FINDING VALUE IN GRAPE POMACE

By

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FINDING VALUE IN GRAPE POMACE

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Abstract: The production of grapes is increasing all around the world. A 7% annual increase in wine grape production, and the waste products associated with wine processing, occurred in 2013 in the US. Pomace is a predominant waste product of wine processing which can be processed into value-added products. The objective of this study was to evaluate potentially valuable components of grape pomace. Vacuum steam distillation was used to obtain essential oil from grape pomaces originating from red ('Merlot') and from white ('Muscat', Riesling', 'Sauvignon Blanc' and 'Traminette') wine grape cultivars. Free and glycosidically-bound aromatic compounds of fresh grape pomace (prior to and after distillation) and of the distillate was evaluated using Gas Chromatography (GC). Pomace remaining was forced air dried at 40^0C and utilized for further determinations. Oil from separated seeds of the above varieties was determined analytically and used to compare oil yield from bulk, mechanically separated seed of 'Riesling' and 'Red Zinfandel' using a mechanical oil press. Separated dry pomace components, seed oils and seed meals were analyzed for phytosterols and policosanols as trimethyl-silyl derivatives by GC. Free aromatics were higher in concentration than bound aromatics in all the pomaces. Phenethyl alcohol predominated in all the grape pomaces in the free aromatic fraction. The glycosidically-bound aromatic fraction was similar in distilled and non-distilled pomaces and the distillates obtained had a similar aromatic profile to the free aromatics of the grape pomace. Phytosterols and policosanols were notably enriched in grapeseed oils with oils containing 8 to 16 times more of these compounds than grape seeds. Mechanically pressed oils only contained about 4 or 5 times more phytosterols and policosinols than the original seeds; some thermal degradation of these compounds appeared to occur, probably due to frictional heat exposure during oil pressing. Phytosterols and policosanols were higher in seeds than skins/pulp and they were notably deplenished in seed residue after oil extraction. The most predominant phytosterol was β-sitosterol; campesterol and stigmasterol were also identified in varying concentrations. Eicosanol, tetracosanol and octacosanol were the major policosanols identified in most of the samples. The overall seed content in dried grape pomace was about 50% on a dry weight basis. The oil content for the grape seeds was in the range of 10-13% and mechanical oil pressing yielded about 70 % of the total oil within the seeds (about 10% of seed weight). Merlot seeds had the highest concentration of oil (about 13%) and Traminette had lowest (10%).

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CHAPTER I

INTRODUCTION

1.1 Wine production

Grapes are the world's largest produced fruit crop. Italy, France, Spain, and the United States (US) are the largest producers for grapes (Kammerer and others 2014). World wine production was at 258 mhl (million hectoliters) in 2012 and increased to about 281 mhl wine in 2013. This increase in wine production was observed in almost all the major wine producing countries. Italy showed a 2% increase (43 mhl in 2012 to 45 mhl in 2013), France showed a 7% increase (41 mhl in 2012 to 44 mhl in 2013), Spain showed a 23% increase (32 mhl in 2012 to 40 mhl in 2013) and the US showed a 7% increase (19 mhl in 2012to 22 mhl in 2013) (US Department of Treasury 2012; US Department of Treasury 2014). There are over 7700 wineries in the US (Fisher 2014). The number of wineries in predominant and other states are listed in the Table 1.

Table 1. Number of wineries in selected states of the US.

State	Number of wineries
California	3674[1]
Washington	689[1]
Oregon	566[1]
New York	320[1]
Virginia	223[1]
Texas	208[1]
Ontario	192[1]
Pennsylvania	174[1]
Ohio	144[1]
Michigan	136[1]
North Carolina	130[1]
Missouri	122[1]
Colorado	106[1]
Illinois	100[1]
Oklahoma	52[2]
New Mexico	48[3]
Kansas	17[3]
Arkansas	12[4]

[1] referenced from Fischer (2014)

[2]referenced from Foley and others (2014)

[3]referenced from Stotz (2014)

[4]referenced from Des Ruisseaux (2013)

California had the highest number of wineries, followed by Washington, Oregon and New York. In the southern US, Texas had the highest number of wineries. Oklahoma had 52 wineries (Foley and others 2014). Neighboring states with fewer wineries than Oklahoma included New Mexico with 48 wineries, Kansas with 25 wineries (Stotz 2014) and Arkansas with 12 wineries (DesRuisseaux 2013). Central New Mexico had the most large wineries in that state (about 20). Arkansas had about 5 large wineries (Robinson and others 2013) and Kansas had 6 large wineries (DesRuisseaux 2013).

1.2 Wine making process

Wine making can be divided into four major steps. The first step is harvesting grapes at optimum time, second is fermenting grapes, third is wine clarification and fourth is wine aging. Although all steps contribute to wine quality, the basic flavor of wine is determined in the first step (Fuente and others 2014).

Grape wines are primarily characterized as red or white, depending on whether fermentation was accomplished in the presence of grape solids (red wines) or was conducted with the grape juice in the absence of grape solids (white wines) (Figure 1). Red wine is made by crushing the grapes and fermenting the juice, pulp, skins and seeds together. At the end of fermentation, a wine press is used to press the wine out and separate it from the red grape pomace. White wine is

prepared from juice after pressing. Fermentation occurs in the absence of pulp, skin and seed. White wine pomace contains pulp, skins and seeds without fermentation whereas red wine pomace contains the same constituents which were exposed to fermentation.

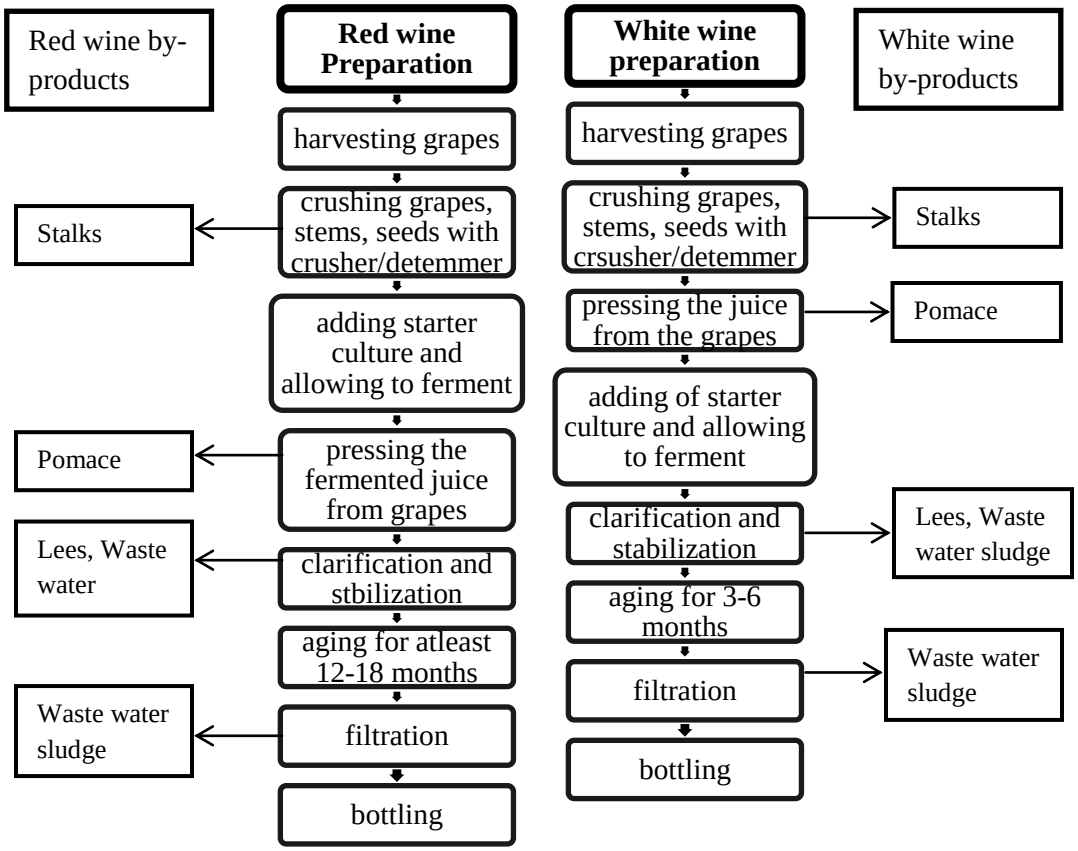

Figure 1. Process and by-products during preparation of red and white wine

1.3 By-products produced from the wine making process

The wine industry produces a substantial quantity of by-products which can be converted to value-added products (Jin and Kelly 2009). White grapes, pressed before fermentation, produce a

moist, sticky, and sugar rich pomace. Red grapes, pressed after fermentation, produce a less sticky and drier pomace. Red grape pomace contains more alcohols than sugars.

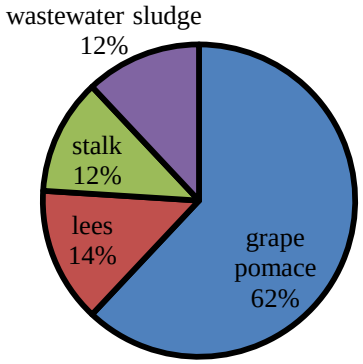

Figure 2. By-products produced from the wine industry. (reproduced from Ruggieri and others 2009)

As seen from figure 2, grape pomace is the main by-product obtained from wine making process. Stalks consist of stems, branches and leaves and are mostly removed by the crusher/de-stemmer. Lees are obtained from clarification of fermented wine and consist of dead or residual yeast and other precipitated particles. Waste water sludge is generated in large volumes from the wine industry. It originates from washing operations of the crusher/de-stemmer, the grape press and after cleaning fermentation tanks and barrels.

1.4 Grape pomace

Grape Pomace accounts for about 14-16% of the fresh weight of harvested grapes (Laufenberg and others 2003), consists of skins, pulp and seeds, and is an important by-product of the wine industry which can be further processed to obtain valuable products (Özkan and others 2004). Grape skins have considerable amounts of bioactive compounds like anti-oxidants, organic

acids, pigments, vitamins, sterols, aromatics, potassium and other minerals (Kammerer and others 2014; Lafka and others 2007; Ruberto and others 2008). Seeds obtained from grape pomace are used to produce grape seed oil, grape seed flour and grape seed extract (Mattick and Rice 1976; Maier and others 2009). Grape seeds constitute about 26% of the fresh grape pomace weight and are considered to be one of the most valuable by-products obtained from grape pomace (Valiente and others 1995). Grape seeds contain about 8-15% oil which is rich in anti-oxidants and has anti-microbial properties (Dalmolin and others 2010). Apart from the above mentioned derivatives, essential oils can also be extracted from the pomace by distillation. These essential oils primarily originate from the grape skins and pulp and are rich in aromatic chemicals which can be used as additives in food products, used to improve aroma of wines, and in cosmetics or beauty products (Bustamante and others 2008).

Grape pomace may also be used to make brandy which is conventionally called *"grappa"* in the US (Szymanski 2012). Large wineries sell their grape pomace to companies that separate and mill grape seeds to get grape seed oil and grape seed meal (Grave 2010). Small wineries often dispose of pomace as a waste product.

1.5 Varieties of grapes used in this study

The different varieties used in this study were 'Merlot', 'Muscat', 'Red Zinfandel', 'Riesling', 'Sauvignon Blanc' and 'Traminette'.

Red Grapes:

- 'Merlot' – The name *'Merlot'* was derived from a French word *Merle*, translated as blackbird. It originated in Bordeaux, France. 'Merlot' is a dark red colored wine grape cultivar. It is one of the most popularly known red wine varieties, used for both varietal and blending wine preparation. 'Merlot' grows best in Washington in the US (Robinson and others 2012).
- 'Red Zinfandel' was first grown within the US in mid-19th century. 'Red Zinfandel' is a dark skinned wine grape cultivar. It grows primarily in California within the US. The wine has fruity flavors like raspberry and cherry (Lorch and others 2014).

White grapes:

- 'Muscat' was derived from the Italian word *Mosc*, meaning "fly", describing the sweet aroma and high sugar levels of 'Muscat' grapes. This cultivar of grape was noted from the time of ancient Greeks, Egyptians and Persians. 'Muscat' is the oldest and most widespread grape in the world. It is believed to have originated from the Middle East (Lorch and others 2014). Grape varieties having high concentrations of terpenols are named as 'Muscat-like' varieties.
- 'Riesling' is of German origin and is grown because of its excellent wine quality, cold hardiness and late harvest dates. Use of this cultivar of grape is dated from 1435 (Prass and Blass 2002). Major advantages of this cultivar is its late harvest dates. A major disadvantage of this cultivar is its susceptibility to Botrytis bunch rot. Leaf removal and fungicides can help overcome disease severity (Boredelon 2009).

- ‘Sauvignon Blanc’ was derived from the French words *Sauvage* (*wild*) and *Blanc* (*white*). ‘Sauvignon Blanc’ is a cultivar from western France and is grown all around the world. ‘Sauvignon Blanc’ is known for its unique “grassy”, “asparagus”, “green apple”, “gooseberries” and “gunflint” aroma (Foley 2010).
- ‘Traminette’ is a cross of ‘Joannes Seyve’ (French American hybrid) and ‘Gewürztraminer’ (Vitis Vinifera), the only hybrid cultivar used in this study. It was originated at University of Illinois by H.C.Barnett. ‘Traminette’ was further researched and introduced into the market by Cornell University in 1996 (Versini and others 1994). It has dominant floral (jasmine and rose) and spicy (nutmeg, black pepper, cinnamon, cloves) characteristics similar to its parent, ‘Gewürztraminer’ (Boredelon 2009).

1.6 Aroma compounds

Many studies have been conducted on identification of grape aromatic compounds from various varieties (García-Carpintero and others 2011a) including compounds originating from non-volatile precursors (Gunata and others 1985a; Rocha and others 2010; Ugliano and others 2006; Ugliano and Moio 2008; Palomo and others 2007).

Aromatic chemicals can be classified into two types, free aromatic compounds and non-volatile glycosidically bound aromatic compounds. Grapes have a mixture of free and bound aromatic compounds found in varying chemical composition and concentrations. Soil, climate, viticulture practices and wine making process influence the composition of free and glycosidically bound aromatic compounds in grapes and wines (Karagiannis and others 2000; Sefton and others 1996). The bound glyco-conjugates do not contribute to grape aroma but can be released with enzyme action or hydrolysis to convert into the free form. Evaluation of bound

aromatics has gained more importance in recent times in aromatic varieties of grapes to increase aroma concentration in grape wine (Loscos and others 2009; Pedroza and others 2010; García-Carpintero and others 2011b). The aroma compounds are present in pulp and skins and their distribution in the berry depends upon cultivar.

Grapes have "neutral" C_6 alcohol aroma compounds which are common to all varieties. They also have their varietal compounds that are responsible for the unique aroma of each grape cultivar (Keyzers and Boss 2009; Schreier 1979). Grape aromas are made up of hundreds of compounds such as monoterpenoids (nerol, citronellol, linalool, geraniol), C_{13} norisoprenoids (β-demascenone and β-ionone), benzoid compounds (pyridine, furan, thiophene), esters (ethyl acetate), volatile phenols (2-phenylethanol) and volatile thiols (4-mercapto-4-methylpentanan-2-one, 3-,ercaptohexan-1-ol) (Moreno-Arribas and Polo 2009; Zalacain and others 2007). Families of compounds could be responsible for the characteristic aroma of grapes such as monoterpenes in 'Muscat', volatile thiols in 'Sauvignon Blanc' and volatile phenols in 'Traminer' (Vilanova and others 2012). Monoterpenoids and C_{13} isoprenoids (such as linalool, α-terpineol, nerol, geraninol) are responsible for a floral scent. Volatile thiols gave a strong "grassy" scent and volatile phenols gave a fruity aroma to the fruit (Ribéreau-Gayon and others 2006).

The aromatic compounds are secondary products of plant metabolism. These compounds are present at lower concentrations in green unripe berries. Their concentration substantially increases as the berry matures and the sugar content is increased (Bueno and others 2003). Production and accumulation of these compounds depend upon cultural practices, climate, soil and geographic location (Cabrita and others 2007). The concentration of bound aromatics could be at lower, similar or at higher levels than free aromatics depending on the cultivar and maturity

of the fruit (Lamorte and others 2008). Most of the bound aromatic compounds are present in grape skins and pulp. Individual analysis of grape free and bound aromatics is complex due to their chemical structure and distribution in the fruit (Bayonove 2003). An enzyme treatment frees the bound aromatic compounds by breaking their aglycone linkage (Sefton 1998). Before an enzyme treatment, grape musts could be subjected to maceration which aids disintegration of the tissues and releases both the free and bound aromatic compounds. Fractionation of free and bound aroma compounds can be conducted though a C_{18} cartridge, which allows separate analysis of free and bound forms (Sefton and others 1996). Bound aromatics can also be separated by Amberlite resins (Williams and others 1982).

1.6.1 Aromatic profile for different varieties of grapes

1.6.1.1 'Merlot'

Eugenol and 4-vinylphenol were detected in high concentrations in bound aromatics (Vilanova and others 2012). The most dominant aromatic compounds which were released by enzyme hydrolysis were 4-vinylguaicanol and 4-vinylphenol (Sefton 1998). The sweet, musty and rose flavors in 'Merlot' were obtained from high concentrations of limonene, linalool, linalool oxide, α-terpineol and β-ocimene (Veverka and others 2012).

1.6.1.2 'Muscat'

In 'Muscat' terpenols, monoterpenes, C_{13} norisoprenoids, benzene derivatives and aliphatic compounds were identified; terpenols was the most dominant fraction (Bayonove 1993). 'Muscat' is the most studied grape cultivar (Gunata and others 1985a). 'Muscat' contained less monoterpenes (of which linalool was the highest) than C_6 alcohols (mostly (E)-2-hexen-1-l and 1-

hexanol). Major aromatic compounds, 1-hexanol, nerol, geraniol and benzene derivatives, were concentrated mostly in the skins and maceration increased release of these aromatic compounds (Palomo and others 2006). During alcoholic fermentation, 2-phenylethanol and 4-vinylguacanol were formed. Among C_6 compounds, isoamyl alcohol and 2-phenylethanol were dominant. An increase in terpeneols (α-terpineol, nerol, geraniol and linalool) was noticed under anaerobic conditions (Bitteur and others 2006). During fermentation, ethyl esters of C_6, C_8 and C_{10} and acetates of higher alcohols were formed contributing to its unique flavor in dry 'Muscat' wines (Karagiannis and others 2000). Grape varieties that show a similar terpenol profile are referred to as 'Muscat-like' varieties (Genovese and others 2013).

1.6.1.3 'Riesling'

Volatile phenols (guaiacol, vanillin, methyl vanilate, ethylguaiacol), monoterpenes (linalool, 4-terpineol), C_{13} nor-isoprenoids (β-demascenon, vitispirane A) were the most predominant volatile compounds identified in 'Riesling' grapes (Sacks 2011). 'Riesling' grapes have fewer terpenes than 'Muscat'. Terpenes identified in 'Riesling' were linalool, geraniol and nerol.

1.6.1.4 'Sauvignon Blanc'

This cultivar had a unique strong and dominant aroma. The typical aromatic descriptors used for 'Sauvignon Blanc' were vegetative, grassy, gooseberry, green pepper, capsicum, tomato leaf, grapefruit and passion fruit (Coetzee and du Toit 2012). The typical aroma of 'Sauvignon Blanc' was characterized by tropical flavors, thiols and greens (asparagus, green pepper, capsicum). Higher alcohols (benzyl alcohol), esters (ethyl acetate), fatty acids (octanoic acid,

decanoic acid, hexanoic acid) and monoterpenes (α-terpeniol, linalool, geraniol) were other compounds that affected aroma of 'Sauvignon Blanc' (Baiano and others 2012). Although volatile thiols were predominant in 'Sauvignon Blanc', the volatile thiols were not limited to this cultivar and contribute to aroma profiles of other varieties such as 'Riseling', 'Colombard', 'Cabernet Sauvignon' and 'Merlot'. Other compound classes detected in 'Sauvignon Blanc' pomace were volatile fatty acids, volatile phenols and carbonyl compounds (Vilanova and others 2012). Glycosidically bound aromatics found in this cultivar included alcohols, C_6 compounds, volatile fatty acids, monoterpenes, C_{13} norisoprenoids, volatile phenols and carbonyl compounds. Bound aromatics usually consist of more monoterpenes than free aromatics (Gómez and others 1995).

The fruity, ester-like character was contributed mostly by acetate esters (Coetzee and du Toit 2012). Higher alcohols and esters had a great influence on the aromatic composition of grapes and gave intense as well as pleasant flavors. The higher alcohols and esters, if present in high concentrations, gave a strong and pungent smell (Coetzee and du Toit 2012). Monoterpene and monoterpene alcohols were known for their floral, fruity and citrus odors caused by linalool, geraniol and α-terpineol. Alpha terpineol was the most abundantly found monoterpene in 'Sauvignon Blanc' after volatile thiols. Terpene concentration increased during the ripening stage and decreased when the fruit was over-ripe (Gunata and others 1985b). Free monoterpenes and glycosidically bound monoterpenes were mostly located in grape skins. Some terpenes were evenly distributed in the skins and pulp of 'Sauvignon Blanc'(Ribéreau-Gayon and others 2006; Marais 1983).

1.7 Phytosterols and policosanols

Phytosterols, with structural properties similar to cholesterol, have the capacity to decrease cholesterol absorption resulting in lowering cholesterol absorption in the human body (Brufau and others 2008; Marangoni and Poli 2010; Sanclemente and others 2009). Phytosterols have a similar base structure of cyclopentanoperhydrophenanthene ring (the steroid nucleus) as that of cholesterol (Ruggiero and others 2013a). Sterol compounds have 27-30 carbon atoms with a carbon side chain (>7 carbon atoms). The structures are closely related and variation can be seen in double bonds. Phytosterols are obtained from isoprenoid biosynthetic pathway, from acetyl coenzyme A via squalene. They are structural components of the plasmalemma (Ruggiero and others 2013a). Plant sterols and stanols help reduce cholesterol absorption and thus reduce concentrations of cholesterol in blood. Many clinical trials have shown that 2mg/day dosage decreases LDL cholesterol (Demonty and others 2009). Also, studies suggest that plant sterols may impart a protection against several types of cancer (Racette and others 2009).

Phytosterols are found in free, esterified and glycosylated forms in certain foods (such as nuts, seeds) (Racette and others 2009). Apart from absorption competition with cholesterol, phytosterols also compete for cholesterol esterase which helps in the breakdown of cholesterol and can be absorbed into small intestine (Shiomi and others 1995). Different types of phytosterols in plants were gramisterol, brassicasterol, campesterol, stigmasterol and β-sitosterol. High concentrations of phytosterols were observed in lipid rich foods such as oils, seeds, cereal grains and nuts. Though about 200 phytosterols have been identified, β-sitosterol, campesterol and stigmasterol were the most dominant phytosterols in plants and in human diets (Ruggiero and others 2013b). Grape phystosterols were mainly present in cuticular wax, berry skins and grape

seeds. Grape seeds are expected to have cholesterol (0.3%), brassicasterol (0.5%), stigmasterol (6.6%), β-sitosterol (87%), and campesterol (1%)(Firestone 2006). β-sitosterol is the most dominant phytosterol present in grape skin and pulp followed by campesterol and stigmasterol (Hollis and others 2009; Dagna and others 1982).

Apart from phytosterols, policosanols are also used as a nutrient supplement. Policosanols are aliphatic primary alcohols isolated from plant waxes. Some policosanols were octacosanol, docosanol, triacontanol. Other policosanols present in lower concentrations were tetracosanol, heptacosanol, hexacsanol and tertratriacontanol (Wang and others 2003; Wang and others 2005). Reduction in cholesterol levels have been observed in animal models. Cholesterol metabolism was influenced by policosanols when they were oxidized to fatty acids, the very long chain fatty acids were implicated as active forms of policosanols (Wang and others 2005). Policosanols have a function in improving muscle endurance, prevention of cardiovascular diseases, reducing blood cholesterol level and influence antioxidant activity (Arruzazabala and others 1993; Carbajal and others 1998; Taylor and others 2011).

1.8 Grape seeds and grape seed oil

Grape seeds constitute to about 5% of the fresh grape fruit weight(Choi and Lee 2009), 36-52% of the dry weight (Maier and others 2009) and are an important part of the grape pomace, a major waste of wine industry. Grape seeds contain about 40% fiber, 16% oil, 11% protein and 7% of phenolic compounds and other complex matter (such as tannins, sugars, minerals) (de Campos and others 2008). Grape seeds are composed of outer seed coat, the endosperm, and the embryo. Seed oil is present in mostly the embryo and also in the endosperm (Boussetta and others 2012). Grape seed oil is an important product obtained by pressing grape seeds. Its high smoke

point (190-230^{0}C) makes it suitable for cooking. Grape seed oil is produced in Italy, Spain, Chile, USA, Australia and France (Vanhanen and Savage 2013).

Grape seed oil is an edible oil which contained about 90% polyunsaturated fatty acids and monounsaturated fatty acids of which linoleic acid (58-78%) and oleic acid (3-15%) were its major components. It also contained minor concentrations of saturated fatty acids (10%) (Mattick and Rice 1976). Vitamin E and essential fatty acids present in grape seed oil also contributed to making it a health beneficial product. The fatty acids help improve cardiovascular health by inhibiting oxidation of low density lipoproteins (Frankel and others 1995) and vitamin E has neuroprotective properties (Maier and others 2009). Studies also suggested that grape seed oil had antimicrobial and antioxidant properties (Delgado Adámez and others 2012). Grape seeds are also known to have polyphenols in large concentrations, mostly procyanidins such as catechin, epicatechin and procyanidin B2 (Cai and others 2011). Unrefined oil contained higher amounts of tocopherols and some other bioactive compounds than refined oil, which helped increase the antioxidant properties of the oil (Passos and others 2010). Resveratrol, a polyphenolic flavanoid, has been found at high levels in grape skins, pulp and seed oils. Beneficial effects on human health included improving cardio vascular health, neuro-protection, anti-oxidant, anti-inflammatory and anti-obesity (Kris-Etherton and others 2004; Catalgol and others 2012; Neves and others 2013). Though resveratrol had many beneficial health effects, its use in the food industry was limited because of its low bioavailability, chemical instability and poor water solubility (Hung and others 2006; Patel and others 2011; Trela and Waterhouse 1996). In grape seed meal, the phenolic compounds are known for their anti-oxidant properties (Gornas and others 2014; Rombaut and others).

In general, red grape seed oil was observed to have higher vitamin E concentration than white grape seed oil (Martino and others 2013; Yilmaz and Toledo 2006). 'Merlot' and 'Muscat' had about 14% and 13% oil respectively (Dwyer and others 2014). 'Sauvignon Blanc' seeds had about 14% oil (Pardo and others 2011) and 'Riesling' had about 13 % oil (Skala and others 2014).

1.9 Objectives

Limited research has been done on cultivars of grapes used for this study which were grown in Oklahoma. The objectives of this study were:

1. To evaluate aromatic chemical extraction from pomaces using vacuum steam distillation.
2. To isolate and characterize free and bound aromatic chemical profiles of pomaces produced from various white and red grapes.
3. To evaluate phytosterols and policosanols obtained from grape pomace skins/pulp, grape seeds, grape seed oil and de-oiled seed meal.
4. To document the concentration of oil present in grape seed from various cultivars grown in Oklahoma and investigate amount of oil that can be extracted mechanically from the seeds.

With this study, we aim to obtain distillate, characterize free and bound aromatic compounds in fresh pomace, as well as phytosterols and policosanols and grape seed oil from dried pomace, to evaluate valuable components in the grape pomaces.

CHAPTER II

MATERIALS AND METHODS

Grape pomaces for this study were obtained from Canadian River Vineyards and Winery at Slaughterville which is located in central Oklahoma. The grapes were first passed through a crusher/de-stemmer (WE273S, Machinery and Equipment Co., San Francisco, California) and then pressed with a Willmes bladder machine (Merlin model, Willmes, Lorsch, Germany), to press most of the juice out of the grapes. Pomaces from white grape varieties ('Merlot', 'Muscat', 'Riesling', 'Savignon Blanc' and 'Traminette') were obtained without prior fermentation. Pomace obtained from the red grape cultivar ('Merlot') was obtained after fermentation. The samples were stored in a freezer at -18^{0}C until processed.

2.1 Extraction of aromatics

The frozen grape samples (before or after vacuum steam distillation) were thawed to room temperature before further processing. Aromatics were extracted from pomace in a fresh state; pomace remaining was dried in a forced air dryer (Proctor Schwartz, Machinery and Equipment Co., San Francisco) at 40^{0}C to approximately 5% moisture, stored in freezer bags and used for phytosterol and policosanol, seed separation and seed oil determination.

2.1.1 Vacuum steam distillation

The distillation apparatus used was a vacuum steam distiller obtained from Eden Labs LLC (Seattle, Washington) (Figure 3 and Figure 4). The distiller had an inner distillation chamber with an outer heating jacket. The jacket was filled with glycol and water. Immersion heaters heated this liquid to provide heat for distillation. The equipment had a vertical sight glass (Figure 3) which indicated the water level inside the distiller; bumping of the water indicated boiling of the water and steam generation during a distillation run. The pressure was monitored from a digital gauge at the vacuum pump (Laboxact, SEM 820, KNF Neuberger Labs, Germany) (Figure 5) and from an analogue gauge measuring pressure inside the distillation chamber. The cone top of the distiller had swing bolt closures which ensured that the distillation chamber had a vacuum tight seal. Steam, generated from water inside the distillation chamber, passed though pomace samples suspended in a basket above the water level and was condensed though a condenser. Tap water was run around the condenser to reduce the temperature of condenser. Distillation water was accumulated in a carboy (Figure 6). Essential oils were condensed by a secondary condenser at the vacuum pump outlet and collected into a round bottom flask which was held on ice (Figure 5).

Prior to each distillation run the distiller was filled with about 10-15 cm of water (which was noted on the sight tube) and preheated to 49^0C. About 10 kg of grape pomace was loaded into a 70 cm basket (to cover the 0.5 cm round hole perforations on the bottom and progressing 45 cm up the circumference of the basket) which was subsequently suspended above the water level. The cone top of the distiller was then securely attached and vacuum was then initiated; distillation runs began when water began to boil (noted from bumping of water inside the sight

tube), usually within 5 min, after vacuum reached 8 KPa at the vacuum pump and 25 inches mercury vacuum inside the distiller. Within 10 min the distiller reached equilibrium vacuum of 4 to 5 KPa (29.5 inches mercury vacuum inside the distiller). Distillation runs were conducted for 2 h. Distillation was halted by release of vacuum. About 200 gm of pomace prior or after distillation was frozen at -18^{0}C to await further processing for aromatic chemical analysis. Essential oils were measured for volume, placed into a brown screw cap bottle and held at 4^{0}C to await GC analysis. The remaining pomace was spread onto trays and dried with intermittent mixing at 40^{0}C in a forced air dryer (Proctor Schwartz, Machinery and Equipment Co., San Francisco). Any lumps of the pomace, if present, were broken apart to obtain a consistently dried pomace. Equilibrium weight was typically reached after 24-48 h of drying, after which the dried pomace was collected into freezer bags and later separated into skin/pulp and seed fractions which were used for phytosterol and policosanol determinations, oil determination and seed percentage.

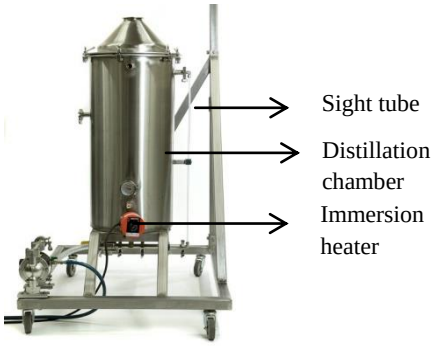

Figure 3. Vacuum Steam Distiller

Figure 4. Vacuum Steam Distillation System

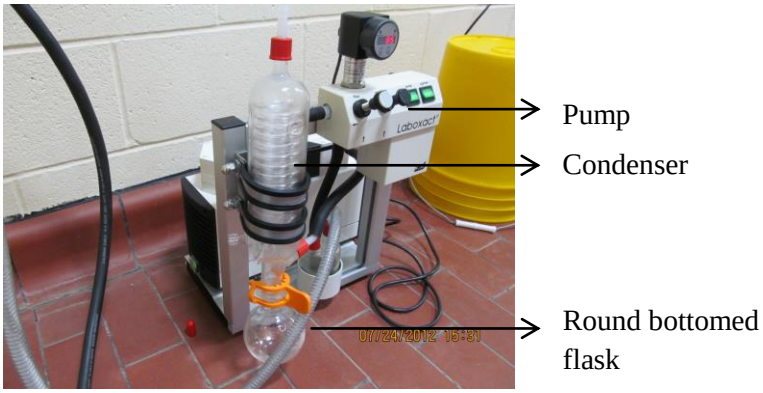

Figure 5. Laboxact SEM 820 Vacuum Pump

Figure 6. Carboy

2.1.2 Extraction of distillate for aromatic analysis

Distillate (400 µl), containing distilled free aromatics from the pomaces, was added to a 2 dram vial (can hold up to 7.5 ml) with 50 µl of 1-heptanol (500 nmol) in dichloromethane added as an extraction internal standard. This solution was thoroughly mixed with a vortex mixer. Two ml of dichloromethane was added to the vial and stirred with a magnetic stirrer for 10 min. Samples were then centrifuged and the lower dichloromethane phase was collected into a new tared vial and weighed. Dichloromethane was evaporated under a stream of nitrogen to approximately 150 µl which was measured by weight. Extract volume was estimated from sample weight using 1.33 gm ml^{-1} as the density of dichloromethane. Ninety five µl of concentrated sample and 5µl of 2-heptanol (50 nmol) (analytical internal standard) were added into a vial, mixed and injected onto the GC.

2.1.3 Maceration and separation for free and bound of aromatics for analysis by GC

Chemicals used:

Standards (purity of standard): 3-methyl-butanol (99%), 1-hexanol (98%), benzaldehyde (99%), s-limonene (96%), cineole (99%), benzyl alcohol (99%), g-terpinene (97%), linalool (97%), 2-phenylethanol (99%), terpinen-4-ol (95%), nerol (97%), geraniol (98%), 4-vinylguaiacol (98%), eugenol (99%), 2-ethyl-1-hexanal (99.5%), α-terpineol (96%), 2-heptanol (95%) and 1-heptanol (98%) were obtained from Sigma Aldrich (St. Louis, MO, USA).

Monohydrate citric acid, tartaric acid and sodium azide were obtained from Sigma Aldrich (St.Louis, MO, USA). Dibasic sodium phosphate was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Polyvinylpolypyrolidone (PVPP) was obtained from ATP Chemicals (Istanbul, Turkey). Sodium Sulfate, Hydrochloric acid and HPLC grade methanol were obtained from EMD chemicals (Billerica, MA, USA). Dichloromethane was obtained from Pharmco Aaper (Farmers Branch, TX, USA). AR 2000 pectinase was obtained from DSM Food Specialties (Heerlen, Netherlands).

2.1.3.1 Buffer solutions

Maceration buffer –Tartaric Acid (5 gm), Polyvinlypolypyrrolidone (PVPP; 10 gm) and Sodium Azide (2gm) were dissolved into approximately 800 ml of distilled water, pH adjusted to 3.2 with Hydrochloric Acid (HCl) and brought to a final volume of 1 liter.

Citrate-phosphate buffer –Citric Acid (0.1M; 2.10 ± 0.001 gm Citric Acid / 100 ml Distilled water) was added to dibasic Sodium Phosphate (0.1M; 2.84 ± 0.002 gm / 100 ml Distilled water) to obtain pH 5.0.

2.1.3.2 Method of extraction for free and bound aromatics

Maceration: The extraction method used for this study was adapted from Genovese and others (2013). One hundred ml of maceration buffer solution was added to 20 gm of grape pomace and mixed with a magnetic stirrer to macerate the tissue for 24 h at room temperature. The samples were centrifuged for 40 min in multiple 50 ml test tubes at 3300 rpm (Model 225, Fisher Centrific centrifuge, Pittsburgh, PA, USA). The supernatants were combined and 100 µl of 1-heptanol (1000 nmol) in dicloromethane was added as an extraction internal standard. The supernatant was utilized as a source of free and bound aromatics.

Separation of free and bound aromatics: Free and bound aromatics were separated using the procedure of Genovese and others (2013). A C_{18} Sep-Pak (AC2 plus short cartridge, Waters Corporation, Milford, MA, USA) was activated with 5 ml of methanol and 10 ml of distilled water. The supernatant was eluted though the activated Sep-Pak. Five ml of dichloromethane was eluted though the Sep-Pak in 1 ml increments to elute free aromatics and collected in a 2 dram vial. Residual water was removed with anhydrous sodium sulfate, and the dichloromethane layer was dried to approximately 250 µl under a slow stream of nitrogen. The exact weight of the concentrated solution was recorded and used to calculate volume based on dichloromethane density (1.33 gm ml^{-1}). A mix of 95 µl of the concentrated sample and 5 µl of 2-heptanol (50 nmol) (as analytical internal standard) was added in a new vial and injected into the GC for

determination of free aromatic chemicals. The C_{18} cartridge containing bound aromatics was eluted with 5 ml of methanol in 1ml increments and collected in a 2 dram vial. Methanol was evaporated to dryness under vacuum in a Speed Vac (SVC-1OOH Savant Instrument Inc., Farmingdale, NY, USA). After drying, 50 mg of AR2000 pectinase and 3 ml of citrate-phosphate buffer (pH 5) was added to deglycosilate the aromatic compounds. This solution was vortexed, incubated at 40^0C in a dry block heater for 24 h and then allowed to cool to room temperature. This solution was applied to an activated Sep-Pak and eluted with 5 ml of dichloromethane in 1 ml increments into a 2 dram vial. Residual water was removed with anhydrous sodium sulfate. The dichloromethane layer was dried to approximately 250 µl under a slow stream of nitrogen. The exact weight of the concentrated solution was used to evaluate volume based on dichloromethane density (1.33 gm ml^{-1}) and 95 µl of the concentrated sample was added to 5 µl of 2-heptanol (50 nmol) (analytical internal standard), mixed and injected onto the GC.

2.1.4 GC analysis of aromatics

One µl of mixture from the vials was injected onto a DB 5 fused silica capillary column (30 x 0.25 mm x 0.25 µm film thickness; J and W Scientific Inc., Rancho Cardova, CA, USA) installed in a Varian Star 3400 CX gas chromatograph (Varian Medical Systems, Polo Alto, CA, USA) equipped with a splitless injection port and an FID detector. Helium was used as a carrier gas at a linear flow velocity of 20 cm sec^{-1}. The initial injector temperature was at 40^0C and temperature was raised at 100^0C min^{-1} and held at 290^0C for 5 min. The detector temperature was at 300^0C. Initial column temperature was 55^0C for 2 min. The temperature was then raised from 55^0C to 75^0C at a rate of 0.5^0C min^{-1} followed immediately by a second temperature gradient

from 75^0C to 145^0C at 5^0C min^{-1}. A final temperature gradient from 145^0C to 280^0C was achieved at 20^0C min^{-1} and held 10 min. The total run was the 75 min.

Peaks were identified by co-elution with authentic standards and quantified relative to the analytical internal standard, 2-heptanol. The sample recovery was corrected relative to 1-heptanol as extraction internal standard.

2.2 Extraction of phytosterols and policosanols

2.2.1 Chemicals used:

Standards (purity of standards): Eicosanol (98%), heneicosanol (98%), tricosanol (98%), tetracosanol (99%), hexacosanol (97%), heptacosanol (98%), octacosanol (99%), campesterol (65%), stignmasterol (95%), β-sitosterol (95%), triacontanol (98%), 5α-cholestane (97%) were obtained from Sigma Aldrich (St. Louis, MO, USA).

Hexane was obtained from Macron Fine Chemicals (Center Valley, PA). Potassium hydroxide, Potassium chloride, pyridine-BSTFA+1%TCMS were obtained from Fisher Chemicals (Pittsburg, PA, USA). Ethanol was obtained from EMD chemicals (Billerica, MA, USA).

2.2.2 Preparation of sample for extraction

Pomace prior to or after distillation was dried to equilibrium weight at 40^0C as previously described. Seeds were hand separated from skins/pulp and both fractions were used for phytosterol and policosanol analysis. About 5 grams of sample was ground in a Warring blender (10 seconds X 3 with pauses to prevent heat buildup during grinding; South Shelton, CT, USA) to reduce the particle size, as a pre-grinding procedure. Samples were then ground to a fine powder

using a UDY cyclone mill (UDY corporation, Boulder, CO) to pass through a 1 mm screen, stored in a brown screw cap bottle and utilized for further analysis. Press cakes obtained from seed oil pressing were ground as described above. Seed oil was utilized without further processing. The moisture content for the samples was determined by drying the ground sample at 70^0C for 24 hours. The data was expressed on a dry weight basis.

2.2.3 Method of Extraction

The method of extraction used for this study was adapted from Liu and others (2010).

Weighing samples for extraction: About 0.5 gm of ground sample was accurately weighed into 2 dram vials and used for analysis. Oil obtained after solvent extraction of approximately 0.5 gm of grape seed was also used for analysis. For the mechanically extracted oil, the oil was stirred with a magnetic stirrer and 0.5 gm of the stirred sample was weighed into a 2 dram vial. All samples were weighed and analyzed in triplicate.

Saponification: Twenty µl of α-cholestane (200 nmol), as an internal standard, was added to the dry sample in the vial along with 4 ml KOH buffer was added to the sample and heated on a heater block at 80^0C for 1 hour for de-esterification. Samples were then allowed to cool to room temperature and 1 ml distilled water, 2 ml hexane and 150 mg potassium chloride was then mixed thoroughly and centrifuged for 15 minutes. The hexane layer was transferred into a new vial, and the sample was re-extracted 3 times with 2 ml, 1 ml and 1 ml of hexane. The hexane layers were combined and rinsed with water until the pH of washed water was 7. Traces of water were removed with anhydrous sodium sulfate and then hexane was completely evaporated under slow stream of nitrogen.

Derivitization: To the dried residue, 100 µl of freshly prepared pyridine-BSTFA with 1% TCMS (1:1 ratio) solution was added. The mixture was heated at 60^0C for 1 hour. After cooling, the liquid was completely dried under nitrogen. To the dried vial, 700 µl hexane was added and sample was injected directly onto the GC for analysis.

2.2.4 GC analysis of phytosterols and policosanols

One µl of sample was injected onto a DB 5 fused silica capillary column (30 x 0.25 mm x 0.25 µm film thickness; J and W Scientific Inc., Rancho Cardova, CA, USA). A Tracor model 540 gas chomatograph (Tracor Instruments, Austin, TX, USA) equipped with a splitless injection port and a FID was used. Helium was used as a carrier gas at a linear flow rate of 20 cm sec^{-1}. The injector temperature was 270^0C and the detector temperature was at 300^0C. Initial column temperature was 50^0C for 2 min. The temperature was then raised from 50^0C to 270^0C at a rate of 2^0C min^{-1}, and a hold at 270^0C for 12 min and a second temperature rise from 270^0C to 310^0C at 10^0C min^{-1} and a hold at 310^0C for a final 24 min period. The total run was 70 min.

Peaks were identified by co-elution of authentic standards and quantified relative to 5α-Cholestane as internal standard.

2.3 Seed oil determination

Solvent Extraction: About 0.5 gm of grape seeds, ground as described for sterol extraction, were weighed in triplicate into 2 dram vials. Four ml diethyl ether was added to the vial and stirred with a magnetic stirrer for 20 min at room temperature. The samples were then centrifuged in a Speed Vac at 3,000 g for 20 min. Supernatant was collected in a new vial. These steps were repeated two more times. The supernatant was filtered using a 0.45 micron Nylon 66 membrane

(Alltech Associates Inc., Illinois) and evaporated in the Speed Vac, leaving the lipids. Grape seed oil content was determined gravimetrically.

Mechanical Extraction: A Tokul oil press (EKOTOK 1, Tokul Agro Products Ind. and Trade Ltd. Co., Izmir, Turkey) was used to press oil from the grape seeds. Moisture content of the seeds were approximately 10%. The seeds were weighed and fed through a funnel (Figure 7 and 8) which allowed the seeds to pass directly through a screw which was rotating. The oil was expelled out through holes that were present through the expeller barrel and press cake was continuously expelled through a 4 mm die at the end of the barrel. The temperature of the press cake was within the range of 60-80^{0}C (Chapuis and others 2014). The expeller die diameter could be adjusted from 1 to 5 mm by replacing it with the other dies. Also, the speed of the screw could be adjusted using an electronically variable speed drive from 0 to 60 hz.

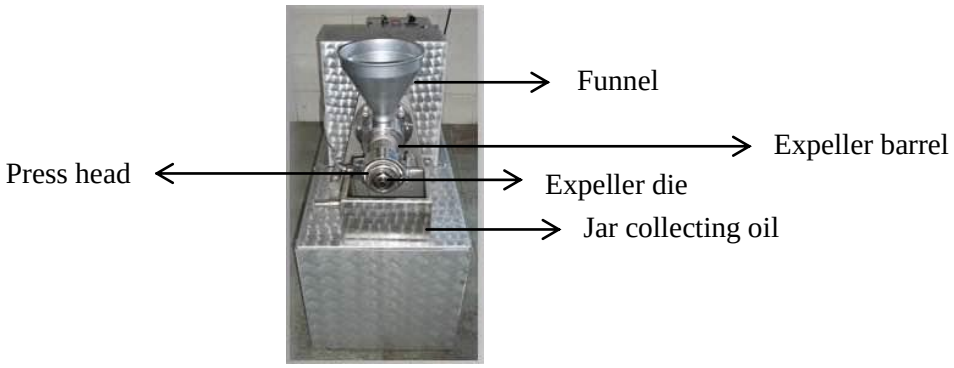

Figure 7. Tokul oil press

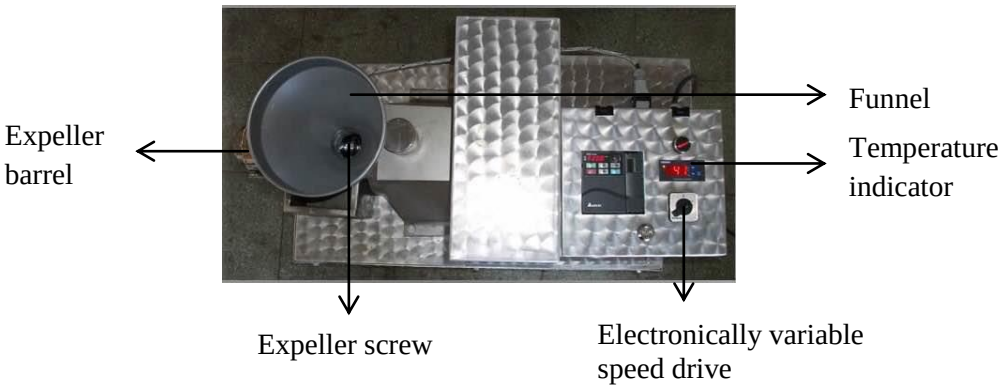

Figure 8. Top view of Tokul oil press

2.4 Statistical analysis

The results obtained were analyzed using Analysis of Variance (ANOVA) and Fisher's Least Significant Difference (LSD) for mean separation was determined using Statistical Analysis System (SAS 9.4, SAS Institute, Cary, NC) at $P \leq 0.05$.

CHAPTER III

RESULTS AND DISUSSION

3.1 Pomace distillation:

Grape pomaces subjected to distillation for 2 h at 4 to 5 KPa pressure and 49^0C produced mean volumes of distillates averaging 7.5 ml kg^{-1} for 'Merlot', 0.8 ml kg^{-1} for 'Muscat', 2.8 ml kg^{-1} for 'Sauvignon Blanc', 1.7 ml kg^{-1} for 'Riesling' and 5.4 ml kg^{-1} for 'Traminette'.

3.1.1 Essential oil:

Essential oils contained free aromatics obtained after distillation. Aromatic chemicals were extracted from the distillates and are discussed by cultivars below (Table 2). There were certain compounds from each cultivar that were predominant in essential oils (benzaldehyde in 'Merlot', geraniol in 'Muscat', citronellol in 'Riesling', limonene in 'Sauvignon Blanc' and phenethyl alcohol in 'Traminette').

'Merlot': Benzaldehyde predominated in 'Merlot' (Table 2). Veverka and others (2012) found benzaldehyde and terpinen-4-ol predominate in 'Merlot'. Among the monoterpenes, α-terpineol, citronellol, nerol and geraniol were identified. Veverka and others (2012) found that α-terpineol predominated in 'Merlot' obtained from grape pomace. Alcohols were also identified in the

‘Merlot’. Other identified compounds in ‘Merlot’, in minor concentrations, were hexanal, 4-vinylguaicanol and eugenol.

‘Muscat’: All of the monoterpenes were identified in ‘Muscat’ except for g-terpineol (Table 2). Marais (1989) documented a similar free terpene concentration (citronellol, nerol and α-terpineol) for ‘Muscat’. Phenethyl alcohol was higher in ‘Muscat’ compared to the other aromatic compounds. Lukić and others (2010) found phenethyl alcohol as one of the predominant aromatic compounds in ‘Muscat’. Other identified compounds in ‘Muscat’ included C_6 compounds such as hexanal, alcohols (such as benzyl alcohol and isoamyl alcohol). Other aromatic compounds identified were benzaldehyde, 2-ethyl-1-hexanal, 4-vinylguaicanol and eugenol. Similar concentrations of isoamyl alcohol, benzaldehyde and eugenol were observed by Gunata and others (1985a) for ‘Muscat’. The compounds identified gave the unique ‘Muscat-like’ flavor to the fruit. In ‘Muscat’, Herraiz and others (1990) found phenethyl alcohol, benzaldehyde, isoamyl alcohol, linalool, α-terpineol, citronellol, nerol, geraniol and limonene (listed in the order of predominance).

Riesling: Similar to ‘Muscat’, monoterpenes also predominated in ‘Riesling’. Citronellol and nerol were highest concentrations among monoterpenes. A similar monote rpene profile in ‘Riesling’ was identified by Yu and Michael (2012). ‘Riesling’ had highest concentration of isoamyl alcohol compared to other grape pomaces. Van Wyk and others (1967) identified isoamyl alcohol, linalool, phenethyl alcohol and benzyl alcohol in their study. Low concentrations (<0.01 μg ml^{-1}) of 4-vinylguaicanol were determined. Eugenol was highest in ‘Riesling’.

‘Sauvignon Blanc’: All the alcohols and monoterpenes were identified in ‘Sauvignon Blanc’. Eugenol predominated in ‘Sauvignon Blanc’ compared to other aromatic compounds. Monoterpenes were present in high concentrations compared to alcohols. Sefton and others (1996) also identified alcohols, monoterpenes and high concentrations of 4-vinylguaicanol in ‘Sauvignon Blanc’. In this study, 4-vinylguaicanol was not identified in essential oil, neither in free fraction of non-distilled pomace.

‘Traminette’: ‘Traminette’ also had all the compounds except benzyl alcohol. Monoterpenes ($\leq$ 0.003 µg ml^{-1}) were present in lower concentrations than alcohols. Limonene, cineole, 2-ehtyl-1-hexanal, linalool, 4-vinylguaicanol and eugenol were the other compounds that were identified in ‘Traminette’.

Table 2. Chemical profile of essential oils obtained from 'Merlot', Muscat', 'Riesling', 'Sauvignon Blanc' and 'Traminette' fresh grape pomaces.[1]

Aromatic compounds	Essential oils				
	Merlot	Muscat	Riesling	Sauvignon Blanc	Traminette
Isoamyl alcohol[2]	0.01f[3]	0.01d	0.02d	0.01d	0.001f
Hexanal	0.001i	0.01d	0.02d	-	0.03b
Benzaldehyde	0.09a	0.01d	-	0.01d	0.01d
Limonene	-	0.001f	-	0.07a	0.02c
Cineole	-	-	-	0.003f	0.01d
2-ethyl-1-hexanal	-	-	-	0.001h	0.03b
Benzyl alcohol	-	0.06c	0.01e	0.0003i	-
g-terpineol	-	-	0.01e	-	0.01d
Linalool	-	0.001f	0.01e	0.001h	0.03b
Phenethyl alcohol[4]	0.07c	0.05c	0.02d	0.03c	0.04a
Terpinen-4-ol	-	0.0001g	0.02d	0.003f	0.01d
α-terpineol	0.05d	0.001f	0.01e	0.002g	0.0001g
Nerol	0.004g	0.004e	0.04c	0.004e	0.001f
Citronellol	0.04e	0.07b	0.06b	0.004e	0.003e
Geraniol	0.0003j	0.09a	0.001f	0.003f	0.003e
4-vinylguaicanol	0.002i	-	0.01e	-	0.01d
Eugenol	0.08b	-	0.08a	0.05b	0.01d
Total	0.35A[5]	0.31B	0.31B	0.20D	0.22C

[1]Units are in μg ml^{-1}, distillation was conducted at 49^0C and 4 to 5 KPa pressure for two hours

[2]Isoamyl alchol = 3-methyl-1-butanol

[3] Means followed by the same lower case letter within each grape cultivar do not differ according to LSD at $p < 0.05$

[4]Phenthyl alcohol = 2-phenyl ethanol

[5] Means for total aromatics followed by the same upper case letter do not differ according to LSD at $p < 0.05$

3.1.2 Free and bound aromatic compounds from non-distilled grape pomaces:

Each grape cultivar contained different predominant free and bound aromatic compounds in the pomace (Table 3). Volatile profiles were comparable to several other studies (Gunata and others 1985a; Zocca and others 2008; Gómez García-Carpintero and others 2012). Phenethyl alcohol was most abundant in free aromatics of all the grape pomaces. Most of the compounds identified in free fraction of non-distilled pomace were identified in their essential oils.

'Merlot': Among free monoterpenes in 'Merlot', citronellol, geraniol, nerol and α-terpineol were identified (Table 3). Citronellol predominated in the free fraction of monoterpenes, contributing to the citrus flavor. Geraniol and α-terpineol were also present in the bound fraction of the pomace. Phenethyl alcohol predominated in free aromatics and was found in lower amounts in bound aromatics. Jiang and others (2013) also found that phenethyl alcohol predominated in free fraction of 'Merlot', followed by 4-vinylguaicanol, isoamyl alcohol and hexanal. The free aromatic compounds identified could possibly be extracted into essential oils by distillation. Most of the free aromatics identified in non-distilled pomace were identified in essential oil but were not in equal proportions. Other identified free aromatic compounds in 'Merlot' non-distilled pomace were isoamyl alcohol, hexanal, benzaldehyde, 4-vinylguaicanol and eugenol. Isoamyl

alcohol, hexanal and 4-vinylguaicanol were identified in bound fraction in 'Merlot' non-distilled pomace. Cineol, linalool and benzyl alcohol were identified only in bound fraction in 'Merlot' non-distilled pomace.

'Muscat: Isoamyl alcohol, benzyl alcohol and phenethyl alcohol were identified in free and bound fraction in 'Muscat' (Table 3). Phenethyl alcohol predominated followed by isoamyl alcohol and benzyl alcohol. Very low concentrations (≤ 0.02 µg gm^{-1}) of alcohols (isoamyl alcohol, benzyl alcohol and phenethyl alcohol) were identified in bound fraction of 'Muscat'. Adams and others (2005) and Yu and Michael (2012) found that isoamyl alcohol and benzyl alcohol gave the oily/whiskey flavor and phenthyl alcohol gave the rose-like flavor. Gunata and others (1985a) found that 'Muscat' had isoamyl alcohol, phenethyl alcohol and benzyl alcohol in the grapes. Among the free monoterpene compounds in 'Muscat', geraniol was highest, contributing to a flowery flavor (Takoi and others 2010). Sánchez-Palomo and others (2009) also found that geraniol was predominant in their study on 'Muscat'. Other monoterpenes (terpinen-4-ol, α-terpineol, nerol, citronellol and gernariol) were identified in low concentrations (≤ 0.01 mg gm^{-1}) (Adams and others 2005; Yu and Michael 2012; Takoi and others 2010). Bound fraction 'Muscat' had higher concentrations of monoterpenes than free fraction. Lukić and others (2012) found that monoterpenes predominated in 'Muscat' marcs. α-Terpineol and nerol were present in almost equal concentrations in bound fraction as observed in free fraction. Very low concentrations of g-terpineol was present compared to other bound monoterpenes in 'Muscat'. Hexanal was higher in free fraction than in bound fraction. Palomo and others (2006) also found that monoterpenes predominated in the bound fraction and alcohols and 4-vinylguaicanol were found in limited quantities. Benzaldehyde, limonene and linalool were other compounds identified in the free

fraction in ‘Muscat’ non-distilled pomace. Limonene, cineole, 2-ethyl-1-hexanal, linalool and 4-vinylguaicanol were other compounds identified in bound fraction in ‘Muscat’.

‘Riesling’: Hexanal, isoamyl alcohol, phenethyl alcohol and benzyl alcohol were identified in ‘Riesling’ (Table 3). Phenethyl alcohol and isoamyl alcohol were at similar concentrations in free and bound fraction. Benzyl alcohol was higher in bound fraction than in free fraction. Van Wyk and others (1967) found that isoamyl alcohol and phenethyl alcohol were the most dominant aromatic compounds identified in free fraction of ‘Riesling’ marc. Terpinen-4-ol, α-terpineol, nerol, citronellol, geraniol and g-terpineol were identified in free form in ‘Riesling’ non-distilled pomace. Citronellol was highest among free monoterpenes identified in ‘Riesling’ non-distilled pomace. Gershenzon and others (2000) found that monoterpenes gave the citrus and flowery flavor. Terpinen-4-ol, α-terpineol, nerol, geraniol and g-terpineol were present in lower concentrations in free fraction of ‘Riesling’ (≤ 0.01 µg gm^{-1}). Only g-terpineol and α-terpineol were identified in bound fraction of ‘Riesling’ non-distilled pomace in lower concentrations than free fractions (≤ 0.01 µg gm^{-1}). Eugenol and 4-vinylguaicanol were the other free aromatic compounds identified in ‘Riesling’ non-distilled pomace. Limonene, cineole, linalool, eugenol and 4-vinylguaicanol were the other aromatic compounds identified in bound fraction in ‘Riesling’ non-distilled pomace.

‘Sauvignon Blanc’: Phenethyl alcohol and isoamyl alcohol were present in higher concentrations in free fraction than bound fraction in ‘Sauvignon Blanc’ (Table 3). Kalua and Boss (2010) found that isoamyl alcohol and phenethyl alcohol contributed as a varietal characteristic of ‘Sauvignon Blanc’ in the free form. Benzyl alcohol was identified only in the bound fraction. Among the monoterpenes (g-terpineol, terpinen-4-ol, α-terpienol, nerol, citronellol and geraniol), α-terpienol and geraniol were present in higher concentrations in free fraction of ‘Sauvignon Blanc’.

Citronellol was predominant among monoterpenes in bound fraction of ‘Sauvignon Blanc’. Benzaldehyde, limonene, cineole, 2-ethyl-1-hexanal, linalool and eugenol were present in higher concentrations in the bound fraction than in the free fraction.

‘Traminette’: ‘Traminette’ also had higher concentration of phenethyl alcohol (0.81 µg gm^{-1}) compared to other free aromatic compounds (Table 3). Hexanal, 2-ethyl-1-hexanal and eugenol were also present in high concentrations. These compounds (hexanal, 2-ethyl-1-hexanal, eugenol and 4-vinyl guaicanol) were present in higher concentrations in free fraction than in bound fraction. Isoamyl alcohol was identified in higher concentration (about 0.01 µg gm^{-1}) in the bound fraction. Isoamyl alcohol, benzaldehyde, limonene, cineole , α-terpienol, nerol and geraniol were present in higher concentrations in the bound fraction than in the free fraction of non-distilled ‘Traminette’ pomace. Linalool, g-terpineol and citronellol were present only in the free fraction, which could be extracted by distillation.

Table 3. Free and bound aromatic compounds for non-distilled 'Merlot', Muscat', 'Riesling', 'Sauvignon Blanc' and 'Traminette' fresh grape pomaces (µg gm^{-1} dry weight).

Aromatic compounds	Free aromatics[1]					Bound aromatics[2]				
	Merlot	Muscat	Riesling	Sauvignon Blanc	Traminette	Merlot	Muscat	Riesling	Sauvignon Blanc	Traminette
Isoamyl alcohol[3]	0.02d[4]	0.20b	0.09c	0.08d	0.01f	0.01d	0.01d	0.06c	0.01g	0.02g
Hexanal	0.03c	0.11c	0.19a	-	0.28b	0.001f	0.01d	0.01d	-	0.07d
Benzaldehyde	0.03c	0.01g	-	0.05f	0.01f	-	-	-	0.03e	0.03f
Limonene	-	0.01g	-	0.12b	0.03f	-	0.001f	0.29a	0.12c	0.05e
Cineole	-	-	-	0.06e	0.001g	0.06b	0.001f	0.001f	0.12c	0.02g
2-ethyl-1-hexanal	-	-	-	0.01h	0.20c	-	0.02c	-	0.003h	0.08c
Benzyl alcohol	-	0.07d	0.01e	-	-	0.002e	0.02c	0.13b	0.01g	-
g-terpineol	-	-	0.001g	0.01h	0.02g	-	0.0004g	0.001f	0.04d	-
Linalool	-	0.02f	-	0.02g	0.01f	0.01d	0.02c	0.06c	0.003h	-
Phenethyl alcohol[5]	0.59a	0.97a	0.09c	0.97a	0.81a	0.002e	0.02c	0.06c	0.03e	0.19a
Terpinen-4-ol	-	0.01g	0.01e	0.02g	0.001g	-	0.003e	0.01d	0.04d	-
α-terpineol	0.0003f	0.01g	0.02d	0.08d	0.001g	0.18a	-	-	0.01g	0.01h
Nerol	0.001f	0.01g	0.02d	0.05f	0.001g	-	0.01d	0.01d	0.15b	0.002f
Citronellol	0.11b	0.01g	0.14b	0.11c	0.05e	-	0.02c	-	0.18a	-
Geraniol	0.004e	0.06e	0.004f	0.06e	0.01f	0.01d	0.09c	-	0.01g	0.13b
4-vinylguaicanol	0.02d	-	0.01e	-	0.02g	0.05c	0.05b	0.01d	0.03e	0.001g
Eugenol	0.02d	-	0.02d	0.01h	0.19d	-	-	0.002e	0.02f	0.01h
Total	0.82C[6]	1.49B	0.61D	1.65A	1.64A	0.32F	0.28G	0.64E	0.81C	0.61D

[1]Free aromatics were the aglycone's found naturally in the pomace obtained after maceration.

[2]Bound aromatics were the glycosylated forms of aromatics in the pomace obtained after maceration. Bound aromatics were analyzed as the aglycone after deglycosilation.

[3]Isoamyl alchol = 3-methyl-1-butanol

[4]Means followed by the same lower case letter within the aromatic type (free or bound) and each grape cultivar do not differ according to LSD at $p < 0.05$

[5]Phenthyl alcohol = 2-phenyl ethanol

[6] Means for total aromatics followed by the same upper case letter do not differ according to LSD at $p < 0.05$

3.1.3 Free and bound aromatic compounds from distilled grape pomaces:

The distilled pomaces had slightly lower concentrations of free aromatic compounds and the bound aromatics in the distilled pomace remained at the same level compared to the non-distilled pomace except for a few compounds (Table 4). Also, the free aromatic compounds that showed a decrease in the distilled pomace were observed in essential oils. Each cultivar had one compound which was predominant in essential oil and was found in lower concentrations in distilled pomace than the non-distilled pomace (such as benzaldehyde in 'Merlot', geraniol in 'Muscat', citronellol in 'Riesling', limonene in 'Sauvignon Blanc' and phenethyl alcohol in 'Traminette').

'Merlot': Isoamyl alcohol, benzaldehyde, phenethyl alcohol, geraniol, 4-vinylguaicanol and eugenol shown at a lower concentration in free fraction of distilled 'Merlot' pomace than non-distilled pomace (≤ 0.01 μg gm^{-1}) (Table 4). Benzaldehyde showed the highest concentration in 'Merlot' essential oil, followed by phenethyl alcohol and others were present in lower concentrations; these compounds were observed in lower concentrations in distilled pomace than non-distilled pomace. Citronellol was observed higher in the free fraction of distilled pomace than in non-distilled pomace, which could have been from deglycosilation during distillation from the bound fraction (lower concentrations were observed in the bound fraction compared to non-distilled pomace). Hexanal was at same concentration in the free and bound fractions of the distilled and non-distilled pomaces. In the bound fraction of 'Merlot' distilled pomace, isoamyl

alcohol, hexanal, cineole, phenethyl alcohol, geraniol and 4-vinylguaicanol were at the same concentration as in non-distilled pomace.

'Muscat': The aromatics obtained in distilled 'Muscat' pomace were identified at lower concentrations (≤ 0.04 μg gm^{-1}) in 'Muscat' non-distilled pomace (Table 4). Nerol was unidentifiable in distilled 'Muscat' pomace but was extracted into the 'Muscat' essential oil. The bound aromatics obtained for distilled 'Muscat' pomace were at same concentrations as obtained in non-distilled 'Muscat' pomace except for benzyl alcohol, nerol geraniol and 4-vinylguaicanol. Benzyl alcohol, nerol, geraniol and 4-vinylguaicanol were present at a lower concentration in bound aromatics of distilled 'Muscat' pomace; these compounds might have changed to free form as they were observed in slightly higher concentrations in distilled pomace than non-distilled pomace and have been identified in the essential oil. Their concentration was lower in bound fraction in distilled pomace than non-distilled pomace.

'Riesling': Hexanal, α-terpineol, citronellol and eugenol were present in the free fraction of distilled pomace and were present in lower concentrations compared to the non-distilled pomace (Table 4). These compounds were identified in essential oil (Table 3). Eugenol was predominant in non-distilled pomace and in 'Riesling' essential oil, followed by nerol and other aromatic compounds were at lower concentration (≤ 0.02 μg gm^{-1}). The bound aromatics for the non-distilled and distilled 'Riesling' pomace were same, except benzyl alcohol and phenthyl alcohol which were lower in the bound fraction (Table 4).

'Sauvignon Blanc': In distilled 'Sauvignon Blanc' pomace lower concentrations of benzaldehyde, limonene, g-terpienol, linalool, phenethyl alcohol and geraniol were observed in the free fraction (Table 4). These compounds (benzaldehyde, limonene, g-terpienol, linalool, phenethyl alcohol) were identified in essential oil along with isoamyl alcohol, 2-ethyl-1-hexanal, benzyl alcohol,

nerol, α-terpineol, citronellol, geraniol and eugenol. In the free aromatic fraction of distilled pomace, isoamyl alcohol, cineole, 2-ethyl-1-hexanal, terpinen-4-ol, α-terpineol, nerol, citronellol and eugenol were at same concentration as that of non-distilled pomace. In the bound aromatic fraction, distilled pomace had lower concentration (about 0.01 µg gm^{-1}) of benzaldehyde, limonene, cineole, 2-ethyl-1-hexanal, g-terpineol, terpinen-4-ol, nerol, citronellol and eugenol. These compounds were also identified in essential oil.

'Traminette': In the free fraction of 'Traminette' distilled pomace, hexanal, limonene, 2-ethyl-1-hexanal and phenethyl alcohol were lower than the non-distilled pomace (Table 4). Isoamyl alcohol, benzaldehyde, cineole, linalool, terpinen-4-ol, α-terpineol, nerol, citronellol, geraniol and eugenol were at same concentrations in as non-distilled 'Traminette' pomace but were also identified in the essential oil. In the bound fraction of 'Traminette' distilled pomace, hexanal, limonene, phenethyl alcohol, α-terpineol and geraniol were about 0.01 µg gm^{-1} lower than non-distilled 'Traminette' pomace. The bound aromatics decreased in distilled pomace and were at same concentration in free fraction. Since, there is a change in bound fraction, the results signify that bound aromatics changed to the free form during the storage period, as also found by Gros and others (2013). Few free aromatic compounds (isoamyl alcohol, benzaldehyde, cineole, linalool, terpinen-4-ol, α-terpineol, neorl, citronellol, geraniol and eugenol) were at same concentrations in both non-distilled and distilled pomaces. But, the same compounds were observed at lower concentration in bound fraction for non-distilled versus distilled pomace. These aromatic compounds may have changed from the bound to the free form during storage.

Table 4. Free and bound aromatic compounds for distilled[1] 'Merlot', Muscat', 'Riesling', 'Sauvignon Blanc' and 'Traminette' fresh grape pomaces (µg gm^{-1} dry weight).

Aromatic compounds	Free aromatics[2]					Bound aromatics[3]				
	Merlot	Muscat	Riesling	Sauvignon Blanc	Traminette	Merlot	Muscat	Riesling	Sauvignon Blanc	Traminette
Isoamyl alcohol[4]	0.01e[5]	0.16b	0.09a	0.08c	0.01e	0.01d	0.01d	0.06d	0.01f	0.02h
Hexanal	0.03c	0.09c	0.01d	-	0.26b	0.001f	0.01d	0.01f	-	0.06e
Benzaldehyde	0.02d	0.002g	-	0.03g	0.003g	-	-	-	0.02e	0.03g
Limonene	-	0.01e	-	0.03g	0.002h	-	0.001f	0.28a	0.13b	0.04f
Cineole	-	-	-	0.06e	0.0002j	0.06b	0.001f	0.002g	0.11c	0.02h
2-ethyl-1-hexanal	-	-	-	0.01i	0.003g	-	0.02c	-	0.002g	0.07d
Benzyl alcohol	-	-	0.01d	-	-	0.003e	0.02c	0.15b	0.01f	-
g-terpineol	-	-	-	0.003k	0.02d	-	0.0004g	0.001h	0.03d	-
Linalool	-	0.01e	-	0.01i	0.01e	0.01d	0.02c	0.06d	0.003g	-
Phenethyl alcohol[6]	0.49a	0.77a	0.08b	0.82a	0.99a	0.001f	0.02c	0.07c	0.03d	0.21a
Terpinen-4-ol	-	0.004f	0.02c	0.02h	0.0002j	-	0.003e	0.01f	0.03d	-
α-terpineol	0.002h	0.01e	0.01d	0.06e	0.002h	0.12a	-	-	0.01f	0.08c
Nerol	0.002h	-	0.02c	0.04f	0.001i	-	0.02c	0.02e	0.13b	0.002j
Citronellol	0.13b	0.001h	-	0.10b	0.01e	-	0.02c	-	0.16a	-
Geraniol	0.001i	0.04d	0.02c	0.07d	0.007f	0.01d	0.10a	-	0.01f	0.12b
4-vinylguaicanol	0.003g	-	0.01d	-	0.02d	0.04c	0.06b	0.01f	0.03d	0.001j
Eugenol	0.005f	-	0.01d	0.005j	0.19c	-	-	0.002g	0.01f	0.01i
Total	0.69e[7]	1.10c	0.27h	1.34b	1.53a	0.26h	0.31g	0.68e	0.72d	0.66f

[1]Distilled pomaces were steam distilled for 2 hours

[2]Free aromatics were the aglycone's found naturally in the pomace obtained after maceration.

[3]Bound aromatics were the glycosylated forms of aromatics in the pomace obtained after maceration. Bound aromatics were analyzed as the aglycone after deglycosilation.

[4]Isoamyl alchol = 3-methyl-1-butanol

[5] Means followed by the same lower case letter within the aromatic type (free or bound) and each grape cultivar do not differ according to LSD at $p < 0.05$

[6]Phenthyl alcohol = 2-phenyl ethanol

[7]Means for total aromatics followed by the same upper case letter do not differ according to LSD at $p < 0.05$

3.2 Phytosterols and policosanols

Phytosterol and policosanol studies were conducted on various fractions of the grape pomace. They were:

(i) Grape skins

(ii) Whole grape seeds

(iii) Solvent extracted oil (obtained from whole seeds) and solvent extracted meal (residue after oil was extracted from seeds)

(iv) Mechanically extracted oil (using oil press)

(v) Press cake (obtained after oil was pressed from seeds).

3.2.1 Seed characterization

Phytosterols and policosanols were prevalent within the lipids and waxes in foods, including grapes (Grosjean and others 2015; Lupi and others 2013; Seo and others 2013). Grape skins were expected to have lower concentrations of phytosterols and policosanols as they had lower amount of lipids and waxes (Seo and others 2013). Since grape seeds had the highest concentration of lipids within pomace components (Wang and others 2003) they were

expected to have the most phytosterols and policosanols. When oil was extracted from grape seeds, phytosterols and policosanols were substantially enriched in the oil and the residual seed meal was notably deficient in the components (Beveridge and others 2005).

(i) Grape skins

Grape skins were less enriched in phytosterols and policosanols in pomace than grape seeds.

Phytosterols – 'Riesling' and 'Traminette' had highest concentrations of phytosterols among the grape skins, followed by 'Merlot', 'Muscat' and 'Sauvignon Blanc' (Table 5). The most dominating phytosterol was β-sitosterol in all grape varieties. Dagna and others (1982) also found that predominant phytosterol in was grape skins β-sitosterol.

Policosanols – 'Merlot' skins had the highest concentration of policosanols, followed by 'Sauvignon Blanc', 'Riesling', 'Traminette' and 'Muscat' (Table 6). Eicosanol predominated in 'Merlot', 'Muscat', 'Sauvignon Blanc' and 'Traminette' skins. 'Merlot' skins also had hexacosanol and octacosanol. Octacosanol predominated in 'Riesling' and also had hexacosanol and heptacosanol in lower concentrations. 'Sauvignon Blanc' had heneicosanol, tetracosanol and tricosanol in lower concentrations and 'Traminette' had heptacosanol and octacosanol.

(ii) Whole seeds

Phytosterols – Phytosterols in seeds were at least twice the concentration present in skins. Highest concentration of phytosterols were identified in 'Sauvignon Blanc' followed by 'Riesling', 'Traminette' and 'Merlot' and lowest in 'Muscat' (Table 5). In all the varieties, β-sitosterol was the most abundant phytosterol. Hollis and others (2009) also found that grape

seeds have β-sitosterol as the predominant phytosterol. 'Riesling' seeds had very low concentration of campesterol and stigmasterol compared to skins.

Policosanols – Policosanols were about 3 times more concentrated in seeds compared to the skins. Highest concentrations of policosanols were observed in 'Sauvignon Blanc' and lowest in 'Riesling' (Table 6). Most dominant policosanol in 'Sauvignon Blanc' was heneicosanol followed by eicosanol, tricosanol and tertracosanol. 'Merlot' and 'Muscat' seeds had almost equal concentrations of policosanols. 'Merlot' had eicosanol, heneicosanol, tertracosanol and tricosanol and 'Muscat' had hexacosanol, heptacosanol and octacosanol. In 'Traminette' hexacosanol, heptacosanol and octacosanol were identified.

(iii) Solvent extracted oil and solvent extracted seed meal: Solvent extracted oil had 8 to 16 times higher concentrations of phytosterols and policosanols than whole seeds and the solvent extracted seed meal was notably depleted in phytosterols and policosanols (Table 5 and 6). Phytosterols –β-sitosterol was the most predominant phytosterol in grape seed oils (Table 5). 'Sauvignon Blanc' solvent extracted oil had highest concentration of phytosterols, followed by 'Merlot', 'Traminette', 'Muscat' and 'Riesling'. Firestone (2006) found that among all phytosterols, β-sitosterol would be present in large concentrations (87%), followed by campesterol (6.6%) and stigmasterol (1%) in grape seed oil. Wang and others (2010) also found that phytosterols predominated in grape seed oil and β-sitosterol was found in high concentrations (84%). In this study, we have obtained about 78% β-sitosterol of total phytosterols in solvent extracted oil. Biglar and others (2012) found that 'Merlot' and 'Riesling' seed oil had about 74% β-sitosterol of total phytosterols. 'Sauvignon Blanc' oil had

the highest concentration of β-sitosterol, followed by ‘Muscat’, ‘Merlot’, ‘Traminette’ and ‘Riesling’.

Policosanols – ‘Sauvignon Blanc’ solvent extracted oil had the highest concentration of policosanols followed by ‘Muscat’, ‘Merlot’, ‘Traminette’ and ‘Riesling’ (Table 6). Eicosanol predominated in ‘Merlot’ seed oil. Heneicosanol predominated in ‘Muscat’ and ‘Sauvignon Blanc’ solvent extracted oil and heptacosanol predominated in ‘Traminette’ solvent extracted oil. ‘Merlot’ and ‘Sauvignon Blanc’ had similar concentrations of policosanols (about 37 µg gm^{-1}). ‘Riesling’ had hexacosanol, heptacosanol and octacosanol.

Table 5. Phytosterols (μg gm^{-1}) for skins, seeds, solvent extracted oil and residual solvent extracted meal obtained from 'Merlot', 'Muscat', 'Riesling', 'Sauvignon Blanc' and 'Traminette' dried grape pomaces.

Cultivar	Fraction	Campesterol	Stigmasterol	β-sitosterol	Phytosterols[1]
Merlot	Skins	35.60b[2]	13.39c	48.84a	97.83L[3]
Merlot	Seeds	45.33c	79.68b	108.42a	233.43G
Merlot	Solvent extracted oil	352.33c	619.67b	1008.54a	1980.54E
Merlot	Solvent extracted meal	16.02c	51.85a	43.93a	111.81L
Muscat	Skins	10.00b	-	39.86a	49.86O
Muscat	Seeds	-	82.41b	126.42a	208.83I
Muscat	Solvent extracted oil	-	654.44a	1004.29a	1658.73D
Muscat	Solvent extracted meal	-	11.28b	66.66a	77.94N
Riesling	Skins	25.10c	37.42b	43.23a	105.75K
Riesling	Seeds	5.10c	7.42b	93.26a	105.78K
Riesling	Solvent extracted oil	471.03b	479.61b	716.03a	1669.67C
Riesling	Solvent extracted meal	25.10c	37.42b	43.23a	105.75K
Sauvignon Blanc	Skins	-	19.99a	16.37b	36.36P
Sauvignon Blanc	Seeds	-	52.92b	185.11a	238.03F
Sauvignon Blanc	Solvent extracted oil	465.68b	-	1609.56a	2075.24A
Sauvignon Blanc	Solvent extracted meal	-	20.04b	102.99a	123.03J
Traminette	Skins	31.39b	20.90c	53.24a	105.53K
Traminette	Seeds	86.12a	52.53b	90.94a	229.59H
Traminette	Solvent extracted oil	747.18b	462.91c	789.23a	1990.32B
Traminette	Solvent extracted meal	19.16b	17.09c	54.09a	90.34M

[1]Phytosterols - the total of campesterol, stigmasterol and β-sitosterol

[2]Means for individual phytosterols followed by the same lower case letter within each cultivar do not differ according to LSD at $p < 0.05$

[3]Means for total phytosterols followed by the same upper case letter do not differ according to LSD at $p < 0.05$

Table 6. Policosanols (µg gm^{-1}) for skins, seeds, solvent extracted oil and residual solvent extracted meal obtained from 'Merlot', 'Muscat', 'Riesling', 'Sauvignon Blanc' and 'Traminette' dried grape pomaces.

Cultivar	Fraction	Eicosanol	Heneicosanol	Tetracosanol	Tricosanol	Hexacosanol	Heptacosanol	Octacosanol	Policosanols[1]
Merlot	Skins	17.41a[2]	-	-	-	6.43c	-	13.05b	36.90I[3]
Merlot	Seeds	-	70.22a	27.52b	-	-	-	-	97.73G
Merlot	Solvent extracted oil	720.30a	-	-	-	-	-	-	720.30D
Merlot	Solvent extracted meal	9.39a	-	-	-	4.05b	-	1.58c	15.02O
Muscat	Skins	5.92a	-	-	5.38a	-	-	-	11.31Q
Muscat	Seeds	11.76c	51.72a	10.43d	24.09b	-	-	-	98.02G
Muscat	Solvent extracted oil	329.68c	-	209.92d	433.57a	351.34b	-	156.66e	1481.17B
Muscat	Solvent extracted meal	4.84c	-	7.20a	5.98b	2.37d	-	-	20.39M
Riesling	Skins	-	-	-	-	5.14c	6.13b	15.72a	27.01K
Riesling	Seeds	-	-	-	-	5.14c	6.14b	15.72a	27.01K
Riesling	Solvent extracted oil	-	-	-	-	135.77c	182.75b	379.61a	698.13E

Riesling	Solvent extracted meal	-	-	-	-	5.15c	6.13b	15.72a	27.02K
Sauvignon Blanc	Skins	10.67a	6.03d	8.29c	9.36b	-	-	-	34.36J
Sauvignon Blanc	Seeds	23.36b	55.86a	9.06d	21.22c	-	-	-	109.53F
Sauvignon Blanc	Solvent extracted oil	252.48d	462.99b	298.44c	725.72a	-	-	-	1739.62A
Sauvignon Blanc	Solvent extracted meal	2.66d	5.29b	8.39a	3.33c	-	-	-	19.67N
Traminette	Skins	15.78a	-	-	-	-	3.83c	6.58b	26.20L
Traminette	Seeds	-	-	-	-	-	31.97a	24.32b	56.30H
Traminette	Solvent extracted oil	677.24a	-	-	-	-	408.59b	314.26c	1400.00C
Traminette	Solvent extracted meal	7.39a	-	-	-	-	4.24b	3.13c	14.76P

[1]Policosanols are the totals of all the identified compounds across the table for each cultivar and fraction

[2]Means for individual policosanols followed by the same lower case letter within each cultivar do not differ according to LSD at $p < 0.05$

[3]Means for total policosanols followed by the same upper case letter do not differ according to LSD at $p < 0.05$

3.2.2 Mechanical seed oil expression

Grape seed oil was expected to have high concentrations of phytosterols (Seo and others 2013; Grosjean and others 2015). The residual cake obtained after extracting the oil was expected to have less phytosterols and policosanols as most of them would be extracted with the oil. Mechanically extracted oil was also expected to have high concentrations of phytosterols and policosanols. More total phytosterols and policosanols were expected in the meal obtained after seed pressing since 25 to 30% of oil typically remained in the press cake (Navas 2009).

'Red Zinfandel' and 'Riesling' used for the mechanical pressing of grape seeds were obtained from a separate batch of seed from that previously used for phytochemical analyses. These grapes were harvested in 2013 and 2014. The grape seeds were separated mechanically and pressed. The moisture content in these seed (about 14%) was adequate for pressing and the seeds were available in large amount, which is required to conduct oil pressing with our oil press. The pomace samples for varieties used for solvent extraction in Tables 5 and 6 had fewer grape seeds and the moisture content was too low (about 5%) for mechanical oil expression. Since skins/pulp for the samples utilized for mechanical oil expression were previously separated and discarded they were not available for analysis.

(iv) Seeds for mechanical oil pressing (Red Zinfandel and Riesling)

Phytosterols – Seeds for mechanical pressing were slightly higher in total phytosterols (>270 µg gm^{-1}; Table 7) than seeds used for solvent oil extraction (Table 5). Seed for mechanical pressing were obtained from a different batch of pomace than those used for drying and pomace component separation. The observed variability was likely normal variation in phytosterol concentration since phytosterol concentration is known to vary in seed from batch

to batch (Pardo and others 2011). These seed contained considerably less phytosterols than mechanically pressed oil (Table 7). 'Riesling' had more phytosterols than 'Red Zinfandel' (Table 7). Stigmasterol and campesterol were at similar concentrations and β-sitosterol was the highest for both 'Red Zinfandel' and 'Riesling'. 'Red Zinfandel' had about 75% β-sitosterol of the total phytosterols and 'Riesling' had about 65% β-sitosterol of the total phytosterols. Jackson (2008) found that 'Red Zinfandel' seeds have 80% of β-sitosterol. Policosanols – About 50% of the policosanols in was octacosanol in 'Riesling' and 'Red Zinfandel'. Policosanols were almost of the same concentration as that of seeds used for solvent extracted oil. Hexacosanol and heptacosanol were also present in 'Red Zinfandel' (Table 8) and eicosanol and heptacosanol were present in 'Riesling'.

(v) Mechanically extracted oil and press cake.

The solvent extracted oil contained more phytosterols and policosanols than mechanically extracted oil (Table 5, Table 6, Table 7 and Table 8). These compounds were also only about half as enriched from the seed into the oil after mechanical pressing (mechanically pressed oil contained 4 times the concentration of phytosterols and policosanols as was contained in seeds; Tables 7 and 8) versus solvent oil extraction (phytosterols and policosanols were 8 to 16 times higher in solvent extracted oil versus the seed feedstocks; Tables 5 and 6). Phytosterols and policosanols are known to be sensitive to degradation at temperatures of 60°C or higher (Fernandes and Cabral 2007). Frictional heat created by the mechanical oil press easily exceeded 60°C. The noted decrease in total phytosterols and policosanols, and the reduced enrichment from seeds into the oil, for mechanically pressed oil was likely caused by their heat degradation and loss.

Phytosterols - Highest phytosterol concentrations were observed in ‘Red Zinfandel’ and ‘Riesling’ was β-sitosterol (Table 7). Press cake had less phytosterols compared to mechanically pressed oil, solvent extracted oil and whole seeds. Highest concentration was observed in ‘Riesling’. Concentrations were approximately halved after solvent extraction of the press cake. The solvent extracted press cakes from mechanical oil pressing contained considerably lower phytosterols than solvent extracted press cakes from seeds not exposed to mechanical pressing, perhaps also indicating thermal loss in the oil press.

Policosanols – Press cake had less policosanols compared to mechanically pressed oil and whole seeds. ‘Riesling’ had more policosanols retained than ‘Red Zinfandel’ (Table 7). Only hexacosanol was retained in ‘Red Zinfandel’ press cake. In ‘Riesling’ press cake, all the compounds were retained but about 8 times lower than in oil.

Table 7. Phytosterols (µg gm^{-1}) from seeds, oil, press cake and solvent extracted press cake obtained after mechanical pressing of 'Red Zinfandel' and 'Riesling' bulk seeds.

Cultivar	Fraction	Campesterol	Stigmasterol	β-sitosterol	Phytosterols[1]
Red Zinfandel	Seeds	34.61b[2]	61.64c	174.60a	270.85D[3]
Red Zinfandel	Oil	201.43b	145.56c	826.83a	1173.82B
Red Zinfandel	Press cake	10.23b	-	52.37a	62.60E
Red Zinfandel	Solvent extracted press cake	12.06b	-	24.02a	36.08G
Riesling	Seeds	56.56c	59.69b	189.74a	305.99C
Riesling	Oil	244.53b	206.23c	764.73a	1215.49A
Riesling	Press cake	15.04b	-	24.52a	39.56F
Riesling	Solvent extracted press cake	4.05b	-	19.50a	23.55H

[1]Phytosterols - total of all the phytosterols for each fraction of the cultivar

[2] Means for individual phytosterols followed by the same lower case letter within each cultivar do not differ according to LSD at $p < 0.05$

[3] Means for total phytosterols followed by the same upper case letter do not differ according to LSD at $p < 0.05$

Table 8. Policosanols (µg gm^{-1}) from seeds, oil, press cake and solvent extracted press cake obtained after mechanical pressing of 'Red Zinfandel' and 'Riesling' bulk seeds.

Cultivar	Fraction	Eicosanol	Hexacosanol	Heptacosanol	Octacosanol	Policosanols[1]
Red Zinfandel	Seeds	-	5.14b[2]	-	6.15a	11.29F[3]
Red Zinfandel	Oil	-	156.54a	-	76.68b	233.22B
Red Zinfandel	Press cake	-	3.27a	-	-	3.27H
Red Zinfandel	Solvent extracted press cake	-	3.81b	-	4.33a	8.14G
Riesling	Seeds	16.94c	-	20.53b	46.41a	83.88C
Riesling	Oil	86.54c	-	102.68b	70.73a	259.95A
Riesling	Press cake	14.42a	-	6.12b	15.08a	35.62D
Riesling	Solvent extracted press cake	-	5.96c	7.15b	13.41a	26.52E

[1]Policosanols - total of all the policosanols for each fraction of the cultivar

[2] Means for individual policosanols followed by the same lower case letter within each cultivar do not differ according to LSD at $p < 0.05$

[3] Means for total policosanols followed by the same upper case letter do not differ according to LSD at $p < 0.05$

3.3 Oil concentration in grape seeds

3.3.1 Solvent extracted grape seed oil

Oil concentration is shown in Table 9. ‘Merlot’ grape seeds had highest concentration of oil and Traminette’ had lowest. Oil percentage was expressed on dry weight basis. The grape seed oil obtained had a light green color.

The seeds obtained were hand separated from the dried grape pomace. ‘Merlot’ grape seeds had about 13% oil and ‘Sauvignon Blanc’ had about 12% oil. Beveridge and others (2005) found that ‘Merlot’ has about 11% oil and ‘Sauvignon Blanc’ had about 13% oil (samples were obtained from grape pomace in Canada). ‘Muscat’ had 13% oil. Results were in agreement with Tangolar and others (2009). They had found that ‘Muscat’ had about 13% oil, samples were grown in Turkey. Baydar and Akkurt (2001) found that ‘Muscat’ seeds have 19% oil and their samples were also from Turkey. In this study, ‘Riesling’ seeds, from Oklahoma, had about 12% oil. Gokturk Baydar and Akkurt (2001) found that ‘Riesling’, from Turkey, had about 15 % oil. The reason for the difference in the values could be the region where the grapes were grown and cultivation practices. Also, the maturity of the grapes affects the concentration of oil present in grape seeds. When the grapes are mature, they have more oil in the seeds than the immature grape seeds (Gokturk Baydar and Akkurt 2001). Grape seed oil can be used as a substitute for linseed oil (Godin and Spensley 1971), in cosmetic products and as an edible oil (Vanhanen and Savage 2013). Grape seeds that had oil containing 8% to 15% are generally used for oil production (Mironeasa and others 2010). The grape varieties used in this study fall within that range of oil content.

Table 9. Oil concentration for ‘Merlot’, ‘Muscat’, ‘Riesling’, ‘Sauvignon Blanc’ and ‘Traminette’ grape seeds obtained from dried grape pomaces.

Cultivar	Oil %
Merlot	12.86c[1]
Muscat	12.60b
Riesling	11.77b
Sauvignon Blanc	11.51ab
Traminette	10.59a

[1]Means followed by the same letter for oil% do not differ according to LSD at $p < 0.05$

3.3.2 Mechanically extracted grape seed oil

The total oil concentration for mechanically extracted oil and press cake obtained after pressing the seed are shown in table 10. About 70% of oil present in the grape seeds was extractable. The press cake obtained had about 4-5% oil which was not extracted. Screw speed and die size could be adjusted to obtain better yield. Also, the moisture content of the seed is important to determine the quantity of oil that could be expelled. Seed that was too moist produced meal that was gummy and would not produce oil as it passes through the press (Rombaut and others 2012). Too much moisture in the seed tied up the oil and inhibited separation from the meal. Too dry seeds will not have sufficient moisture for meal lubrication as it is forced past the die. Due to high pressure and friction, the temperature of the screw would increase and the seed meal would burn and come out through the holes on the barrel. As the expeller die diameter is made smaller, force needed to push press cake through the press increased, increasing the pressure to expel oil out of the seeds. Though solvent extraction yielded more oil, mechanical cold oil pressing of oils is preferred due to lower cost (Rombaut and others 2015; Da Porto and others 2013). At increased temperature, the phytosterols, policosanols, antioxidants, phenols and fatty acids present could break down to

unwanted products. One other advantage of having a press to extract oil is to have a consistent product with continuous feed and is easy to maintain (Esposto and others 2013).

Table10. Seed and press cake oil concentration obtained after mechanical pressing of 'Red Zinfandel' and 'Riesling' bulk seeds.

Cultivar	Tissue type	Oil%
Red Zinfandel	Seed	14.63a[1]
Red Zinfandel	Press cake	4.28b
Riesling	Seed	14.46a
Riesling	Press cake	4.92b

[1]Means followed by the same letter for oil% do not differ according to LSD at $p < 0.05$

3.4 Pomace seed content

The seeds were obtained from different pomaces. Data is tabulated in table 11. The seeds were hand separated and corrected to moisture content to obtain data on dry basis. Baydar and others (2007) and Khanal and others (2009) have found in their study that grape pomace had about 50% seeds. Hand separation was a lengthy process. Two different types of sieves were used: one to separate the large skins and the other to separate smaller particles than seeds. The stems and twigs had to be separated from the seeds to obtain seed percentage. Seed separation equipment (such as The Clipper Eclipse 324, Indiana, USA) could be used on an industrial basis to obtain faster results. These seeds had 4-5% moisture. To obtain a mechanically pressed oil from these seeds, the moisture has to be adjusted to about 10%.

Table 11. Seed percentage for ‘Merlot’, ‘Muscat’, ‘Riesling’, ‘Sauvignon Blanc’ and ‘Traminette’ dried grape pomaces.

Cultivar	Seed %[1]
Merlot	49.53ba[2]
Muscat	53.35a
Riesling	52.16ac
Sauvignon Blanc	48.83c
Traminette	51.63ac

[1]Seed percentage is expressed on dry weight basis

[2]Means followed by the same letter for seed % do not differ according to LSD at $p < 0.05$

CHAPTER IV

CONCLUSION

This work provided baseline information related to phytochemicals present in grape pomaces and which could represent valuable components from this current Oklahoma waste product. Some general conclusions are presented below, in order of the stated objectives:

Objective 1: To evaluate aromatic chemical extraction from pomaces using vacuum steam distillation. Aromatics were mostly located in skins/pulp and were obtained from fresh pomaces with total concentrations ranging from approximately 1 to 2 mg gm^{-1}; the 2 h vacuum steam distillation process reduced aromatics by < 10 to almost 20 % and obtained distillates ranging from 0.8 to 7.8 mg kg^{-1} which contained 0.2 to 0.3 mg l^{-1} total aromatics. Further work to improve this low yield of aromatics could include longer distillation time, improving efficiency of distillate condensation by cooling the condenser as well as the round bottom flask and/or by reducing the bed volume of pomace to improve steam contact during distillation.

Objective 2: To isolate and characterize free and bound aromatic chemical profiles of pomaces produced from various white and red grapes. The free and the bound aromatic analysis showed that the free aromatic compounds present in non-distilled pomace were found at lower concentrations in distilled pomaces but the bound fraction remained at about the same concentration. Free aromatics were present in higher concentration than bound

volatiles ranging from 1.49 to 0.61 µg gm^{-1} and bound volatiles ranged from 0.64 to 0.28 µg gm^{-1}. 'Riesling' showed the most decrease in free aromatics of distilled pomace compared to non-distilled pomace. But 'Merlot' essential oil showed highest amount of aromatics. The reason for this unexpected data is unknown. Further work to better preserve these aromatics could be continued from this project. Methods to capture the aromatic compounds could include improving the condensation technique of essential oil.

Objective 3: To evaluate phytosterols and policosanols obtained from grape pomace skins/pulp, grape seeds, grape seed oil and de-oiled seed meal. Phytosterols and policosanols were mostly located in grape seed oil. Grape seed oil had about 8-16 times of phytosterols and policosanols than in grape seeds, indicating substantial enrichment into the oil fraction. A mechanical oil press could extract about 70% of the oil present in the grape seeds. Solvent extracted oil had twice the concentration of phytosterols and policosanols compared to mechanically pressed oil. The high temperature of the press cake and oil during oil expelling probably caused thermal degradation of the phytosterols and policosanols in the oil. Future work to optimize oil extraction from seeds and yet reduce temperature during the seed pressing operation should include oil press mechanical variables of expeller die size (influencing back-pressure on the press cake) and expeller screw speed (influencing volume of product expelled per unit time and interacting with frictional heat produced during operation). Grape seed moisture content strongly interacts with the press mechanical variables by influencing the press cake plasticity, thus frictional force required to pass out of the press. Using a larger size expeller die and adjusting the screw speed, in addition to adjustment of seed moisture content to increase/decrease press cake plasticity during the

pressing operation could decrease the frictional force and temperature within the expeller barrel and might help retain the phytochemicals. Reconstituting and/or adjusting the moisture into the grape seeds gives a scope for a new study that can be continued from this project.

Objective 4: To document the concentration of oil present in grape seed from various cultivars grown in Oklahoma and investigate amount of oil that can be extracted mechanically from the seeds. 'Merlot' grape seeds had highest amount of oil (13%) and 'Traminette' had lowest (10%). About 70% of the oil in grape seeds could be extracted mechanically (about 8-10% of grape seed weight). The press cake, representing 80 to 90 % of the remaining weight can also be further processed to obtain value-added products. Frictional heat during oil pressing can degrade valuable phytochemicals in the oil and the press cake so the work suggested in the previous paragraph to optimize and decrease heat generated during the oil pressing step could add value to the press cake. While we did not investigate further use of the press cake in this study, grape seed flour and grape seed extract represent possible uses for the press cake. Grape seed extract can be obtained from the press cake by extracting with 50% ethanol to produce a product containing 33% total phenolics (Vayupharp and Laksanalamai 2012). Purification of this extract with chromatographic purification would yield a dry extract containing 95% Oligomeric Proanthocyanidin (Ramirez-Lopez and DeWitt 2011). This grape seed extract is rich in catechins and proanthocyanidins and is used in meat industries for its anti-oxidant and anti-microbial properties (Lau and King 2003; Meeprom and others 2011; Özvural and Vural 2014). Grape seed flour is obtained by milling grape seed cake. Further work can be conducted on the grape seed cake by breaking down, separating and sizing the press cake which could aid milling. Grape seed flour can be used in baking

industries as gluten-free ingredient which can be added to baked goods to improve flavor, color and nutrition. Methods to preserve the potency of phytochemicals while producing the grape seed flour would need to avoid degradative conditions, such as exposure to high temperature and prolonged exposure to oxygen to maintain its potency for food uses.

Based on this work some values for products generated from Oklahoma wine grape pomaces can be derived. Oklahoma has about 439 acres of commercially operating vineyards, producing about 2.5 tons of grapes per acre (about 1054 tons in total). Grape pomace would produce a pomace accounting to 14 % of fresh weight. Grape seeds represent 26% of fresh grape pomace and 50% of dry weight. Oklahoma produces about 38 tons of seed. This seed would yield about 3,500 kg seed oil and about 25 tons of de-oiled meal. Grape seed oil would cost about $5 per liter. The de-oiled seed powder may be ground and sold for $4 for 2 pounds. The de-oiled meal would carry a value of $200,000. A grape seed extract would carry a value of about $282,000 if extracted and sold in bulk. The seed separating equipment and oil press account to about $27,000 making it economic for the industries to obtain products on a commercial scale.

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