduction

The grape processing or wine industries produce a large number of residues or by-products such as grape marc, pomace, and grape seeds. These by-products or waste products are a rich source of polyphenolic compounds that possess antioxidant compounds and provide various health benefits. The phenolic compounds produced from grape pomace include anthocyanidins, cyanidin, malvidin, flavonols include quercetin, myricetin, catechin, epichin, and epicatechin which act as antimicrobial, antiviral, and anti-inflammatory agents and used as pharmaceutical and therapeutically agents (Sirohi et al., 2020).

Recovery of these valuable bioactive chemicals from grape by product adds value to the biowaste. Phenolic compounds and antioxidants can be extracted from plant by-products with basic processes or technologies like maceration, solid-solvent extraction, and Soxhlet based apparatus as well as novel technologies. In conventional extraction such as Soxhlet and heat reflux, the bioactive component from the analytical compound can be separated with the help of several chemicals e.g. diethyl ether, hexa methanes, dichloromethane , ethyl acetate, but most commonly used is ethanol (Singh et al., 2011). Heat reflux and Soxhlet are mostly applied for phenolic and antioxidants extractions as they are convenient and cost-effective mechanism (Routray & Orsat, 2013). But, these methods take long extraction time and give poor retention of the bioactive components. Contrary to these extraction methods use toxic non polar and organic solvent chemicals e.g. ethanol which cause threat to human health and environment. Hence, to reduce or

eliminate these problems, novel extraction technologies with PI techniques can be applied to boost the extraction system efficiency which makes the process simpler and safe for human safety and the surroundings (DiNardo et al., 2018).

Novel extraction technologies used for the extraction of bioactive components reduce the expenses, enhance human health, and lessen the risk of hazards due to simple extraction methods. Process Intensification is the approach applied to omit all the drawbacks or limitations associated with conventional extraction technologies. In the process intensification approach, two novel methods microwave-assisted extraction (MBE) and ultrasound-assisted extraction (UAE) are used for the extraction. MBE and UAE are the novels and simple technologies that are relatively inexpensive and give efficient extraction by enhancing transfer of mass and contact between the interfacial area and decrease the extraction time (Singh, 2010).

Microwave-assisted extraction is a novel technology used to extract bioactive components as phenols and antioxidants from plant materials such as potatoes (Singh et al., 2011), grape pomace, and grape seeds (de Melo, Silvestre, Portugal, & Silva, 2017). Microwaves use EM radiations to transmit energy to the polar media which is transformed into heat by conduction of electrons or dipole-dipole interaction (Routray & Orsat, 2012). Cell disruption takes place due to consistent heating occurred by dipole rotation in MBE. Increased pressure improves the porous structure of the biological material and enhances the solvent penetration through the material which further increases the yield (Baghdikian et al., 2016). MBE of biological materials causes quick heating, reduces the thermal gradient, uses less solvent, and increases the extraction yield (Strati & Oreopoulou, 2011).

Ultrasound-assisted extraction (UAE) is reliable set-up that uses sound waves with ranging from 20KHz-100MHz for viable bio compounds from biological matrix. In this range, 20 KHz is the

most preferred one .UAE uses the cavitation effect in the liquid medium formed by expansion and contraction of the sound waves which further expand and collapse in the medium. During compression, cavitation bubble disrupts causing rupturing of biological material and enhances the solvent transfer into biological material and increases the yield of solute in the solvent. Hence, this cavitation effect and disruption of solid material shorter the extraction time and increases the yield of active compounds into the solvent as compared to the conventional technologies (Khaw, Parat, Shaw, & Falconer, 2017).

The major objectives of this study were to increase the extraction yield of polyphenols and antioxidants from grape pomace and to reduce the extraction time as well as environmental hazards caused by the use of toxic solvents. This work reported the range of MBE and UAE for securing TP and antioxidants from grape pomace in comparison to conventional extraction technology such as HE. Based on the results of both the novel technologies process intensification approach was applied to optimize the operating parameters of the microwave extraction which mitigates the cons of traditional technologies such as time, temperature, solvent, cost, and energy requirements.

4.2. Materials and Methods

4.2.1 Pomace and Chemicals

The grape Pomace (GPE) sample was procured from a winery named Andrew Peller Inc. located at 697 S Service Rd, Grimsby, ON in the Niagara region. GPE was purplish-brown the residue obtained during the processing of grapes into wine. Being fermented during the process of winemaking, its strong odor resembled that of the wine with pH 4.24. Four varieties of red grapes (Baco Noir, Pinot Noir, Merlot, and Cabernet Franc) were used to make wine and leftover pomace was packed in 400 gm sample bags. These were stored in a deep freezer at approximately -25°C.

Chemicals used: Regents such as Folin-Ciocalteu, sodium bicarbonate (NaHCO3), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) were procured from Sigma Aldrich. Sodium Acetate, Sodium hydroxide, ferric chloride was purchased from Thermo-Fisher Scientific. Ascorbic acid, sodium hydroxide, sodium acetate buffer, and hydrochloric acid were bought from Fisher Scientific (Fisher Scientific, Ottawa, Ontario). HPLC grade methanol and ethanol solvents, Gallic Acid and Ascorbic Acid were supplied by Thermo-Fisher Scientific. Water as a solvent was utilized from the Milli-Q system.

4.2.2 Preparation of samples

For sample preparation, 100 gm of deep-frozen pomace was thawed at room temperature. Homogenization of the freshly thawed grape pomace was done by crushing it in pestle and mortar. For extraction of bioactive components, 2g of homogenized pomace sample was taken with 20 ml of solvent (ethanol, and water) and left at 20 C for 5 minutes to settle down. Further, these solutions were treated with different extraction methods such as Heat Reflux extraction, UAE, and MBE.

4.3 Heat Reflux Extraction

The extraction of bioactive components from grape pomace was done at three different temperatures for a constant period. Triplicates of all the prepared samples were kept at 30,50 and 70°C for 90 minutes respectively. Working with temperature as low as 30°C gives the advantage of restricting degradation due to heat sensitivity, though low temperatures may alter extraction rates too. The higher temperature of the range 70°C helps in better extraction rates but there are chances of degradation of compounds. This wide range helped in analyzing the extraction-degradation relationship. After keeping the samples for 90 minutes in a hot water bath, they were kept at room temperature for a while. Then triplicates were centrifuged at 10,000 rpm using Servell

Enclosed Superspeed Centrifuge (USA). The supernatant (Extract) was collected and kept at 4°C for further analysis.

Solvent	Temperature	Time
Methanol	30,50, and 70°C	90 minutes
Ethanol	30,50, and 70°C	90 minutes
Water	30,50, and 70°C	90 minutes
Ethanol: Water (1:1)	30,50, and 70°C	90 minutes

Table 4.1: Design of Time temperature variations for various solvents used.

4.4 Microwave and Ultrasound-assisted Extraction

The second study determined the effect of varying temperatures (30, 50, and 70°C) and time (4,8, and 12 min for microwave extraction of the total phenolics and antioxidants from grape pomace. These time temperature combinations were based on pre studies and in relation to the boiling temperatures of the solvents considered. Ethanol and methanol have boiling temperatures in the range of 65-70 C which was taken as the highest point. Lowest temperature of 30C is taken in in consideration of a low temperature just higher than the room temperature so that anthocyanins can be protected from degradation. Prepared grape pomace samples with green solvents such as ethanol and water were placed in the microwave digester at the desired temperature and set time. Microwave digester works with a range of temperature time combinations. The system adjusts it according to the requirements pre-set in the system.

In another experiment, the grape samples dissolved in green solvents were put into an ultrasonic water bath for required temperature and time at 50 kHz frequency for 4, 8, and 12 minutes. The ultrasonic bath is first heated to reach the desired temperature then afterwards ultrasonic waves are passed for the required time .

After each extraction experiment, the obtained homogenates were centrifuged at 5000 rpm for 10 minutes. After centrifugation, the obtained supernatant was filtered through a 0.45 micron filter paper to remove the extraneous material.

Solvent	**Temperature**	**Time**
MBE (3X3 full factorial design)		
Ethanol	30,50, and 70°C	4, 8, and 12 minutes
Water	30,50, and 70°C	4, 8, and 12 minutes
Ethanol: Water(1:1)	30,50, and 70°C	4, 8, and 12 minutes
UAE (3X3 full factorial design)		
Ethanol	30,50, and 70°C	4, 8, and 12 minutes
Water	30,50, and 70°C	4, 8, and 12 minutes
Ethanol: Water(1:1)	30,50, and 70°C	4, 8, and 12 minutes

Table 4.2: Factorial Design of time temperature variations for various solvents used.

4.5 Determination of Total Phenolic Content

The Total Phenolic Content of the grape pomace extract was determined by a protocol reported by (Singh et al., 2011). 1 mL extracts obtained after the heat reflux treatment, added to 7.5 mL double distilled water and 0.5 mL of Follin Ciocalteau Reagent to make 9 mL of total volume. After 5

minutes of resting time, 1 mL of 5% of Sodium Bicarbonate Solution was added into the mixture and incubated at 23°C for 90 minutes. After incubation of 90 minutes, the absorbance of the solution mixture was measured at 765 nm using a UV-visible spectrophotometer (Thermo Scientific GENESYS 20, Canada).

Standard curves of pure Gallic Acid for the estimation of total phenolic content was measured at different concentrations (0.02,0.04.0.06,0.08 and 0.1 mg/mL) for all four solvents with their intercept at (0,0).

Solvent	**Equation**	**R^2**
Ethanol	$y = 6.9809x$	R^2=0.9803
Ethanol & Water (1:1)	$y = 5.7264$x	R^2=0.9929
Water	$y = 3.4136$x	R^2=0.9796
Methanol	$y = 9.4555$x	R^2=0.9909

Table 4.3: Gallic Acid Standard curve equations for various solvents.

Total Phenolic Content was expressed as Gallic Acid Equivalents (GAE) in mg per g of fresh weight. Here, x is the concentration of Total Phenols in terms of mg GAE per g of fresh weight.

4.6 Determination of Radical Scavenging activity

Free radical scavenging activity was calculated with a modified version of the method proposed by (Brar et al., 2020). Fifty µL of the extract was added to 1.5 mL of DPPH solution. The solution is kept for 20 minutes during which its color fades. The discoloration was observed due to a reduction in free electrons in DPPH. This reduction helps in determining the Radical scavenging activity of DPPH by measuring absorption at 517 nm by a spectrophotometer.

$$Scavenging\ \ activity(\%) = 100 \times \left(\frac{Abs_{control}^{517nm} - Abs_{sample}^{517nm}}{Abs_{control}^{517nm}} \right)$$

Here, $Abs_{control}$ is the absorption of blank and Abs_{sample} is the absorption of the extract at 517 nm.

4.7 Determination of ferric reduction antioxidant potential (FRAP)

The ferric reducing antioxidant potential (FRAP) method measures antioxidant capacity spectrophotometrically by Ferric Reducing Antioxidant Power Assay. The procedure being followed is taken from the modified method suggested by (Routray & Orsat, 2013). Due to low pH, colorless Ferric ion (Fe+3 of TPTZ radical) reduces to form a blue colored complex (Fe+2 - tripyridyltriazine). FRAP was prepared using fresh 10mM TPTZ, 2.5 ml of HCl (40 mM), 2.5 ml of ferric chloride solution (20 mM) in 25 mL of sodium acetate buffer (300 mM). Twenty microliters of extract were mixed with three hundred microliters of DDW. To this, 2 ml of FRAP was added and incubated at 37°C for 30 minutes. The absorbance of all the samples was recorded using a spectrophotometer at 595nm.

Ascorbic Acid Equivalent (AE) was used as a standard for the solvent (0.02,0.04.0.06,0.08 and 0.1 mg/mL) and its absorbance at 595 nm. The following curves were used for the quantification of FRAP in terms of AE per gm fresh sample.

Table 4.4: Ascorbic Acid Standard curve equations for various solvents.

Solvent	**Equation**	**R^2**
Ethanol	$y = 3.4891\ x$	R^2=0.9978
Ethanol & Water (1:1)	$y = 3.28\ x$	R^2=0.9941
Water	$y = 2.9036\ x$	R^2=0.9901

Methanol	$y = 7.6175x$	R^2=0.9909

Here, y is the absorbance at 595 nm and x is the AE concentration of the sample.

4.8 Statistical Analysis

All the quantitative measurements were taken in triplicates and results were presented as mean ± standard deviation. ANOVA (Analysis of Variance) and Duncan's Tukey post hoc analysis were carried out using MINITAB software (version 17.5) and significant difference between different samples were measured at a 95% significance level ($p<0.05$).

4.9. Results and Discussion

For the recovery of antioxidant and total phenolic from the grape pomace and their utilization in the pharmaceutical, nutraceutical industry, and food processing industry the choice of extraction technology and optimization of operating parameters is an important step. To this purpose, two novel technologies such as MBE and UAE were used to extract bioactive components from grape pomace and their extraction yield and operating parameters were compared with the conventional extraction such as HRE.

4.9.1. Microwave-assisted extraction

The total phenolic content extracted by different solvents from grape pomace by microwave-assisted extraction is reported in Table 4.5. The total phenolic content of fresh grape pomace extracted by microwave radiations gave yield in the range of 20.5 to 40.32mg GAE/ g FW. The maximum yield of phenolic content was achieved at a temperature of 70°C for 8-12 minutes with ethanol and methanol solvents. There was an increase in total phenolic content was observed with an increase in temperature from 30-70°C with all the solvents. It can be observed because, with an increase in temperature of the microwave-assisted extraction system, more amount of energy is

being supplied to the operating system which results in the subsequent increase in the extraction kinetics of the grape pomace (DiNardo, Subramanian, & Singh, 2019). On the other hand, prolonged exposure of the biological material to the microwave radiations could degrade the phenolic compounds which explained the fact that further increase in temperature from 70°C could give less amount of phenolic compounds (Krishnaswamy, Orsat, Gariépy, & Thangavel, 2013). Hence, 70°C was the optimized temperature for the extraction of phenolic components from the grape pomace.

Concerning time, treatment time of 4 minutes gave a significantly different amount of phenolic compounds as compared to the 8 and 12 minutes. From ANOVA analysis, it can be determined that treatment temperature, time, and their interaction were significant factors ($p \leq 0.05$) for response variable TPC. A combination of all the factors resulted in the highest concentration of total phenolics from grape pomace at 8 minutes and further decreased when the time was increased to 12 minutes at 70°C. Similar results were also observed by (Singh et al. 2011) that explained the fact that with an increase in microwave power and temperature, a decrease in ascorbic acid was observed during the MBE of phenolics from potato peels.

Table 4.5: Total Phenolic Content of grape pomace extract by MBE at different temperatures with different solvents (mg GAE/ g FW)

Solvent	Temperature	TPC (4 minutes)	TPC (8 minutes)	TPC (12 minutes)
		mg GAE/g FW	mg GAE/g FW	mg GAE/g FW
Ethanol	30	31.632±0.79	30.28±0.56	30.21±0.78
	50	32.77±0.38	35.81±0.30	36.67±0.15
	70	40.32±0.15	39.04±0.24	38.97±0.42
Methanol	30	33.96±0.03	32.65±0.25	33.82±0.16
	50	35.18±0.22	34.10±0.22	36.61±0.11
	70	39.43±0.12	40.26±0.08	39.88±0.06
Water	30	11.15±0.66	8.53±0.23	10.94±0.14
	50	10.05±0.18	11.85±0.38	12.76±0.41
	70	22.52±0.37	18.80±.37	21.40±0.48
Ethanol: Water (1:1)	30	17.46±0.38	18.56±0.81	19.20±0.12
	50	20.56±0.11	18.38±0.16	17.17±0.22
	70	18.03±0.12	20.23±0.38	18.06±0.43

DPPH free radical scavenging activity of grape pomace was observed between 9.09 to 47.27% as represented in table 4.6. The antioxidant activity of grape pomace extract gave a higher yield at 70°C for 4 and 8 minutes. A further decrease in antioxidant activity was observed when

temperature and time were increased to 12 minutes at a particular temperature (50 and 70°C). The previous studies also gave similar results in the case of antioxidants extracted from potato peels (Singh et al., 2011), grape marc (Krishnaswamy et al., 2013), blueberry leaves (Routray & Orsat, 2014), and European plums (DiNardo, Brar, Subramanian, & Singh, 2019). ANOVA analysis for DPPH scavenging activity of grape pomace could be attributed that time and temperature were the significant factors for the response variable. On the other hand, the maximum antioxidant yield was observed with alcoholic solvents like ethanol and methanol.

Table 4.6: Radical Scavenging Activity of DPPH free radical for grape pomace extract by MBE at different temperatures with different solvents

Solvent	**Temperature**	**DPPH (%)**	**DPPH (%)**	**DPPH (%)**
		(4 minutes)	**(8 minutes)**	**(12 minutes)**
Ethanol	30	33.29±0.63	31.67±0.26	34.86±2.27
	50	39.68±0.61	38.42±0.49	40.44±0.49
	70	43.59±0.49	42.90±0.17	41.59±2.09
Methanol	30	36.73±3.58	35.34±3.75	38.07±3.28
	50	42.22±4.10	41.13±4.14	42.87±4.33
	70	45.57±3.86	44.12±3.91	43.29±2.71
Water	30	9.11±0.96	9.09±0.55	10.77±0.93
	50	11.10±0.79	11.78±1.68	11.40±0.38
	70	11.49±0.68	11.12±0.55	12.02±0.53
Ethanol:	30	17.05±0.34	15.66±0.43	17.81±0.67
Water (1:1)	50	18.23±0.71	17.39±0.46	20.15±0.50

	70	20.65±0.46	20.12±1.97	21.44±1.21

Antioxidant activity by FRAP gave a yield of antioxidant compounds between 24.12-45.12 mg AE/g FW. It can be observed that with an increase in temperature antioxidant activity was also increased and significantly affected with temperature. However, with an increase in time at a particular temperature antioxidant activity was decreased. Previous studies also proved that with an increase in temperature beyond 60°C and an increase in time, antioxidant activity was significantly affected. Hence, results proved that methanol gave a maximum yield of antioxidant activity at 60°C for 8 minutes.

Table 4.7: Ferric complex (Fe3+) reduction antioxidant potential (FRAP) for grape pomace extract by MBE at different temperatures with different solvents

Solvents	**Temperature (°C)**	**FRAP (mg AE/g) (4 minutes)**	**FRAP (mg AE/g) (8 minutes)**	**FRAP (mg AE/g) (12 minutes)**
Ethanol	30	28.80±1.55	30.61±0.43	32.38±0.37
	50	33.15±1.66	31.57±0.87	35.15±0.93
	70	36.35±0.16	35.34±1.67	38.30±0.81
Water	30	24.28±0.62	26.05±1.31	27.09±0.19
	50	29.27±0.17	25.77±0.26	23.82±0.36
	70	25.19±0.19	28.75±0.62	25.25±0.69
Methanol	30	37.03±2.88	37.57±4.59	37.73±0.67

	50	42.59±1.09	44.34±1.38	43.16±2.23
	70	43.40±0.88	45.52±0.43	48.10±0.56
Ethanol:	30	25.45±0.54	25.96±1.16	27.64±0.83
Water (1:1)	50	30.03±0.30	29.82±1.50	31.14±1.67
	70	34.19±0.63	35.06±1.24	36.07±0.98

4.9.2. Ultrasound-assisted extraction

UAE is another green technology to extract bioactive components in a short period with less energy consumption. The objective of this study was to compare the extraction yield of MBE and UAE with conventional extractions methods such as HRE. Ultrasound extraction technology is also considered as a process intensification technique as it consumes very less energy as compared to conventional extraction methods and increases the yield of bioactive components by cavitation process with the production of bubbles which further ruptures the surface cell and extraction the component. Ultrasonic treatment time and temperature also play a major role in the extraction. The frequency of the ultrasonic systems used for this study was 50 kHz, chosen on the behalf of previous studies which gave maximum yield at this frequency. In ultrasonic extraction, due to bubble explosions, less time is required for the extraction. Therefore, extraction time of 4, 8, and 12 minutes was selected for this study to determine the optimum temperature.

Total phenolic content for ultrasound-assisted extraction gave yield in the range of 8.49-33.87mg GAE/g FW for all the extraction temperature and extraction time. The data in Table 4.8 represented the total phenolic content extracted with different time and temperature on ultrasound-assisted extraction. It can be observed from the data with an increase in temperature for ultrasonic-assisted extraction, an increase in total phenolic content was observed. However, no significant difference

between the extraction time was observed at different temperatures. Hence, Our results are in agreement with the results reported by (DiNardo, Subramanian, et al., 2019) that with an increase in extraction temperature of the ultrasound extraction system, an increase in total phenolic content was observed in the Yellow European plums. ANOVA analysis associated with data represented in the table revealed that temperature was a significant factor for the extraction of antioxidants and phenolics from yellow European plums. On the other hand, it was determined from the ANOVA analysis that time is not a significant factor for the extraction yield of total phenolics ($p>0.05$). On the other hand, a decrease in the extraction yield of bioactive compounds was observed with an increase in extraction time from 8 to 12 minutes. Hence, the extraction time of 8 minutes gave significant yield at 70°C.

Table 4.8: Total Phenolic Content of grape pomace extract by UAE at different temperatures with different solvents (mg GAE/ g FW)

Solvent	**Temperature**	**TPC (4 minutes)**	**TPC (8 minutes)**	**TPC (12 minutes)**
		mg GAE/g FW	mg GAE/g FW	mg GAE/g FW
Ethanol	30	23.39±0.35	23.82±0.04	24.51±0.32
	50	25.02±0.32	26.57±0.21	25.45±0.23
	70	31.39±0.08	32.37±0.43	31.44±0.32
Water	30	9.76±0.47	8.49±0.58	10.98±0.89
	50	12.30±0.52	13.81±0.66	11.37±0.47
	70	17.18±0.30	18.94±0.36	16.45±1.13
Methanol	30	30.31±0.38	29.22±0.23	28.99±0.26

	50	31.04±0.52	30.89±0.08	30.21±0.21
	70	32.30±0.58	33.04±0.34	32.52±0.09
Ethanol :	30	16.35±1.10	17.31±0.44	16.29±0.20
Water(1:1)	50	16.47±0.33	17.20±0.17	18.04±0.20
	70	23.60±0.22	25.23±0.52	24.06±0.84

DPPH radical scavenging activity of grape pomace extract extracted with UAE represented in table 4.9 for different extraction temperature and time. Radical scavenging activity of all the extracts varied from 10.02-31.8% for all the processing temperatures and time. Operating the ultrasound-assisted extraction at 50°C for 8 minutes gave the maximum yield, which increased from 4 to 8 minutes and further decreased after 8 minutes. With further increase in time beyond 8 minutes resulted in a decrease in scavenging activity. Hence, extraction temperature did not give any significant difference after 8 minutes' extraction time. (Goula et al., 2016) reported similar results with an increase in extraction time at 50 and 70°C reduced the extraction yield of antioxidants and phenolics. ANOVA analysis of ultrasound-assisted extraction yield with respect time and temperature gave similar results as that of total phenolics. Hence, time is not a significant factor for antioxidant activity yield with ultrasound-assisted extraction as giving p-value >0.05.

Table 4.9: Radical Scavenging Activity of DPPH free radical for grape pomace extract by UAE at different temperatures with different solvents

Solvent	**Temperature**	**DPPH (%) (4 minutes)**	**DPPH (%) (8 minutes)**	**DPPH (%) (12 minutes)**
Ethanol	30	29.79±0.21	25.97±0.56	26.14±0.62

	50	27.52±0.463	26.76±0.59	27.99±1.02
	70	28.30±0.67	30.01±0.62	32.26±0.97
Water	30	10.48±0.58	10.67±0.33	11.99±0.67
	50	12.70±0.79	13.22±1.65	12.51±0.48
	70	12.76±0.86	12.32±0.64	13.25±0.56
Methanol	30	30.20±0.72	27.67±4.23	30.81±3.73
	50	35.15±4.74	34.19±4.84	36.23±4.51
	70	39.60±4.35	37.22±4.67	41.12±2.93
Ethanol : Water (1:1)	30	16.53±0.26	16.76±0.70	17.22±0.69
	50	18.58±0.47	17.79±0.69	17.70±0.57
	70	19.57±0.51	18.98±2.00	20.30±1.23

Antioxidant activity by ferric ions reduction potential of UAE yield 16.2-27.1 mg AE/g FW for all the extraction temperature and time represented in table 4.9. FRAP antioxidant activity gave similar results as that of DPPH scavenging activity that with an increase in temperature antioxidant activity was increased and with an increase in extraction time, antioxidant activity was decreased. FRAP results revealed that maximum antioxidant activity was observed at 50°C for 8 minutes with ethanol and methanol solvents. Hence, beyond 50°C temperature, there was not a significant difference observed in the antioxidant activity of the grape pomace extract. ANOVA analysis of FRAP results revealed that time is also not a significant factor for the extraction yield of antioxidant activity with ultrasound-assisted extraction as it gives p-value >0.05.

Table 4.10: Ferric complex (Fe3+) reduction antioxidant potential (FRAP) for grape pomace extract by UAE at different temperatures with different solvents

Solvents	Temperature (°C)	FRAP (mg AE/g) (4 minutes)	FRAP (mg AE/g) (8 minutes)	FRAP (mg AE/g) (12 minutes)
Ethanol	30	22.02±1.78	21.11±0.86	21.06±1.61
	50	23.69±2.01	27.27±1.16	27.65±1.37
	70	28.94±0.38	30.71±1.44	29.38±2.37
Water	30	22.04±1.07	22.90±0.78	22.27±0.09
	50	23.36±0.55	24.16±0.35	24.85±2.19
	70	25.71±1.46	25.65±1.07	26.46±1.79
Methanol	30	33.12±0.70	33.58±0.40	34.13±0.23
	50	35.31±0.22	35.94±0.95	36.23±0.86
	70	36.95±0.26	37.05±0.50	37.06±0.48
Ethanol:Water (1:1)	30	18.03±0.76	17.88±1.14	16.97±0.86
	50	21.29±0.57	19.91±0.23	22.71±0.46
	70	24.89±1.12	24.23±0.15	26.37±0.60

4.10. Conclusion

Process intensification is the major step in novel technology to increase the yield and reduce the time and energy requirements. Two novel technologies microwave-assisted extraction and ultrasound technology were investigated to increase or intensify the yield and process of the conventional extraction method. These novel technologies omit the drawbacks associated with

conventional heat reflux such as long extraction time, large solvent, and energy consumption with a greater yield of bioactive components i.e. phenolic compounds and antioxidants. Different experiments with varying parameters such as time and temperature were carried out using MBE and UAE to determine the best-optimized yield of bioactive components.

Figure 4.1 compares extraction yield of DPPH for different solvents at 70 C.

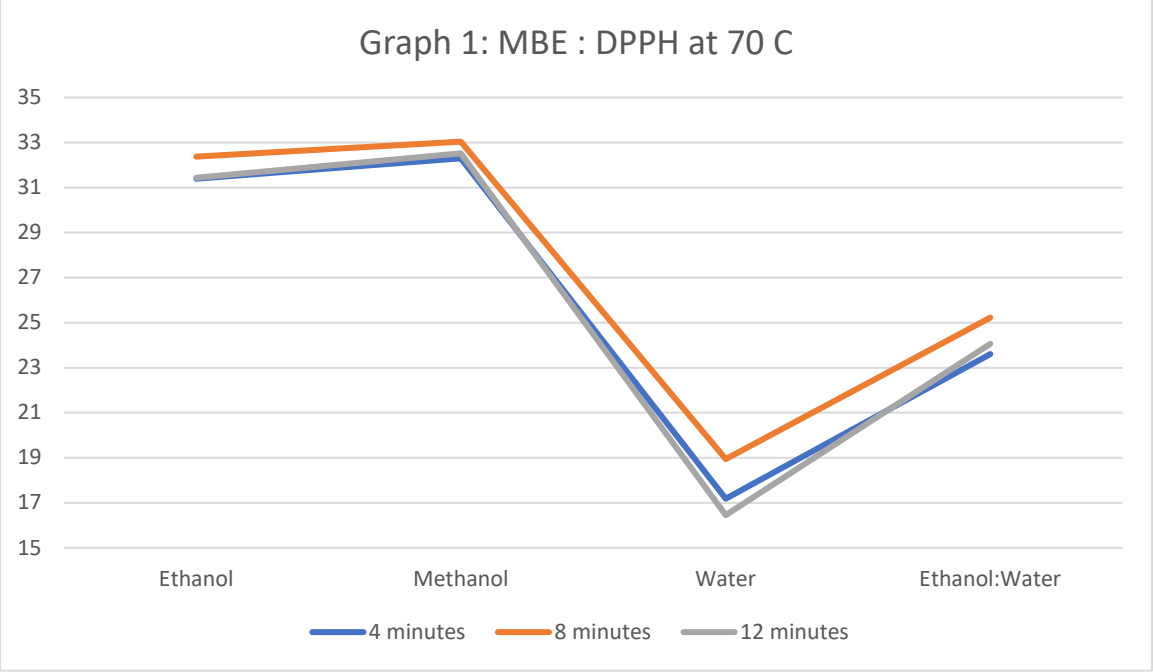

Figure 4.1: Compare DPPH extraction yield in MBE for different solvents at 70 C

The highest extracted rates are obtained for 8 minutes time in microwave based extraction. This is in relevance to the degradation of phenols when subjected to longer duration of heating.

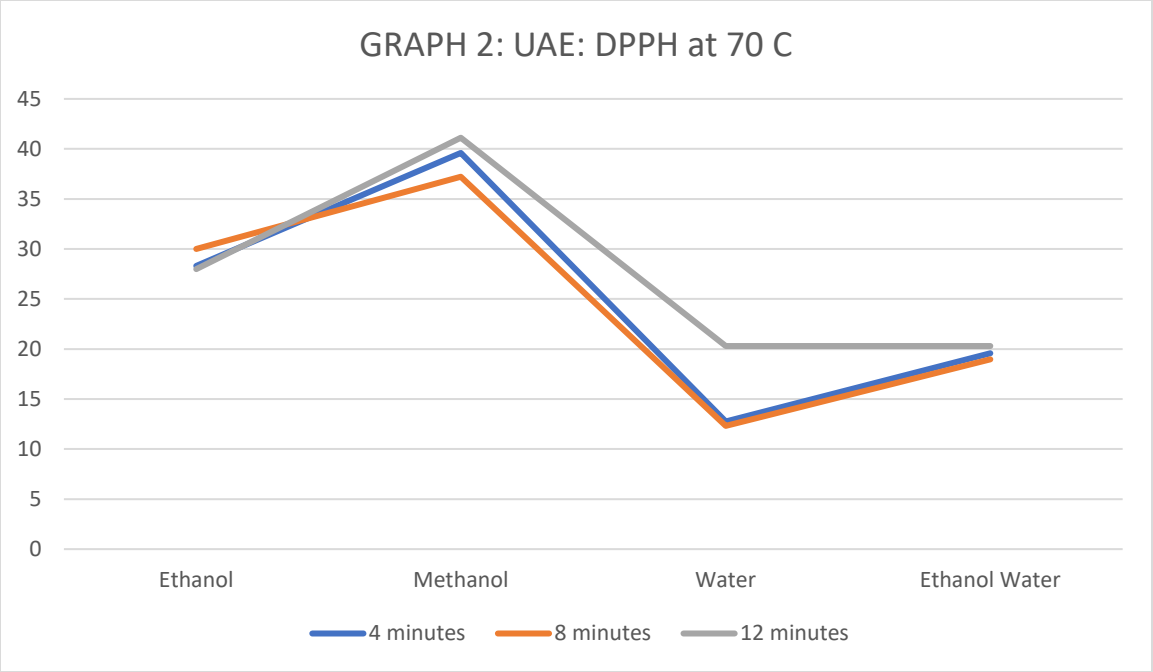

Figure 4.2: Compare extraction yield of DPPH in UAE for different solvents at 70 C

The highest extracted rates are obtained for 12 minutes time in case of ultrasonic assisted extraction.

Figure 4.3 compares extraction yield for different solvents for FRAP.

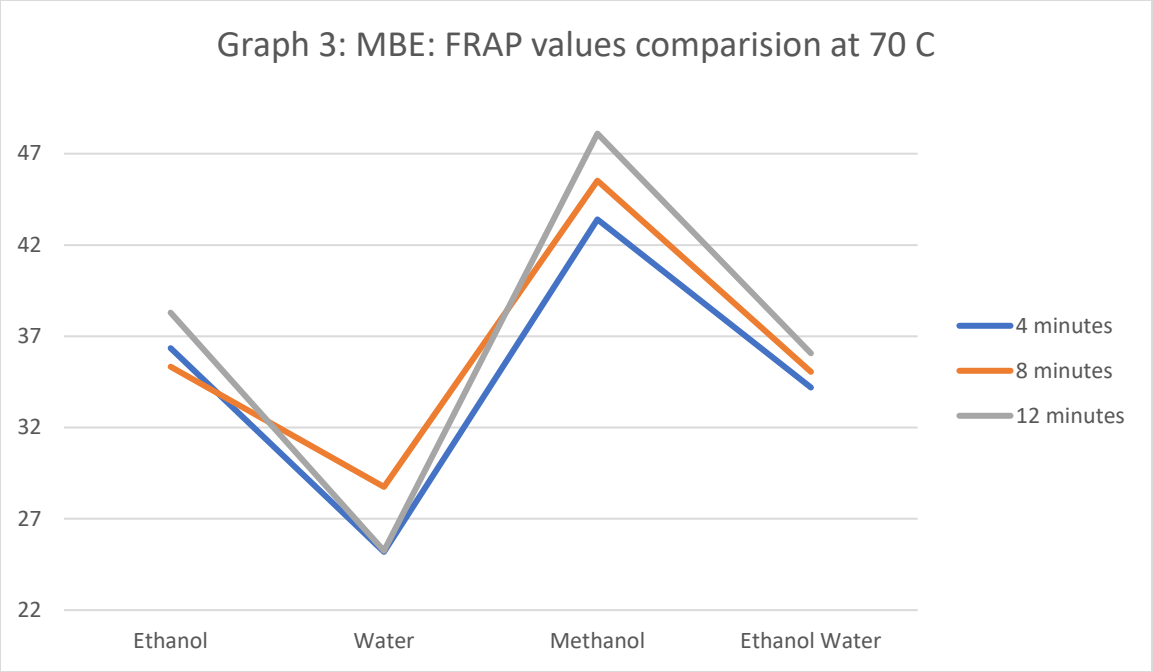

Figure 4.3: Compare extraction yield of FRAP in MBE for different solvents at 70 C

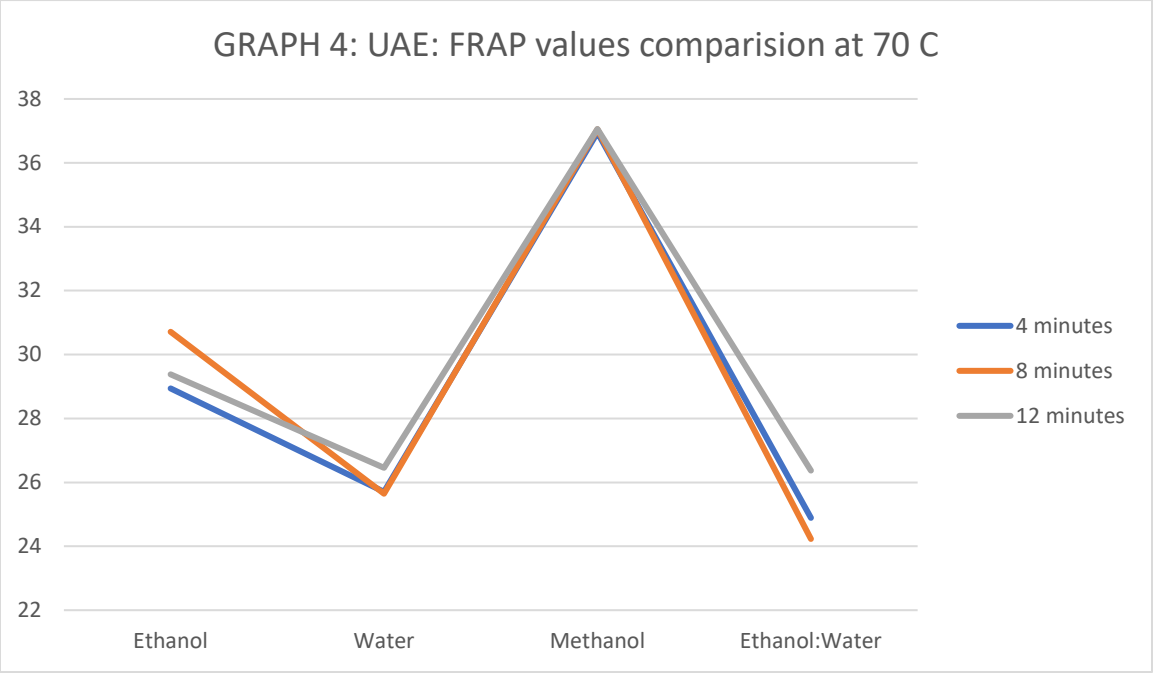

Figure 4.4: Compare extraction yield of FRAP in UAE for different solvents at 70 C

Here, Highest FRAP values were obtained for methanol for 12 minutes of extraction time. This progression is relevant to the relationship of extraction with time. As the time increases, extraction rates increase significantly. Hence, from the results of this study, it concluded that temperature of 70°C for 8 minutes with ethanol and methanol solvents, gave the highest yield of antioxidants and total phenolics for both advanced processes over conventional HRE.As the temperature increases, there is significant increase in extracted content of phenols for both MBE & UAE as shown by the figure

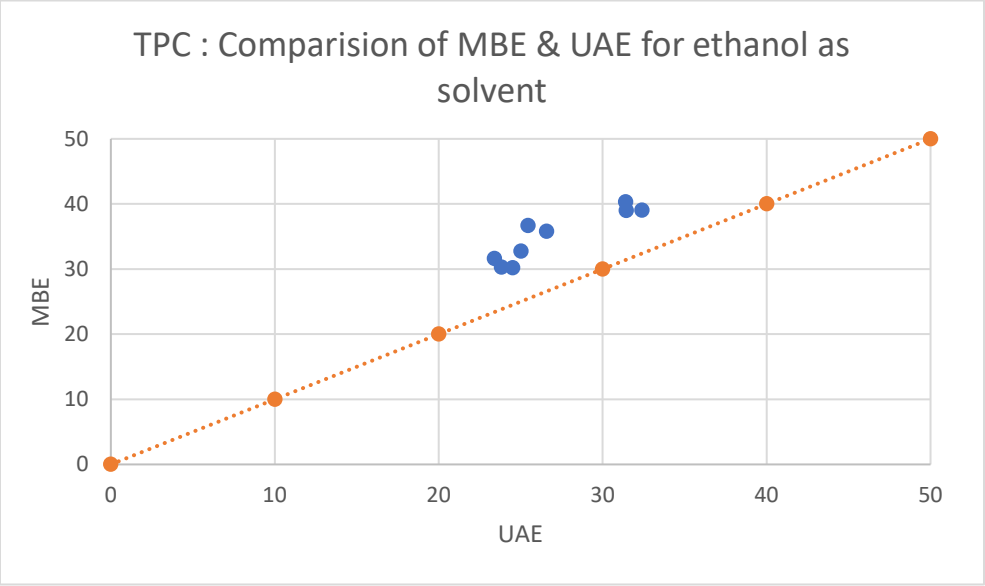

Figure 4.5: Comparison of increase in temperature with TPC for type of extraction

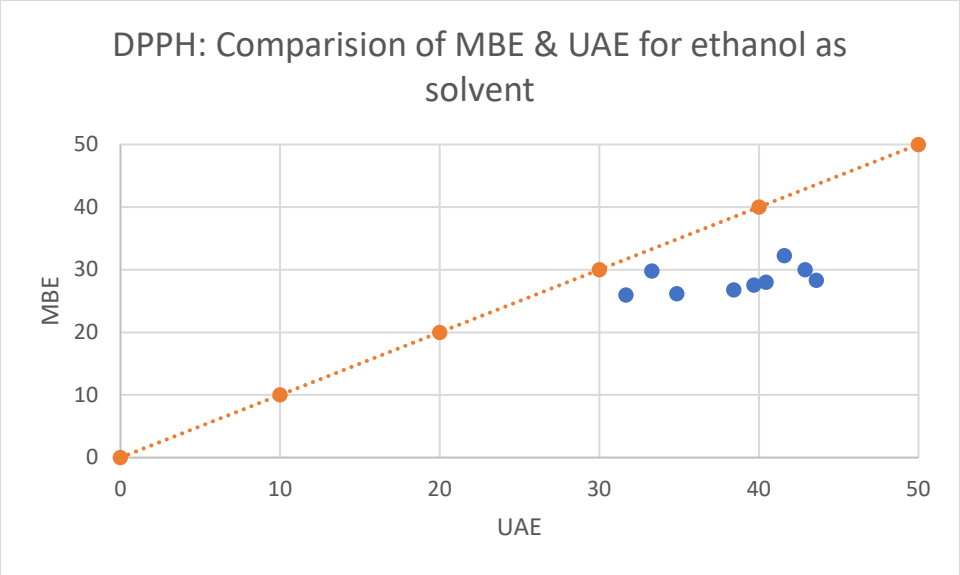

Figure 4.6: Comparison of increase in temperature with DPPH for type of extraction

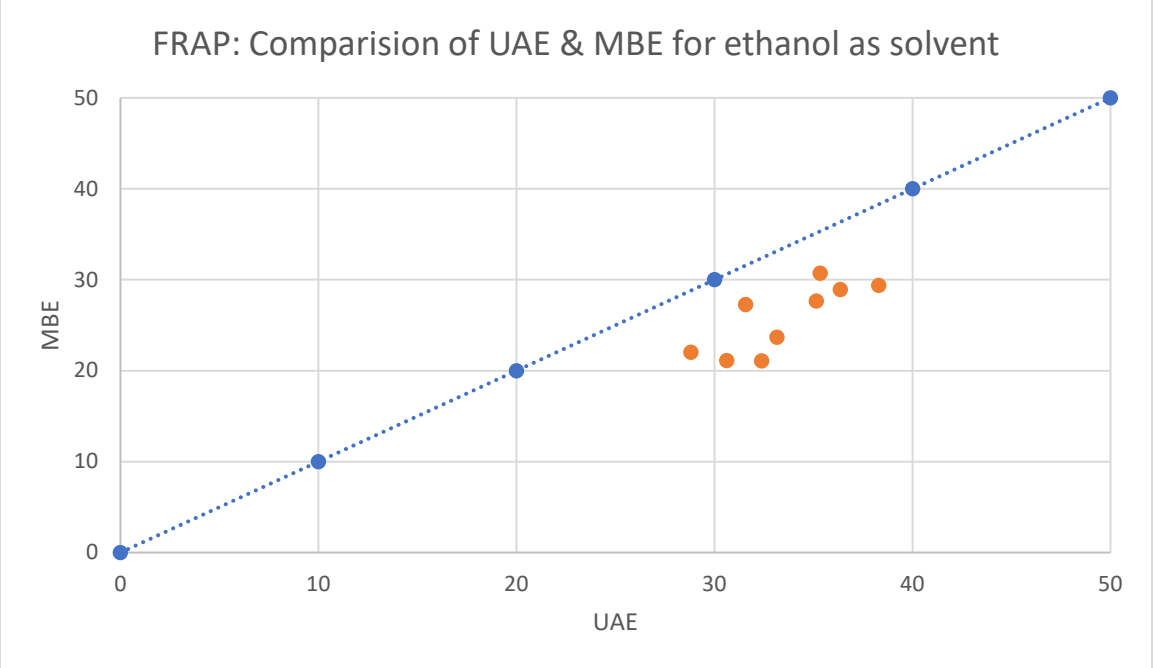

Figure 4.7: Comparison of increase in temperature with FRAP for type of extraction

Comparing the two extraction methods for the TPC, with increase in temperature, a direct increase in TPC in MAE and UAE reported. However, TPC was highest in MAE. All the values obtained above the average values. Hence, it is inferred that microwave technique was more effective in extracting total phenols in comparison UAE. This can be due to rapid volumetric heating of the solution in microwave in a short time resulting in less extraction time and more yield. Similar observations were seen in FRAP and DPPH, higher values of content in MAE extraction. Similar observations were made in a study on enzyme assisted ultrasonic extraction and microwave extraction in water chestnut. Researchers observed 1.48% higher yield in MAE. Extraction of flavonoids was reported to increase with MAE (Reddy, 2020). Thus, in future studies, MAE can be effectively studied for wide range of compounds and in combination with UAE and it will be interesting to see how the yield changes in the combination strategy. Highest FRAP values for methanol for 8 minutes of extraction time. As UAE is related to cavitation, increase in temperature after a certain time degrades the chemical composition of ferric reducing agents. As the time increases, extraction rates starts decreasing significantly.

CHAPTER 5
SUMMARY, CONCLUSION AND FUTURE WORK

Global culture of sustainability is evolving. There is an increasing shift from animal derived products to the plant-based ones. Previous plant-based production lines are being extended to utilize their wastes to extract viable biobased phytochemicals. Cosmetics and pharmaceuticals have emerged as the main consumer of these bioactive compounds. In this drift, fruits have emerged as a main source of phytochemicals. The basic idea lies in the fact that high polyphenols and antioxidants activity make grapes a good source for wine production. Yeast surviving naturally on the grape skin makes it a prime contender to perform fermentation. Wine marc or grape pomace is the residue of wine production. It consists of grape skin, seeds and stalks. This is used as raw material for distilled products called as grapy, which is a type of brandy. But most of it goes to landfill. Utilizing grape pomace has high potential for value addition.

In this study, the aim was to compare different solvents and extraction technique for determining maximum total phenolic content and antioxidant activity on two different samples of grape pomace. In one scenario, fresh grape pomace was ground and used for the study. In second scenarios, to increase the extraction area, fresh grape pomace was freeze dried and subjected to similar parameters as that of fresh one.

The first objective of the study was to determine the solvent which would be suitable for heat-based extraction at three different temperatures (30, 50 and 70 °C) for as constant time of 90 minutes. Type of solvents included in the study were both polar(water) and nonpolar (ethanol and methanol). This study was performed with a mixture of water and ethanol too, this would help in reducing the cost involved in removing nonpolar solvent at the end of extraction and would help in extracting polar solutes too. Effect of different solvents on total phenolic yield and antioxidant

activity of bioactive compounds were studied. Extraction temperature of 70 °C proved to be the best for methanol as solvent.

As methanol is not considered a GRAS (Generally Recognized As Safe), ethanol and water were considered for further studies. Second objective of the study was to determine the optimal conditions for the maximum extraction of polyphenols and antioxidants using microwave and ultrasound assisted extraction processes. Three different temperatures (50, 60, and 70 °C) and time were tested. It was observed that the temperature of 70 °C at 8 minutes gave highest possible viable polyphenols for both the aforementioned processes as compared to the conventional heat reflux.

Proceeding further the analysis of GP extract can be performed by High Pressure Liquid Chromatography (HPLC). The basic principle of HPLC lies in the reduction in the movement of solute(extract) in the stationary phase (reverse phase column). But the separation of different constituents of solute depends on a mobile phase involved in the process, here called as eluent. Different constituents are obtained at different times. Eluent or solvents are specific for anthocyanins, phenolic acids and anthoxanthins.

For Anthocyanins; Mobile phase is W:Water-formic acid(9:1) and A:Acetonitrile-formic acid(9:1).The gradient is maintained between the two eluents. Generally, 10%-25% A for the interval of 10 minutes, 25-31% A for 5 minutes and 31-40% for 5 minutes helps in extracting anthocyanins.

For Phenolic Acids; Mobile phase is W: Water- Acetic acid(2:8) and A: Acetic Acid-Water(0.5 %) and Acetonitrile(5:5) .The gradient follows in pattern as follows: 10%-15% A for 10 mins, 15% A isocratic for 3 minutes, 15%-25% A for 7 minutes and 25%-50% A for 30 minutes. Absorptions are recorded for hydroxybenzoic acid (280 nm) and hydroxycinnamic acid (320 nm).

Quantitative analysis can be performed by standard curves of malvidin and quercetin.

Future work: The work reported in this study analyzes the viability of grape pomace as a nutraceutical source for food and pharmaceutical industries. In this study the total phenolic compound obtained from grape pomace was quantified, but still more work is required to identify and quantify predominant phytochemicals that are present in grapes including anthocyanins and resveratrol. Future work should also evaluate the effectiveness other advanced extraction technique such as super-critical fluid extraction and also investigate encapsulation strategies to be used for delivery of phytochemicals obtained from grape pomace into different food, pharmaceutical and cosmetic products.

REFERENCES:

Al-juhaimi, F., Ghafoor, K., Özcan, M. M., Jahurul, M. H. A., Babiker, E. E., Jinap, S., … Zaidul, I. S. M. (2018, October 1). Effect of various food processing and handling methods on preservation of natural antioxidants in fruits and vegetables. *Journal of Food Science and Technology*. Springer. https://doi.org/10.1007/s13197-018-3370-0

Aliakbarian, B., Fathi, A., Perego, P., & Dehghani, F. (2012). Extraction of antioxidants from winery wastes using subcritical water. *Journal of Supercritical Fluids*, *65*, 18–24. https://doi.org/10.1016/j.supflu.2012.02.022

Álvarez, A., Poejo, J., Matias, A. A., Duarte, C. M. M., Cocero, M. J., & Mato, R. B. (2017). Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity. *Food and Bioproducts Processing*, *106*, 162–170. https://doi.org/https://doi.org/10.1016/j.fbp.2017.09.007

Amico, V., Chillemi, R., Mangiafico, S., Spatafora, C., & Tringali, C. (2008). Polyphenol-enriched fractions from Sicilian grape pomace: HPLC–DAD analysis and antioxidant activity. *Bioresource Technology*, *99*(13), 5960–5966.

Aybastıer, Ö., Işık, E., Şahin, S., & Demir, C. (2013). Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. *Industrial Crops and Products*, *44*, 558–565.

Baghdikian, B., Filly, A., Fabiano-Tixier, A. S., Petitcolas, E., Mabrouki, F., Chemat, F., & Ollivier, É. (2016). Extraction by solvent using microwave and ultrasound-assisted techniques followed by HPLC analysis of Harpagoside from Harpagophytum procumbens and comparison with conventional solvent extraction methods. *Comptes Rendus Chimie*,

19(6), 692–698. https://doi.org/10.1016/j.crci.2016.02.020

Balachandran, S., Kentish, S. E., Mawson, R., & Ashokkumar, M. (2006). Ultrasonic enhancement of the supercritical extraction from ginger. *Ultrasonics Sonochemistry*, *13*(6), 471–479. https://doi.org/https://doi.org/10.1016/j.ultsonch.2005.11.006

Ballard, T. S., Mallikarjunan, P., Zhou, K., & O'Keefe, S. F. (2009). Optimizing the Extraction of Phenolic Antioxidants from Peanut Skins Using Response Surface Methodology. *Journal of Agricultural and Food Chemistry*, *57*(8), 3064–3072. https://doi.org/10.1021/jf8030925

Baumann, A. R., Martin, S. E., & Feng, H. (2009). Removal of Listeria monocytogenes biofilms from stainless steel by use of ultrasound and ozone. *Journal of Food Protection*, *72*(6), 1306–1309.

Beres, C., Costa, G. N. S., Cabezudo, I., da Silva-James, N. K., Teles, A. S. C., Cruz, A. P. G., … Freitas, S. P. (2017). Towards integral utilization of grape pomace from winemaking process: A review. *Waste Management*, *68*, 581–594. https://doi.org/10.1016/j.wasman.2017.07.017

Bhalla, T. C., & Joshi, M. (1994). Protein enrichment of apple pomace by co-culture of cellulolytic moulds and yeasts. *World Journal of Microbiology and Biotechnology*, *10*(1), 116–117. https://doi.org/10.1007/BF00357577

Bonfigli, M., Godoy, E., Reinheimer, M. A., & Scenna, N. J. (2017). Comparison between conventional and ultrasound-assisted techniques for extraction of anthocyanins from grape pomace. Experimental results and mathematical modeling. *Journal of Food Engineering*, *207*, 56–72. https://doi.org/10.1016/j.jfoodeng.2017.03.011

Brar, H. S., Subramanian, J., Nair, G. R., Singh, A., & Engineering, B. (2020). Effect of chemical pre-treatment on drying and Physio-Chemical characteristics of Yellow European Plums . *International Journal of Fruit Science*, *00*(00), 1–28. https://doi.org/10.1080/15538362.2020.1717403

Cáceres, C. X., Cáceres, R. E., Hein, D., Molina, M. G., & Pia, J. M. (2012). Biogas production from grape pomace: Thermodynamic model of the process and dynamic model of the power generation system. *International Journal of Hydrogen Energy*, *37*(13), 10111–10117. https://doi.org/https://doi.org/10.1016/j.ijhydene.2012.01.178

Casagrande, M., Zanela, J., Pereira, D., de Lima, V. A., Oldoni, T. L. C., & Carpes, S. T. (2019). Optimization of the extraction of antioxidant phenolic compounds from grape pomace using response surface methodology. *Journal of Food Measurement and Characterization*, *13*(2), 1120–1129. https://doi.org/10.1007/s11694-018-00027-x

Celli, G. B., Ghanem, A., & Brooks, M. S.-L. (2015). Optimization of ultrasound-assisted extraction of anthocyanins from haskap berries (Lonicera caerulea L.) using Response Surface Methodology. *Ultrasonics Sonochemistry*, *27*, 449–455.

Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, *34*, 540–560.

Chen, M., Zhao, Y., & Yu, S. (2015). Optimisation of ultrasonic-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from sugar beet molasses. *Food Chemistry*, *172*, 543–550.

Czochanska, Z., Foo, L. Y., & Porter, L. J. (1979). Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry*, *18*(11), 1819–1822.

Da Silva, J. M. R., Darmon, N., Fernandez, Y., & Mitjavila, S. (1991). Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *Journal of Agricultural and Food Chemistry*, *39*(9), 1549–1552.

de Lucas, A., Martinez de la Ossa, E., Rincón, J., Blanco, M. A., & Gracia, I. (2002). Supercritical fluid extraction of tocopherol concentrates from olive tree leaves. *The Journal of Supercritical Fluids*, *22*(3), 221–228. https://doi.org/https://doi.org/10.1016/S0896-8446(01)00132-2

de Melo, M. M. R., Silvestre, A. J. D., Portugal, I., & Silva, C. M. (2017). *Emerging technologies for the recovery of valuable compounds from coffee processing by-products*. *Handbook of Coffee Processing By-Products: Sustainable Applications*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-811290-8.00005-0

DiNardo, A., Brar, H. S., Subramanian, J., & Singh, A. (2019). Optimization of microwave-assisted extraction parameters and characterization of phenolic compounds in Yellow European Plums. *Canadian Journal of Chemical Engineering*, *97*(1), 256–267. https://doi.org/10.1002/cjce.23237

DiNardo, A., Subramanian, J., & Singh, A. (2018). Investigation of Antioxidant Content and Capacity in Yellow European Plums. *International Journal of Fruit Science*, *18*(1), 99–116. https://doi.org/10.1080/15538362.2017.1381873

DiNardo, A., Subramanian, J., & Singh, A. (2019). Intensification of phenolic extraction from yellow European plums by use of conventional, microwave-, and ultrasound-assisted

extraction. *Separation Science and Technology (Philadelphia)*, *00*(00), 1–14. https://doi.org/10.1080/01496395.2019.1567547

Dolatowski, Z. J., Stadnik, J., & Stasiak, D. (2007). Applications of ultrasound in food technology. *Acta Scientiarum Polonorum Technologia Alimentaria*, *6*(3), 88–99.

Dwyer, Kyle, Farah Hosseinian, and Michel R. Rod. (2014). The market potential of grape waste alternatives. *Journal of Food Research*, 3(2), 91-91.

El Gharras, H. (2009). Polyphenols: food sources, properties and applications - a review. *International Journal of Food Science & Technology*, *44*(12), 2512–2518. https://doi.org/10.1111/j.1365-2621.2009.02077.x

El Hassani, S. E. A., Driouich, A., Mellouk, H., Bejjany, B., Dani, A., & Digua, K. (2019). Extraction of phenolic from Moroccan grape pomace: Optimization of decoction extraction of phenolic compounds using response surface methodology. *Mediterranean Journal of Chemistry*, *7*(6), 423–432. https://doi.org/10.13171/mjc76198121610hm

Elbadrawy, E., & Sello, A. (2016). Evaluation of nutritional value and antioxidant activity of tomato peel extracts. *Arabian Journal of Chemistry*, *9*, S1010–S1018. https://doi.org/10.1016/j.arabjc.2011.11.011

Escribano-Bailón, M., Guerra, M. V, Rivas-Gonzalo, J., & Santos-Buelga, C. (1994). Procyanidin composition in skin and seed from grapes. *Polyphenol Comun*, *94*, 225–227.

Ferreira, S. R. S., Nikolov, Z. L., Doraiswamy, L. K., Meireles, M. A. A., & Petenate, A. J. (1999). Supercritical fluid extraction of black pepper (Piper nigrun L.) essential oil. *The Journal of Supercritical Fluids*, *14*(3), 235–245.

https://doi.org/https://doi.org/10.1016/S0896-8446(98)00092-8

Fontana, A. R., Antoniolli, A., & Bottini, R. (2013). Grape pomace as a sustainable source of bioactive compounds: extraction, characterization, and biotechnological applications of phenolics. *Journal of Agricultural and Food Chemistry*, *61*(38), 8987–9003.

Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Mayor, L., Ballesteros, R., Conidi, C., & Cassano, A. (2015). Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (Citrus paradisi L.) solid wastes. *LWT-Food Science and Technology*, *64*(2), 1114–1122.

García-Lomillo, J., & González-SanJosé, M. L. (2017). Applications of wine pomace in the food industry: approaches and functions. *Comprehensive Reviews in Food Science and Food Safety*, *16*(1), 3–22.

Gassara, F., Brar, S. K., Pelletier, F., Verma, M., Godbout, S., & Tyagi, R. D. (2011). Pomace waste management scenarios in Québec—Impact on greenhouse gas emissions. *Journal of Hazardous Materials*, *192*(3), 1178–1185. https://doi.org/https://doi.org/10.1016/j.jhazmat.2011.06.026

González-Centeno, M. R., Knoerzer, K., Sabarez, H., Simal, S., Rosselló, C., & Femenia, A. (2014). Effect of acoustic frequency and power density on the aqueous ultrasonic-assisted extraction of grape pomace (Vitis vinifera L.)–a response surface approach. *Ultrasonics Sonochemistry*, *21*(6), 2176–2184.

González-Paramás, A. M., Esteban-Ruano, S., Santos-Buelga, C., de Pascual-Teresa, S., & Rivas-Gonzalo, J. C. (2004). Flavanol content and antioxidant activity in winery byproducts. *Journal of Agricultural and Food Chemistry*, *52*(2), 234–238.

Gonzalex-San Jose, M. L., & Diez De Bethencourt, C. (1987). Presencia de pigmentos antociánicos en el reino vegetal con referencia especial al genero Vitis. In *Anales de edafologia y agrobiologia* (Vol. 46, pp. 157–162).

Goula, A. M., Thymiatis, K., & Kaderides, K. (2016). Valorization of grape pomace: Drying behavior and ultrasound extraction of phenolics. *Food and Bioproducts Processing*, *100*, 132–144. https://doi.org/10.1016/j.fbp.2016.06.016

Guerra-Rivas, C., Gallardo, B., Mantecón, Á. R., del Álamo-Sanza, M., & Manso, T. (2017). Evaluation of grape pomace from red wine by-product as feed for sheep. *Journal of the Science of Food and Agriculture*, *97*(6), 1885–1893.

Hammi, K. M., Jdey, A., Abdelly, C., Majdoub, H., & Ksouri, R. (2015). Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian Zizyphus lotus fruits using response surface methodology. *Food Chemistry*, *184*, 80–89.

Hawthorne, S. B., Grabanski, C. B., Martin, E., & Miller, D. J. (2000). Comparisons of Soxhlet extraction, pressurized liquid extraction, supercritical fluid extraction and subcritical water extraction for environmental solids: recovery, selectivity and effects on sample matrix. *Journal of Chromatography A*, *892*(1), 421–433. https://doi.org/https://doi.org/10.1016/S0021-9673(00)00091-1

Hayat, K., Zhang, X., Chen, H., Xia, S., Jia, C., & Zhong, F. (2010). Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity. *Separation and Purification Technology*, *73*(3), 371–376. https://doi.org/https://doi.org/10.1016/j.seppur.2010.04.026

He, B., Zhang, L.-L., Yue, X.-Y., Liang, J., Jiang, J., Gao, X.-L., & Yue, P.-X. (2016).

Optimization of ultrasound-assisted extraction of phenolic compounds and anthocyanins from blueberry (Vaccinium ashei) wine pomace. *Food Chemistry, 204*, 70–76.

Hilger, H., & Humer, M. (2003). Biotic landfill cover treatments for mitigating methane emissions. *Environmental Monitoring and Assessment*, *84*(1–2), 71–84.

Hiranvarachat, B., & Devahastin, S. (2014). Enhancement of microwave-assisted extraction via intermittent radiation: Extraction of carotenoids from carrot peels. *Journal of Food Engineering*, *126*, 17–26. https://doi.org/10.1016/j.jfoodeng.2013.10.024

Hogervorst, J. C., Miljić, U., & Puškaš, V. (2017). 5 - Extraction of Bioactive Compounds from Grape Processing By-Products. In C. M. B. T.-H. of G. P. B.-P. Galanakis (Ed.) (pp. 105–135). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-809870-7.00005-3

Husni, A., Shin, I.-S., You, S., & Chung, D. (2009). Antioxidant properties of water and aqueous ethanol extracts and their crude saponin fractions from a far-eastern sea cucumber, Stichopus japonicus. *Food Science and Biotechnology*, *18*(2), 419–424.

Jara-Palacios, M. José, Hernanz, D., Cifuentes-Gomez, T., Escudero-Gilete, M. L., Heredia, F. J., & Spencer, J. P. E. (2015). Assessment of white grape pomace from winemaking as source of bioactive compounds, and its antiproliferative activity. *Food Chemistry*, *183*, 78–82. https://doi.org/10.1016/j.foodchem.2015.03.022

Jara-Palacios, M José, Escudero-Gilete, M. L., Hernández-Hierro, J. M., Heredia, F. J., & Hernanz, D. (2017). Cyclic voltammetry to evaluate the antioxidant potential in winemaking by-products. *Talanta*, *165*, 211–215.

Jara-Palacios, M José, Hernanz, D., González-Manzano, S., Santos-Buelga, C., Escudero-Gilete,

M. L., & Heredia, F. J. (2014). Detailed phenolic composition of white grape by-products by RRLC/MS and measurement of the antioxidant activity. *Talanta*, *125*, 51–57.

Jara-Palacios, María José. (2019). Wine Lees as a Source of Antioxidant Compounds. *Antioxidants*, *8*(2), 45.

Joana Gil-Chávez, G., Villa, J. A., Fernando Ayala-Zavala, J., Basilio Heredia, J., Sepulveda, D., Yahia, E. M., & González-Aguilar, G. A. (2013). Technologies for Extraction and Production of Bioactive Compounds to be Used as Nutraceuticals and Food Ingredients: An Overview. *Comprehensive Reviews in Food Science and Food Safety*, *12*(1), 5–23. https://doi.org/10.1111/1541-4337.12005

Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (Vitis vinifera L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry*, *52*(14), 4360–4367.

Kassama, L. S., Shi, J., & Mittal, G. S. (2008). Optimization of supercritical fluid extraction of lycopene from tomato skin with central composite rotatable design model. *Separation and Purification Technology*, *60*(3), 278–284. https://doi.org/https://doi.org/10.1016/j.seppur.2007.09.005

Khaw, K. Y., Parat, M. O., Shaw, P. N., & Falconer, J. R. (2017). Solvent supercritical fluid technologies to extract bioactive compounds from natural sources: A review. *Molecules*, *22*(7). https://doi.org/10.3390/molecules22071186

Krishnaswamy, K., Orsat, V., Gariépy, Y., & Thangavel, K. (2013). Optimization of Microwave-Assisted Extraction of Phenolic Antioxidants from Grape Seeds (Vitis vinifera). *Food and Bioprocess Technology*, *6*(2), 441–455. https://doi.org/10.1007/s11947-012-0800-2

Kubrakova, I. V, & Toropchenova, E. S. (2008). Microwave heating for enhancing efficiency of analytical operations. *Inorganic Materials*, *44*(14), 1509–1519.

Kumanda, C., Mlambo, V., & Mnisi, C. (2019). From Landfills to the Dinner Table: Red Grape Pomace Waste as a Nutraceutical for Broiler Chickens. *Sustainability*, *11*(7), 1931. https://doi.org/10.3390/su11071931

Ky, I., Lorrain, B., Kolbas, N., Crozier, A., & Teissedre, P.-L. (2014). Wine by-products: phenolic characterization and antioxidant activity evaluation of grapes and grape pomaces from six different French grape varieties. *Molecules*, *19*(1), 482–506.

Laghari, A. Q., Memon, S., Nelofar, A., & Laghari, A. H. (2011). Extraction, Identification and Antioxidative Properties of the Flavonoid-Rich Fractions from Leaves and Flowers of Cassia <i>angustifolia</i> *American Journal of Analytical Chemistry*, *02*(08), 871–878. https://doi.org/10.4236/ajac.2011.28100

Larrauri, J. A., Rupérez, P., & Saura-Calixto, F. (1997). Effect of Drying Temperature on the Stability of Polyphenols and Antioxidant Activity of Red Grape Pomace Peels. *Journal of Agricultural and Food Chemistry*, *45*(4), 1390–1393. https://doi.org/10.1021/jf960282f

Lei, F., & VanderGheynst, J. S. (2000). The effect of microbial inoculation and pH on microbial community structure changes during composting. *Process Biochemistry*, *35*(9), 923–929. https://doi.org/https://doi.org/10.1016/S0032-9592(99)00155-7

Leite, P., Salgado, J. M., Venâncio, A., Domínguez, J. M., & Belo, I. (2016). Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase production by solid-state fermentation. *Bioresource Technology*, *214*, 737–746. https://doi.org/https://doi.org/10.1016/j.biortech.2016.05.028

Louli, V., Ragoussis, N., & Magoulas, K. (2004). Recovery of phenolic antioxidants from wine industry by-products. *Bioresource Technology*, *92*(2), 201–208. https://doi.org/10.1016/j.biortech.2003.06.002

Luque de Castro, M. D., & Priego-Capote, F. (2010). Soxhlet extraction: Past and present panacea. *Journal of Chromatography A*, *1217*(16), 2383–2389. https://doi.org/https://doi.org/10.1016/j.chroma.2009.11.027

Lutterodt, H., Slavin, M., Whent, M., Turner, E., & Yu, L. L. (2011). Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chemistry*, *128*(2), 391–399.

Majd, M. H., Rajaei, A., Bashi, D. S., Mortazavi, S. A., & Bolourian, S. (2014). Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (Phlomidoschema parviflorum) leaves using response surface methodology. *Industrial Crops and Products*, *57*, 195–202.

Makris, D. P., Boskou, G., & 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*(2), 125–132. https://doi.org/https://doi.org/10.1016/j.jfca.2006.04.010

Manca, M. L., Marongiu, F., Castangia, I., Catalán-Latorre, A., Caddeo, C., Bacchetta, G., … Manconi, M. (2016). Protective effect of grape extract phospholipid vesicles against oxidative stress skin damages. *Industrial Crops and Products*, *83*, 561–567. https://doi.org/10.1016/j.indcrop.2015.12.069

Medina, K., Boido, E., Dellacassa, E., & Carrau, F. (2005). Yeast Interactions with

Anthocyanins during Red Wine Fermentation. *American Journal of Enology and Viticulture*, *56*(2), 104 LP – 109. Retrieved from http://www.ajevonline.org/content/56/2/104.abstract

Montealegre, R. R., Peces, R. R., Vozmediano, J. L. C., Gascueña, J. M., & Romero, E. G. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis*, *19*(6–7), 687–693.

Muangrat, R., Pongsirikul, I., & Blanco, P. H. (2018). Ultrasound assisted extraction of anthocyanins and total phenolic compounds from dried cob of purple waxy corn using response surface methodology. *Journal of Food Processing and Preservation*, *42*(2), e13447.

Oliveira, D. A., Salvador, A. A., Smânia, A., Smânia, E. F. A., Maraschin, M., & Ferreira, S. R. S. (2013). Antimicrobial activity and composition profile of grape (Vitis vinifera) pomace extracts obtained by supercritical fluids. *Journal of Biotechnology*, *164*(3), 423–432. https://doi.org/10.1016/j.jbiotec.2012.09.014

Otero-Pareja, J. M., Casas, L., Fernández-Ponce, T. M., Mantell, C., & Ossa, J. M. de la E. (2015). Green Extraction of Antioxidants from Different Varieties of Red Grape Pomace. *Molecules* . https://doi.org/10.3390/molecules20069686

Pan, X., Niu, G., & Liu, H. (2002). Comparison of microwave-assisted extraction and conventional extraction techniques for the extraction of tanshinones from Salvia miltiorrhiza bunge. *Biochemical Engineering Journal*, *12*(1), 71–77. https://doi.org/https://doi.org/10.1016/S1369-703X(02)00039-6

Pérez-Magariño, S., & González-San José, M. L. (2004). Evolution of flavanols, anthocyanins,

and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *Journal of Agricultural and Food Chemistry*, *52*(5), 1181–1189.

Pintać, D., Majkić, T., Torović, L., Orčić, D., Beara, I., Simin, N., … Lesjak, M. (2018a). Solvent selection for efficient extraction of bioactive compounds from grape pomace. *Industrial Crops and Products*, *111*(January), 379–390. https://doi.org/10.1016/j.indcrop.2017.10.038

Pintać, D., Majkić, T., Torović, L., Orčić, D., Beara, I., Simin, N., … Lesjak, M. (2018b). Solvent selection for efficient extraction of bioactive compounds from grape pomace. *Industrial Crops and Products*. https://doi.org/10.1016/j.indcrop.2017.10.038

Pourmortazavi, S. M., & Hajimirsadeghi, S. S. (2007). Supercritical fluid extraction in plant essential and volatile oil analysis. *Journal of Chromatography A*, *1163*(1), 2–24. https://doi.org/https://doi.org/10.1016/j.chroma.2007.06.021

Rada, E. C., Ragazzi, M., Fiori, L., & Antolini, D. (2009). Bio-drying of grape marc and other biomass: a comparison. *Water Science and Technology*, *60*(4), 1065–1070. https://doi.org/10.2166/wst.2009.559

Ramić, M., Vidović, S., Zeković, Z., Vladić, J., Cvejin, A., & Pavlić, B. (2015). Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from Aronia melanocarpa by-products from filter-tea factory. *Ultrasonics Sonochemistry*, *23*, 360–368.

Raviyan, P., Zhang, Z., & Feng, H. (200

Reddy, A. M. (2020). *Recent improvements in the extraction, cleanup and quantification of bioactive flavonoids.* Studies in Natural Products Chemistry (Vol. 66, pp. 197-223). Elsevier.

Ultrasonication for tomato pectinmethylesterase inactivation: effect of cavitation intensity and temperature on inactivation. *Journal of Food Engineering*, *70*(2), 189–196.

Raynie, D. E. (2006). Modern extraction techniques. *Analytical Chemistry*, *78*(12), 3997–4004.

Reverchon, E. (1997). Supercritical fluid extraction and fractionation of essential oils and related products. *The Journal of Supercritical Fluids*, *10*(1), 1–37. https://doi.org/https://doi.org/10.1016/S0896-8446(97)00014-4

Roberts, J. S., & Gerard, K. A. (2004). Development and evaluation of microwave heating of apple mash as a pretreatment to pressing. *Journal of Food Process Engineering*, *27*(1), 29–46.

Routray, W., & Orsat, V. (2012). Microwave-Assisted Extraction of Flavonoids: A Review. *Food and Bioprocess Technology*, *5*(2), 409–424. https://doi.org/10.1007/s11947-011-0573-z

Routray, W., & Orsat, V. (2013). Preparative extraction and separation of phenolic compounds. In *Natural Products* (Vol. 188, pp. 2013–2045). https://doi.org/10.1038/188440b0

Routray, W., & Orsat, V. (2014). MBE of phenolic compounds from blueberry leaves and comparison with other extraction methods. *Industrial Crops and Products*, *58*, 36–45. https://doi.org/10.1016/j.indcrop.2014.03.038

Salinas-Moreno, Y., García-Salinas, C., Ramírez-Díaz, J. L., & Alemán-de la Torre, I. (2017). Phenolic compounds in maize grains and its nixtamalized products. *Phenolic Compounds—*

Natural Sources, Importance and Applications.

Sharma, A., & Gupta, M. N. (2004). Oil extraction from almond, apricot and rice bran by three-phase partitioning after ultrasonication. *European Journal of Lipid Science and Technology*, *106*(3), 183–186.

Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., & Jiang, Y. (2005). Extraction of polyphenolics from plant material for functional foods - Engineering and technology. *Food Reviews International*, *21*(1), 139–166. https://doi.org/10.1081/FRI-200040606

Shirsath, S. R., Sonawane, S. H., & Gogate, P. R. (2012). Intensification of extraction of natural products using ultrasonic irradiations—A review of current status. *Chemical Engineering and Processing: Process Intensification*, *53*, 10–23.

Singh, A. (2010). Optimization of microwave-assisted extraction of antioxidants from potato peels. *ProQuest Dissertations and Theses*, *M.Sc.*(August).

Singh, A., Sabally, K., Kubow, S., Donnelly, D. J., Gariepy, Y., Orsat, V., & Raghavan, G. S. V. (2011). Microwave-assisted extraction of phenolic antioxidants from potato peels. *Molecules*, *16*(3), 2218–2232. https://doi.org/10.3390/molecules16032218

Sirohi, R., Tarafdar, A., Singh, S., Negi, T., Gaur, V. K., Gnansounou, E., & Bharathiraja, B. (2020). Green processing and biotechnological potential of grape pomace: Current trends and opportunities for sustainable biorefinery. *Bioresource Technology*, *314*(July), 123771. https://doi.org/10.1016/j.biortech.2020.123771

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food*

Engineering, *81*(1), 200–208. https://doi.org/10.1016/j.jfoodeng.2006.10.021

Stój, A., Szwajgier, D., Baranowska-Wójcik, E., & Domagała, D. (2018). Gentisic Acid, Salicylic Acid, Total Phenolic Content and Cholinesterase Inhibitory Activities of Red Wines Made from Various Grape Varieties. *South African Journal of Enology and Viticulture; Vol 40, No 1 (2019)*. https://doi.org/10.21548/40-1-2885

Strati, I. F., & Oreopoulou, V. (2011). Process optimisation for recovery of carotenoids from tomato waste. *Food Chemistry*, *129*(3), 747–752. https://doi.org/10.1016/j.foodchem.2011.05.015

Szolar, O. H. J., Rost, H., Braun, R., & Loibner, A. P. (2002). Analysis of Polycyclic Aromatic Hydrocarbons in Soil: Minimizing Sample Pretreatment Using Automated Soxhlet with Ethyl Acetate as Extraction Solvent. *Analytical Chemistry*, *74*(10), 2379–2385. https://doi.org/10.1021/ac015739l

Tchabo, W., Ma, Y., Engmann, F. N., & Zhang, H. (2015). Ultrasound-assisted enzymatic extraction (UAEE) of phytochemical compounds from mulberry (Morus nigra) must and optimization study using response surface methodology. *Industrial Crops and Products*, *63*, 214–225.

Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D. A., & Garcia-Viguera, C. (2014). Natural bioactive compounds from winery by-products as health promoters: A review. *International Journal of Molecular Sciences*, *15*(9), 15638–15678. https://doi.org/10.3390/ijms150915638

Theagarajan, R., Malur Narayanaswamy, L., Dutta, S., Moses, J. A., & Chinnaswamy, A. (2019). Valorisation of grape pomace (cv. Muscat) for development of functional cookies.

International Journal of Food Science & Technology, *54*(4), 1299–1305. https://doi.org/10.1111/ijfs.14119

Tournour, H. H., Segundo, M. A., Magalhães, L. M., Barreiros, L., Queiroz, J., & Cunha, L. M. (2015). Valorization of grape pomace: Extraction of bioactive phenolics with antioxidant properties. *Industrial Crops and Products*, *74*, 397–406. https://doi.org/https://doi.org/10.1016/j.indcrop.2015.05.055

Tsali, A., & Goula, A. M. (2018). Valorization of grape pomace: Encapsulation and storage stability of its phenolic extract. *Powder Technology*, *340*, 194–207.

Ugliano, M., Bartowsky, E. J., McCarthy, J., Moio, L., & Henschke, P. A. (2006). Hydrolysis and transformation of grape glycosidically bound volatile compounds during fermentation with three Saccharomyces yeast strains. *Journal of Agricultural and Food Chemistry*, *54*(17), 6322–6331.

Unterkofler, J., Muhlack, R. A., & Jeffery, D. W. (2020). Processes and purposes of extraction of grape components during winemaking: current state and perspectives. *Applied Microbiology and Biotechnology*, 1–19.

Vilkhu, K., Mawson, R., Simons, L., & Bates, D. (2008). Applications and opportunities for ultrasound assisted extraction in the food industry—A review. *Innovative Food Science & Emerging Technologies*, *9*(2), 161–169.

Villamena, F. A. (2013). *Molecular Basis of Oxidative Stress*. (F. A. Villamena, Ed.), *Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis*. Hoboken, NJ, USA: John Wiley & Sons, Inc. https://doi.org/10.1002/9781118355886

Villano, D., Fernandez-Pachon, M. S., Moya, M. L., Troncoso, A. M., & Garcia-Parrilla, M. C. (2007). Radical scavenging ability of polyphenolic compounds towards DPPH free radical. *Talanta*, *71*(1), 230–235. https://doi.org/10.1016/j.talanta.2006.03.050

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, *8*(3), 303–313.

Wang, X., Wu, Y., Chen, G., Yue, W., Liang, Q., & Wu, Q. (2013). Optimisation of ultrasound assisted extraction of phenolic compounds from Sparganii rhizoma with response surface methodology. *Ultrasonics Sonochemistry*, *20*(3), 846–854.

Xu, J., Wang, W., Liang, H., Zhang, Q., & Li, Q. (2015). Optimization of ionic liquid based ultrasonic assisted extraction of antioxidant compounds from Curcuma longa L. using response surface methodology. *Industrial Crops and Products*, *76*, 487–493.

Xu, Y., Burton, S., Kim, C., & Sismour, E. (2016). Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four virginia-grown grape varieties. *Food Science and Nutrition*, *4*(1), 125–133. https://doi.org/10.1002/fsn3.264

Yan, L., & Kim, I. H. (2011). Effect of Dietary Grape Pomace Fermented by Saccharomyces boulardii on the Growth Performance, Nutrient Digestibility and Meat Quality in Finishing Pigs. *Asian-Australas J Anim Sci*, *24*(12), 1763–1770. https://doi.org/10.5713/ajas.2011.11189

Yang, B., Zhao, M., Shi, J., Yang, N., & Jiang, Y. (2008). Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chemistry*, *106*(2), 685–690. https://doi.org/https://doi.org/10.1016/j.foodchem.2007.06.031

Zhou, B., Feng, H., & Luo, Y. (2009). Ultrasound Enhanced Sanitizer Efficacy in Reduction of Escherichia coli O157 : H7 Population on Spinach Leaves. *Journal of Food Science*, *74*, M308-13. https://doi.org/10.1111/j.1750-3841.2009.01247.x

Zollinger, M., Krebs, W., & Brandl, H. (2006). Bioaerosol formation during grape stemming and crushing. *Science of The Total Environment*, *363*(1), 253–259. https://doi.org/https://doi.org/10.1016/j.scitotenv.2005.05.025