Effect of organic production methods on antioxidant activity and concentrations in grapes, grape juice and wine; results from meta-analyses, and farm and retail surveys

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Thesis submitted for the degree of Doctor of Philosophy

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December 2017

ABSTRACT

A wide range of studies have investigated effects of organic farming practices on the composition of nutritionally relevant compounds in crop plants. Many studies focused on antioxidant concentrations, since plant polyphenols and other phytochemicals with antioxidant activity have been linked to a reduction in a range of chronic diseases.

However, relatively few studies have compared the nutritional composition of grape and grape products such as grape juice and wine. Grapes are known to contain substantial amounts of secondary metabolites (e.g. phenolic compounds) with antioxidant activity.

The main aim of the study reported here was to investigate whether and to what extent organic management practices and variety choice affect quality parameters in grape and grape products, using a range of approaches including (a) a systematic literature review and meta-analysis of published data, (b) a basket studies/retail survey in the UK and (c) a farm survey in central Crete. The systematic literature review and meta-analysis of published data was conducted to identify significant composition differences of organically and conventionally produced grape and its products. For example, significantly higher concentration of total flavonoids ($P=0.017$ and $P=0.006$) and anthocyanins ($P=0.024$ and $P<0.001$) were detected in organic compared to conventional grapes/grape products by both unweighted and weighted meta-analysis.

The retail survey of table grapes available in UK supermarkets and farm survey of grapes and wine produced by organic and conventional grape and wine producers in Crete were both conducted over a 2 year period and aimed at identifying effects of agronomic practices used in organic versus conventional farming systems and potentially confounding factors (e.g. variety choice, climatic conditions) on the phytochemical composition in grapes/grape products.

The retail survey of table grapes was based on collecting organic and conventional white, red and black grapes from 3 big UK supermarkets (Tesco, Sainsbury's and Waitrose) at regular intervals in both the winter (when grapes are mainly from Southern Africa) and summer (when grapes are mainly from Mediterranean area) seasons in 2 years. For total antioxidant activity, total phenolic and anthocyanin contents there were no consistent effects of production system (organic vs conventional) but a range of significant interactions between production system and grape variety were identified. For some parameters there were also interactions between production systems and/or grape variety, and year. A significant

interaction (*P=0.043)* between management system and variety choice was detected for the black grape variety Midnight Beauty, which had a higher concentration of total anthocyanins in organic samples compared to conventional. Additionally a positive association between total phenolic content and antioxidant activity was observed while analysing black grape varieties. The interaction between management system and production year indicated higher concentration of total phenolic content and antioxidant activity in organic samples compared to conventional in one of the experimental years (2015). A significant interaction between variety choice and production year was detected for total antioxidant activity mainly in white (*P=0.002)* and red (*P=0.004)* grape varieties. For total antioxidant activity there was also significant three-way interaction (between management system, variety choice and production year), but only in the red grape varieties (*P=0.042*). A significant 2-way and 3-way interactions between factors (e.g. management system and variety choice, and production year) was also detected for a range of individual anthocyanins (e.g cyanidin 3-O-glucoside, malvidin 3-O-glucoside, peonidin 3-O-glucoside).

The farm survey in Crete focused on the most widely grown local red and white grape varieties, as well as wines made from these local varieties. A significant interaction between management system and variety choice (*P=0.013)* and between variety choice and production year (*P<0.001)* were detected only for total antioxidant activity, but there were also significant main effect of factors (management system, variety choice and production year).

Similar to previous studies, a positive association between total phenolic content and antioxidant activity was also detected for analysed grape samples.

Different to previous reports on differences in phenolic compounds between grape varieties, the concentration of total phenolic in one of white grape varieties (Vidiano) was slightly higher than in the red grape variety (Kotsifali). However, in wine samples it was confirming findings from previous studies with white wine samples having lower concentration of total phenolic comparing to red wine samples. For wine samples, only significant interaction (*P=0.047*) (between production system and variety choice) was detected for the total phenolic content, where the concentration was higher in conventional compared to organic red wine samples, but no significant difference in white wine.

The results indicate that variety choice and pedo-climatic conditions in different years may be a strong confounding factor in studies comparing the nutritional composition of organic and conventional grapes and grape products. Future studies should therefore investigate the reasons why some varieties produce higher levels of specific antioxidants in organic, while

other varieties have higher levels in conventional production systems. Also, future studies should investigate to what extent switching to organic table grape/wine consumption may provide additional health benefits.

This thesis is dedicated to my parents, Akif and Gulaba, who believed in and supported me with their blessings.

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ACKNOWLEDGMENTS

I am always grateful to my family for offering me all the support, love and understanding that helped me to be able to continue through all these years and accomplish my target.

I would like to thank my supervisor Prof. Carlo Leifert for his precious patience, advice and guidance from the beginning till the end of my study and for every support he has offered me through these years.

I also would like to thank my supervisors Prof. Chris Seal, Dr. Gavin Steward and Dr. Edward Okello for their valuable help and scientific contribution to my thesis.

I would like to thank Panagiotis Pakos for his precious help for my study design by finding farmers, interviewing farmers and providing any help during sample collection in Crete. Also, would like to thank Nikos Volakakis for his great help with accommodation, storage of samples, providing working station and with everything I needed during stay in Crete.

I would like to thank Eleni Chatzidimitriou for her precious help during sample collection in Crete, laboratory training, assistance and patience through experimental years of my study; Dr. Marcin Baranski for his patience and precious help with statistical analyses of data presented in this thesis, especially for meta-analyses; Dr. Leonidas Rampelos for his precious support and guidance during writing-up period.

I would like also to thank laboratory technician team of school for their help during my study and Andrew Porter from NUPPA for his valuable help in LCMS analysis.

I also would like to thank my colleagues Alex Macpherson, Amelia Magistrali, Natasha Auch and Craig Tallentire for their help during proofreading of my thesis and support.

I am very grateful to my friends Pattanapong Tiwassing (Oak), Enas Safur, Juan Wang and especially Jorge Altamirano Flores for their great support and making these years of my life happier.

Finally, I am thankful to everyone who made me to accomplish this stage of my life with their patience, understanding, love and blessing.

Chapter 1. Introduction

1.1 Grapevine taxonomy, morphology and usage

Taxonomy

Grapevine is the common name for the deciduous woody plants of the genus *Vitis* and grapes are fruit (botanically berries), growing on the deciduous woody vines of *Vitis* plants. A range of *Vitis* species are cultivated throughout the world and consumed fresh as table grapes or processed into a range of products (e.g. wine, raisins, jams/jellies, juice, grape seed oil, vinegar or distilled spirits such as cognac, grappa or raki). The genus *Vitis* is a member of the *Vitacea* family and typically members of this family are woody, tree-climbing vines, although a few have a shrubby growth habit. The genus *Vitis* consists of two subgenera, Euvitis and Muscadinia. These subgeneras have different (a) morphological features (e.g. seed shape, berry number per cluster, smooth bark) and (b) numbers of chromosomes (38 and 40 respectively). The subgenus Muscadinia includes species *M. rotundifolia*, *M. munsoniana* and *M. popenoei* (Keller, 2015). However only a few varieties of *M. rotundifolia*, known as "muscadines", are cultivated for table grape, jelly and wine (Olmo, 1986).

The subgenus Euvitis includes *V. vinifera* and *V. labrusca* which are the most widely planted/grown grape species in the world and are mainly use as table grapes, and for wine, distilled spirit, grape juice and raisin production (Creasy and Creasy, 2009). The original area of domestication of *V. vinifera* grapes, which is now planted all over the world, is thought to be mountain ranges of southern Caucasia, an area which is now north-west Turkey, northern Iraq, Azerbaijan and Georgia (Mullins *et al.*, 1992; Grassi *et al.*, 2006). In contrast *V. labrusca* originates from North America (Creasy and Creasy, 2009).

Morphology

The grapevine root system consists of large branches, root tips and root hairs, which accounts for the majority of nutrient and water uptake by the plant (Pratt, 1971). Another important part of the root is the root cap, which covers and protects the continuously dividing root tip meristem cells from physical obstacles while the root tip penetrates the soil. The starch granule cells within the root tip are thought to play a "navigation" role in directing the expansion of roots and the below ground distribution of the root system (Wilkins, 1966). Cell division, elongation and differentiation are stimulated by several hormones (e.g. auxin, cytokinin, gibberellin) during growth (Kramer and Bennett, 2006; Wang and Li, 2008; Yamaguchi, 2008). Through a far-reaching and highly brunched structure, mature grapevine

roots can grow to a depth of 30 m and more, substantially decreasing the potential of water stress (Keller, 2010). This characteristic feature means that grapevine is well-suited plant for semi-arid climate conditions, such as the Mediterranean area (Chaves *et al.*, 2010). The vascular system consists of the xylem and phloem layers, which are responsible for water and nutrient circulation from roots to leaves (xylem) and from leaves to the rest of the plant (phloem) (Chaves *et al.*, 2010).

The above ground part of the mature vine is called the trunk, from which new shoots/canes arise in spring and which provides the base for new shoot and canopy growth, while acting as carbohydrate storage tissue (Creasy and Creasy, 2009). Grapevines are deciduous and lose their leaves in autumn, entering a dormant stage in winter and then producing new shoots, leaves and flowers/fruit in the spring. New shoots develop from nodes on the trunk and form canes with many new nodes on them. Usually leaves, tendrils and buds appear just above the nodes. Prompt, dormant and latent buds are the common bud types for grapevines. Dormant buds are overwintering compound buds which develop in spring and usually contain primary and secondary buds (Keller, 2010). The primary bud is the main bud from which flower clusters and fruits develop in the following season, however secondary buds are less fruitful and usually develop if the primary bud has been injured (Rombough, 2002). Prompt buds are usually considered as a secondary crop with small, less fruitful clusters, whereas latent buds can be dormant for some years (Keller, 2010) Nodes also produce tendrils, which support shoots and assist climbing for the vine and leaves. The leaves are the main part of the plant for photosynthesis and food production (Figure 1.1) (Rombough, 2002).

Figure 1.1 Grapevine tendrils and leaves inserted (opposing each other) from nodes (Keller, 2010)

The grapevine flower is a panicle inflorescence (Pratt, 1971) and florets of cultivated vines are usually perfect with pistil (stigma, style and ovary) and the stamen (anther and filament) (Figure 1.1.2) (Creasy and Creasy, 2009). The flowering period depends on environmental conditions and is affected by both temperature and light with flowering being delayed by cool, cloudy weather in spring. Unlike many plants, grapevine flowers have a cap (calyptra) instead of petals, which drops during flowering (Figure 1.2).

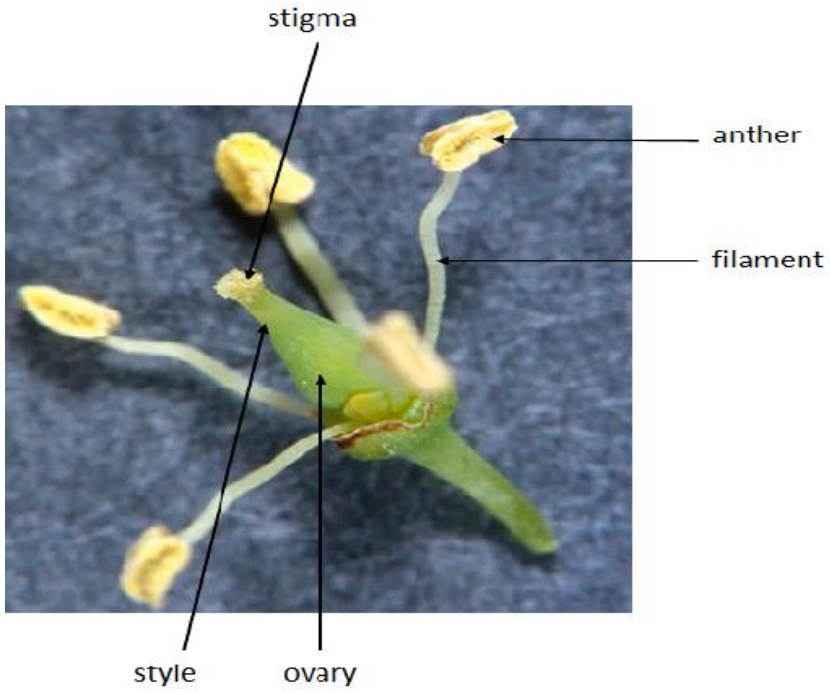


Figure 1.2 Individual parts of grape flower (Stewart, 2001)

The fruit of grapevines is a true berry, the bulk of which is flesh. The percentage of fruit set depends on density of the flower clusters (e.g. described as loose, well-filled, compact, very compact) (Rombough, 2002). Many factors, such as temperature, water supply, deficiency in certain nutrients, and diseases may affect fruit set of the grapevine (Creasy and Creasy, 2009). Varieties of the Euvitis subgenus usually have a maximum of four seeds (Winkler; Winkler; Winkler, 1935), while Muscadine grapes may have up to six seeds (Olien, 1990). The berry has a protective waxy surface, called the cuticle, which limits water loss and infection from bacterial and fungal diseases (Possingham *et al.*, 1967; Blanke and Leyhe, 1988). The epidermal layer of cells, or skin, of the berry also has a protective function, and contains colour and important flavour compounds.

Usage of grapes

Grapes are the most popular fruit for wine production and other alcoholic drinks (e.g. brandy, raki, grappa), besides being consumed as a table grape. There are also many other food products made from grape, such as grape juice, dried fruit (raisins) and vinegar (Creasy and Creasy, 2009).

The Food and Agriculture Organization (FAO, 2009) statistics suggest that around 76,000 square kilometres of agricultural land is used for grape production and this area has been increasing by 2% per year. Approximately 21% of global grape production is for table grapes and 2% for dried fruit, while 71% is processed into wine. Although there are no reliable statistics for the use of specific varieties it is thought that the most widely grown variety is Sultana, also known as "Thompson Seedless", which is grown on at least 3,600 km^2 (880,000 acres). The second most popular variety is thought to be Airén and other popular varieties include Cabernet Sauvignon, Sauvignon blanc, Cabernet Franc, Merlot, Grenache, Tempranillo, Riesling, and Chardonnay (FAO, 2009)

Wine production can be described as a "natural" method of fruit storage, which preserves many of the nutrients of grapes by protecting them against microbial spoilage and oxidation (Creasy and Creasy, 2009). The basic process of wine making is relatively simple, requiring virtually no additives, although nowadays a variety of compounds/substances that are intended to improve/modify the appearance, aroma, storability and taste of wines are added during the wine-making process (Amerine, 1980). The maturity level which grapes are harvested for wine making differs depending on the type of wine produced. Grapes for sparkling wine production, for example, are collected at a relatively early maturity stage or lower Brix° (level of sugar content of an aqueous solution) compared to grapes used for still table wines (Martínez-Lapuente *et al.*, 2012; Jones *et al.*, 2014). Grapes for sweet desert-type wines are picked very late in the season when the sugar levels are highest. The quality of wine is linked to the variety of grape and process related parameters including pH (acidity), sugar and alcohol content and flavour compounds, which all combine to generate the aroma and quality of the wine (Creasy and Creasy, 2009; Moreno-Arribas and Polo, 2009). It can be assumed that fresh fruit consumption was the primary use of grapes by humans. Today table grapes remain an important product for grapevine producers, with some specific cultivars (e.g. "Sultana", "Thompson Seedless", "Oval Kishmish") (Winkler et al., 1974) becoming popular table grapes throughout the world. Sugar concentration, acidity, colour, texture and aroma/ flavour parameters are important quality characteristics for table grapes. Also, uniformity of

fruit colour within the cluster has become an important table grape quality feature, especially in the supermarket supply chains (Clydesdale, 1993). Another important sensory parameter for customers is fruit texture, with most consumers preferring a crisp rather than a softer skin and flesh (Cliff *et al.*, 1996; Sato and Yamada, 2003). Table grapes are handpicked and there are usually several harvest times over a 4-8 week period in vineyards (Winkler, 1974).

Raisins or dried grapes are an important part of grape production but there are fairly few cultivars used for dried grape/raisin production, with "Sultana" being the most popular (Winkler, 1974). The production of raisins is highly reliant on the weather condition, as it usually involves placing bunches of grapes onto racks outdoors and drying them in the sun. Any significant precipitation or high humidity during the drying period will reduce raisin quality or lead to crop loss due to microbial spoilage (Creasy and Creasy, 2009). Raisins dried in the sun are usually dark coloured due to the oxidation of phenolic compounds. However, lighter-coloured raisins (sultanas or sultana raisins) can also be produced. Raisin production requires a special procedure where fresh fruit is dipped in a hot solution of caustic soda and sodium sulphite for a short period of time, then subjected to gaseous sulphite, which gives the raisin a yellow/golden colour. Usually after the dipping process, tiny cracks appear on the berry skin, which enhance the drying rate during air drying (Winkler, 1974). "Sultana" is the most popular variety for raisin production and this is thought to be due to its noticeable and natural bloom, its meaty texture and characteristic flavour, and also the fact that the sultana raisins are not sticky and have a low inclination to cake/stick together in storage (Winkler, 1974).

Grapes are suitable for the production of jellies, jams and other preserves. Grape juice is widely used in the bottled drinks industry, as a natural sweetener or on its own as grape juice (Winkler, 1974; Olien, 1990). However, compared to wine and table grape production, the amount of global grape production used for juice and other products is very small. Grape juice concentrate is often used as a food ingredient and has the benefit of being a means of adding a "natural" form of sugar to other products. More commonly, white grape concentrates are utilized as sweeteners and juice stock whereas red grape concentrates are used as food colourings (Clydesdale *et al.*, 1978).

The residues (grape skins and flesh) from juice and wine production have traditionally been used for the production (fermentation followed by distillation) of spirits (e.g. grappa or raki) and more recently for extraction of compounds such as polyphenols and other compounds

with antioxidant properties for use as nutritional supplements, natural health products or cosmetics (Teissedre *et al.*, 1996; Nuttall *et al.*, 1998; Yilmaz and Toledo, 2004).

1.2 Cultivation and Fertilisation:

The establishment of vineyards and subsequent management/cultivation of grapevines is a highly knowledge and labour-intensive process. Grape yield and quality are affected by a range of pedo-climatic and agronomic parameters, the most important of which are summarised in separate sections below.

Temperature

Heat accumulation, winter minimum temperatures, water availability and soil characteristics are the common features that are considered, while establishing vineyard site. Temperature is vital for grape existence and production of the preferred cultivar and composition of the grape. Therefore longstanding weather information at the specific site makes it easier to make a definite decision about the site's suitability for viticulture. Grapes can be cultivated in both hot and cold areas, but this most of the time depends on variety (Creasy and Creasy, 2009). However, higher temperature is always favourable by plants for more rapid phenological succession than cooler conditions (Alleweldt *et al.*, 1984; Chuine *et al.*, 2004; Wolfe *et al.*, 2005). When temperatures go below 10°C a dormancy period starts, which allows the plant to survive even if temperatures drop below 0°C (Creasy and Creasy, 2009). Temperatures below zero can cause chilling injury, which are reversible if they lasts for a short period (Jackson, 2008). The primary buds' tissues are the most sensitive parts effected by winter and spring frost, whereas secondary buds are the most cold-hardy. Frost tolerance can reach up to well below -30°C depending on variety (Jackson, 2008). Temperature control is relatively non-specific for bud activation, however it is crucially important for flowering and the fruit period. Flowering usually starts when the temperature riches 20°C and above, followed by pollen germination and fertilization at higher temperatures. Style penetration and fertilization can last a few days at lower temperatures (15°C), but only for a few hours when to temperature is higher (30°C) (Staudt, 1982). Even though high temperature is desirable for pollen germination and early fertilization, ovule fertility, seed number per berry and berry weight is more successful at lower temperatures (Jackson, 2008). Berry maturation, fruit composition and subsequently wine quality are also significantly influenced by temperature. Thus, higher temperatures usually increase sugar and reduce malic acid levels, which are important for taste, colour stability and aging of fruit. Consequently, depending on sugar and malic acid

levels the average temperature range for grape maturation is between 20-25^{0}C and lower for anthocyanin synthesis (Kliewer and Torres, 1972). Very high temperatures (above 40°C) usually affect photosynthetic process (by reducing) which is important for growth and fruit ripening (Zsófi *et al.*, 2009). In this case, evaporation plays an important role for plant's normal physiological activities by minimizing overheating (Keller, 2010).

Irrigation

Most grape-growing areas of the world depend on natural rainfall to reserve water to their vines, but many are also reliant on water brought to the vines. Grapevines are quite adaptable to semi-arid condition, because of the deep and wide growing root system, which can navigate water source from deeper levels of soil (Creasy and Creasy, 2009). However, vines need to be watered (25-50 mm) during the first few weeks after planting, which will help roots to grow deeper (Rombough, 2002). Irrigation is widely used for vineyards in semi-arid regions and is a valuable management tool to improve both grape yield and quality. However, the relative impact of establishing irrigation systems depends on the soil depth, type, texture, structure and drainage since these parameters all affect the availability of irrigation water to the crop and levels of water loss (Goldammer, 2013). All methods/technologies for irrigation are used in vineyards including flood/channel, sprinkler and drip/tape irrigation systems (Figure 1.3 [A-C]).

In semi-arid regions with limited water supply drip irrigation systems are increasingly used. Drip irrigation is the most efficient systems with respect to water use efficiency but also the most expensive to install and maintain (Goldammer, 2013). This system is comprised of long plastic water tubes that track down under each row of vines in the vineyard with individual drippers/emitters at appropriate intervals (Creasy and Creasy, 2009). In regions with high temperatures and low water availability, irrigation tubes may be permanently established/buried below ground, delivering water directly to the root zone and further minimising water loss/increasing water use efficiency (Camp, 1998).

Flood irrigation is another commonly used method with low-cost advantage, however it needs frequent maintenance of vineyards floor flattening and is not successful for precise water application (Creasy and Creasy, 2009).

A B C

Figure 1.3 Vineyards irrigation methods: A-flood/channel (Creasy, 2011); B-sprinkler (Impey, 2017) ; C-drip/tape (TaylorWines, 2017)

Sprinkler irrigation is effective for frost management (heat releases while freezing) or bud-break delay, however it can cause growth of fungus, due to the wet leaves and humidity (Rombough, 2002; Creasy and Creasy, 2009). Poor and excessive water sources are unfavourable for grapevines, in terms of fruit quality and yield, so careful management of water is essential for commercial production (Goldammer, 2013).

Soil conditions

Grapevines can grow on a wide range of different soil types, and soil type is thought to have relatively small effect on grape/wine quality (Rankine *et al.*, 1971; Wahl, 1988). When assessing soils it is important to consider several parameters, such as texture, water availability, pH and nutrient level. Soil texture and structure is usually characterised by soil aggregates, size of particles and spaces (pores) between them (Proffitt and Campbell-Clause, 2012). Mostly vineyard soil types are categorized as clay, loam and sandy soil (Jackson, 2008). Water holding capacity of the soil is essential to consider when deciding on soil type, as uptake of nutrients is higher in wet soils compared to dry soils. Clay soils have many small pores, which slow water movement, whereas sandy soils have many large pores, which make water flow faster. However, loams are considered more fertile, as they can retain water and consequently sufficient nutrients for vine growth (Proffitt and Campbell-Clause, 2012). Nevertheless, once the vine is established, it will most probably adapt to the soil type (Rombough, 2002).

Soil nutrient availability significantly relates to the soil pH level (Creasy and Creasy, 2009; Proffitt and Campbell-Clause, 2012). For example, some macro nutrients, such as phosphorus and potassium are less available for vines at lower pH levels, whereas lower pH levels are

favourable for higher availability of micro nutrients like iron and zinc (Marschner, 2011). It is mainly because of their reaction with other available soil macro and micro nutrients and the formation of insoluble compounds that are poorly available for plant uptake. The optimal availability of most soil nutrients can be observed in the 5.5 to 8 pH range (Proffitt and Campbell-Clause, 2012).

Cover crops

One of the options for managing the soil surface in vineyards is to establish cover crops, an approach that is widely used in organic production. Commonly used cover crops include legumes (e.g. clover, alfalfa/medicago/lucerne, bean or peas), and grasses (e.g. barley, brome, rye, blue and fescue) and mixtures of legumes and grasses (Creasy and Creasy, 2009). Cover crops can be effective as a tool to (a) improve soil N levels, (b) minimise nutrient (N, P and K) loss, (c) improve soil structure and water holding capacity, (d) reduce completion from weeds and/or (d) minimise soil erosion (Hartwig and Ammon, 2002; King and Berry, 2005) However, they may compete with the grapevine for nutrients and in semi-arid environments also for water (Creasy and Creasy, 2009). Cover crops also buffer soil temperature and prevent the heating up of the soil during the day, but increase the drop in temperature of the soil during the night. This may increase the risk of spring frost damage (Dethier and Shaulis, 1964). Cover crops may be cut and left on the soil as mulches. This practice reduces competition from weeds and soil erosion, decreases evaporation and water losses from soil and improves soil structure, water infiltration rates and root system development and branching (Creasy and Creasy, 2009). However, mulching may also increase vegetative growth at the expense of grape yield, providing a shelter for rodents and increase production costs (Penfold, 2004). Besides cover crops there are several other organic fertilizers, such as animal manures, compost and green manure crops that are used as a source of N-P-K and micronutrients (Goldammer, 2013). Organic fertilizers mainly help the long-term health of the vineyards and have more variable nutrient compositions. However they can provide inadequate amounts of specific nutrients at particular growth stages and often these nutrients are not readily available before microorganisms break them down (Proffitt and Campbell-Clause, 2012). Fertilizers are usually applied to the young vines, in small quantities, near the newly developing roots, so they can have access to required nutrients. Later fertiliser application depends on growth cycle nutrient requirements of the vine (Proffitt and Campbell-Clause, 2012).

1.3 Crop protection in organic and conventional grape production

1.3.1 Weed management

Weeds may affect grapevines by competing for water and nutrients and offer a favourable environment for pests (e.g. omnivorous carabids, crickets), as well as disease (e.g. *Botrytis*) if not properly controlled (Lampkin, 1990; Ingels *et al.*, 2005). Competition can be tolerated by grapevines depending on weed community, climate, soil condition and cultivar (Monteiro and Lopes, 2007; Baumgartner *et al.*, 2017). Extreme weed growth can also result in (a) competition for light (especially when orchards are first planted) and (b) reduced airflow and increased humidity within the orchard (Creasy and Creasy, 2009). Weed control usually starts before the first plant establishment and continues through the whole life cycle of the vine, so it has to be monitored efficiently year-round (Goldammer, 2013; Susaj *et al.*, 2013).

The principal methods for weed control differ between conventional and organic grape production systems. In conventional production weed control usually relies primarily on herbicides (Rombough, 2002), while organic farming regulations prohibit the use of synthetic chemical herbicides (Regulation, 1991). Organic weed management therefore relies on ground cover crops (see above), mulching and mechanical or thermal weed control. Mechanical methods are quicker than classic methods (e.g. sheep or geese grazing, power cutting tools), which is especially helpful in regions where frost is an early-season circumstance, as weeds prevent soil surface from daytime heat absorption (Creasy and Creasy, 2009). There are several mechanical implements that are used for in and between rows weed control, such as rotary tillers, mowers and disc ploughs (Goldammer, 2013) (Figure 1.4).

Figure 1.4 Mechanical weed control machines: A-in-raw mower; B-Disc plough (Goldammer, 2013)

There are mowers that accumulate the clippings as a mulch under the vines within the rows rather than leaving them between the rows. This can help with suppressing weed growth under the vines as well as improving soil organic matter content in the soil next to the vines (Creasy and Creasy, 2009). Mulching may also be used to suppress weeds between rows and a range of materials can be used for mulching including plastic sheets/mesh, composted wood chips, cereal straw, green waste etc. (Rombough, 2002). Mulching prevents/reduces weed growth by being a physical barrier and preventing wind/water dispersed weed seed to reach the soil surface. It also prevents sufficient light to reach the soil surface and trigger the germination of weed seeds present in the soil or on the soil surface (Rombough, 2002; Goldammer, 2013).

Thermal weed control methods are also used in organic grape production systems and these include flame or hot steam weeding systems. The use of thermal weed control methods is controversial within the organic farming movement and is likely to be restricted or prohibited in the future, due to the high fossil fuel use and negative carbon footprint associated with these methods (Goldammer, 2013).

1.3.2 Control of major diseases and pests

Grapevines are attacked by a range of diseases (fungal, bacterial and viral) and invertebrate pests (Creasy and Creasy, 2009).

Fungal disease

Powdery mildew, downy mildew and *Botrytis*-bunch rot are the commercially the most important fungal diseases in many regions (Creasy and Creasy, 2009). The severity of different diseases depends on climatic conditions/the geographical location. For example, while downy mildew and *Botrytis* are the most important crop protection challenges in areas with high precipitation/humidity in Northern Europe and America, they rarely cause problems in semi-arid regions such as the southern Mediterranean and desert areas of California (Rombough, 2002; Dufour, 2006).

Downy mildew (*Plasmopara viticola*) is native to the American fungus and very common in areas with high rainfall and humid climatic conditions. It tends to be most severe when a damp winter and spring is followed by a hot summer with rainfall (Pearson and Goheen, 1988).

After overwintering in dead or infected leaves, the fungus becomes active two to three weeks prior to blooming. Infection starts when conditions are rainy and warmer (above 10°C), then disease develops actively as the temperature increases (20 to 25°C) (Goldammer, 2013). Usually spores spread during warm, humid nights which make condition more severe by allowing diseases to have a secondary cycle of spreading (Rombough, 2002). Primary symptoms are especially visible in young leaves, with a covering of the upper leaf surface with light yellow spots, which are yellow-brown in older leaves. Later it appears as an oily spot on the lower surface of the leaf. Infection can also appear on other parts of the plant (e.g. young shoots, tendrils, clusters) and cause plant parts to curl and dry. However, the disease is not effective after berry ripening (Rombough, 2002; Goldammer, 2013) (Figure 1.5)

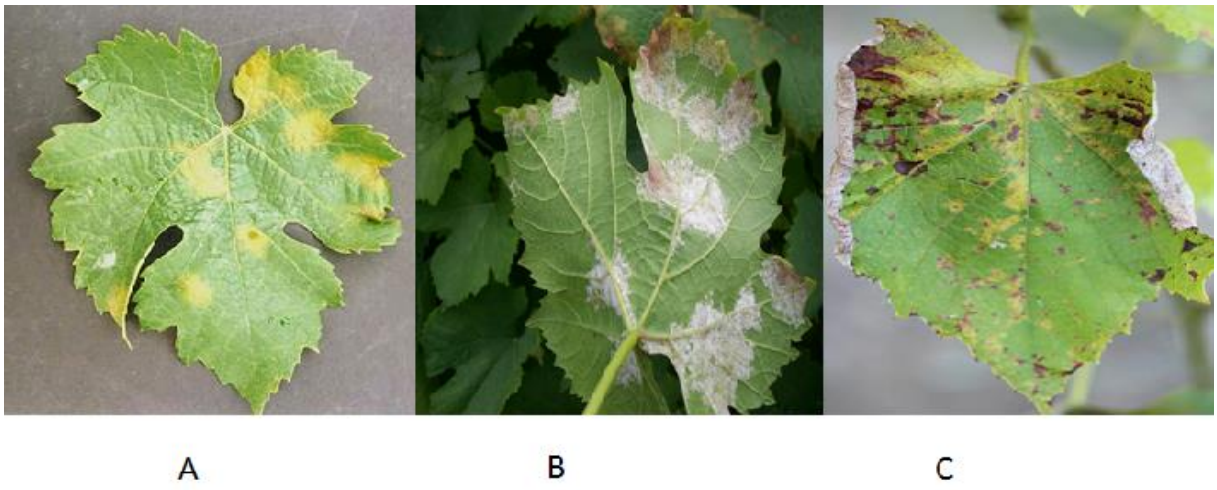


Figure 1.5 Different stages of Downy mildew infection: A-intial infection (early yellow spots); B-Masses of spots (oily spots on lower surface of leave); C-necrotic stage (browning, curling and drying) (OSU, 2017)

Native American grape varieties (*Muscadinia rotundifolia, V. aestivalis*) are the most resistant varieties, whereas *V. vinifera* varieties are the most vulnerable to downy mildew. Disease management can be successful, if site conditions are monitored, such as improving sun exposing, air circulation by pruning and training system, reducing water stand by compost application and mycorrhizal inoculation (Rombough, 2002). However, in highly susceptible cases the use of fungicides is important. Liquid copper or Bordeaux mix and Trilogy (neem seed derivative) are the main recommended organic fungicides (Dufour, 2006). Spraying usually starts before infection period begins (2-3 weeks before bloom) and continuous space till veraison (2-4 weeks after fruit set) (Goldammer, 2013) .

Powdery mildew (*Uncinula nector*) infects the leaves and other green parts of the plant, by covering the upper surface of leaves with a greyish or white mycelium which produces a large numbers of conidia (asexual spores) which gives infected leaves the typical "powdery" appearance (Rombough, 2002) (Figure 1.6).

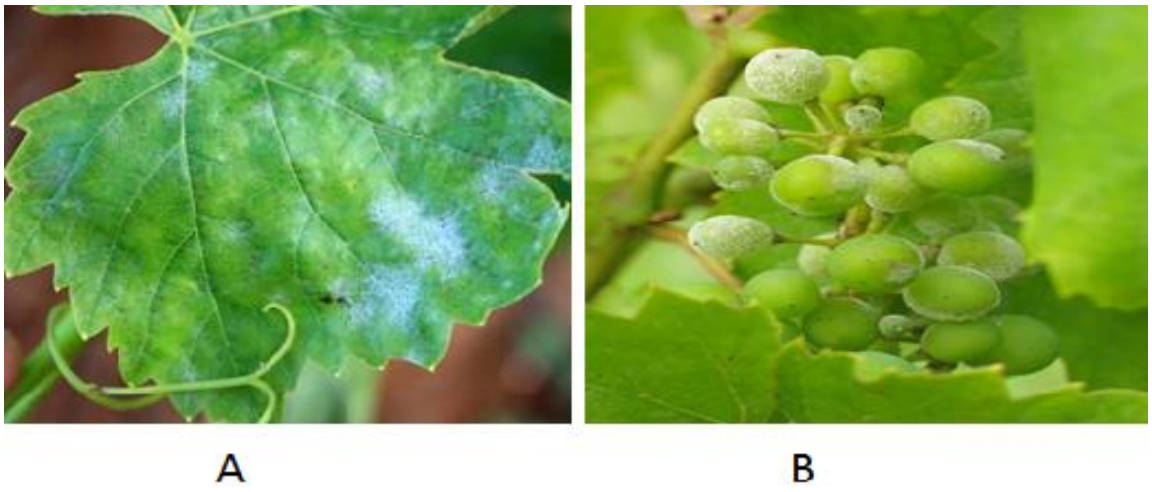


Figure 1.6 Powdery Mildew infection on leaf (A) (Osborn, 2013) and berry (B) (Petruzzello, 2017)

Powdery mildew overwinters and releases spores when the temperature reaches 10°C with rain. After primary infection in the leaves, a secondary phase starts, which means the fungal mycelium, grows across the surface of the leaves and extracts nutrients from the leaf epidermal cell layer via haustoria. The fungus affects yield and grape quality by reducing photosynthesis in leaves (Pearson and Goheen, 1988; Rombough, 2002). The favourable temperatures for infection and disease development are 21-29°C, however at higher temperatures (32-35°C) reproduction slows or stops completely (Rombough, 2002; Goldammer, 2013). Berries are usually infected or susceptible during the cluster/pre-bloom period, but are highly resistant during the ripening period (Creasy and Creasy, 2009). The most resistant cultivars are Native American followed by French-American hybrids varying in levels of resistance. However, most of *V.vinifera* varieties are highly susceptible (Dufour, 2006). Sufficient air circulation, good sun exposure and the use of resistant/tolerant varieties can all contribute to reduce powdery mildew severity, however the use of organic fungicides are essential for optimal protection (Rombough, 2002) In organic viticulture sulphur-based fungicides are the main crop protection products used for powdery mildew control, but a range of other treatments are also available. These include, bicarbonates (sodium/potassium), mineral oils (e.g. JMS Stylet-Oil) and biological control agents (e.g. AQ-10 Biofungicide) (Rombough, 2002; Dufour, 2006). However, at high temperature (<30-35 °C) sulphur

fungicides may cause phytotoxicity in some grape varieties. Sulphur fungicides are also known to kill and reduce populations of beneficial insects in orchards and it has therefore been recommended to replace the use of sulphur fungicides with other crop protection products such as bicarbonates (Thomas *et al.*, 1993; Kauer *et al.*, 2000; Emmett *et al.*, 2003). Usually spraying starts before blooming and continues until veraison (Goldammer, 2013).

***Botrytis* bunch rot** or **grey mould** (caused by *Botrytis cinerea*) is often found to attack ripe or nearly ripe and tightly clustered berries, and can cause severe reductions in yield and fruit quality (Rombough, 2002).

Cool temperature (13-25°C) and wet conditions are essential for the spore germination, which infects buds in the early development stage (Rombough, 2002). The conidia/spores of *Botrytis* are easily wind dispersed and can stay inactive during periods of dry weather condition only to re-appear in cool and wet periods (Creasy and Creasy, 2009). Berries that have been injured by other disease or insects are susceptible to infection and spores may stay there until sugar levels increase (Goldammer, 2013). Infected berries are covered with grey cotton-like mycelia, which cause colour change in white (brownish) and purple (reddish) varieties (Goldammer, 2013). Young leaves usually display a V-shaped area of infection (Emmett *et al.*, 1992). The typical "grey mould" symptoms are shown in Figure 1.7.

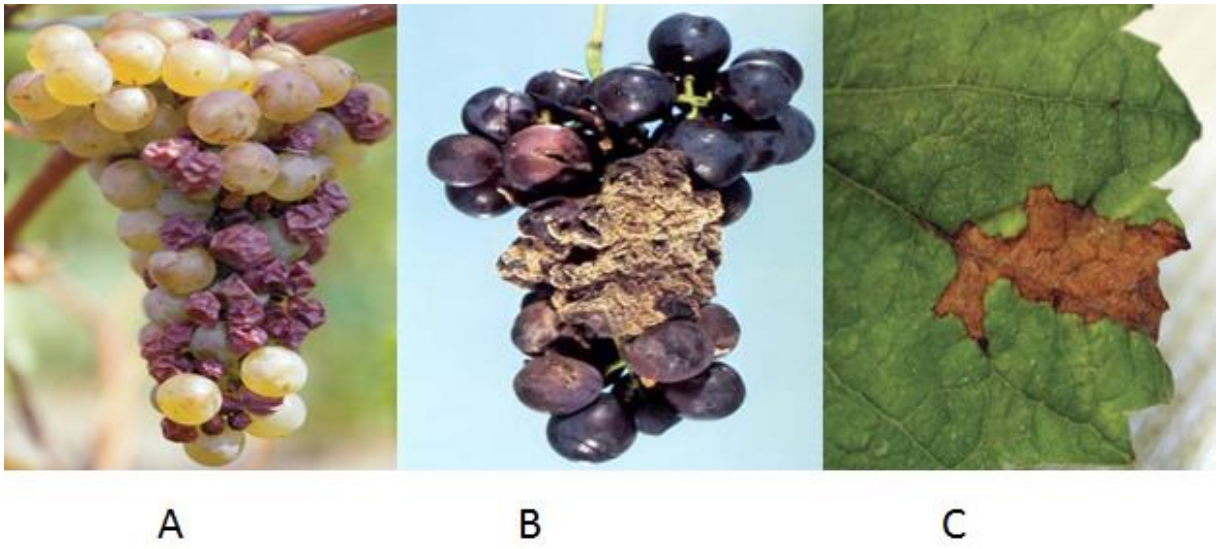


Figure 1.7 Botrytis bunch rot (grey mould) infection of berries: A- white variety; B-purple variety; and infected leaf (C) (Ellis, 2008; MSU, 2014)

Botrytis may cause substantial yield loss, and can induce colour change, oxidative damage and early aging in grapes. The infection can rendering table grapes unmarketable and generate off-flavours/poor sensory quality in wine (Ribéreau-Gayon, 1983; Smart and Robinson, 1991). A range of preventative management approaches can be used for *Botrytis* control. These include (a) careful monitoring of vines/grapes for symptoms and immediate removal of infected leaves close to the ripening cluster-zone, (b) improving air circulation (e.g. by pruning that exposes grape cluster to the sun and circulating air) (Bettiga *et al.*, 1989; Intrieri *et al.*, 2008; Tardáguila *et al.*, 2008).

Fungicides used to control powdery or downy mildew in organic vineyards (Cu and S-fungicides) are not very effective against *Botrytis*. Several biofungicides (e.g. Trichodex, Mycostop) based on antagonistic fungi were shown to have an effect against *Botrytis cinerea* and are commercially available, but it is unclear to what extent they are used by organic farmers (Dufour, 2006).

Other grapevine diseases

Grapevine may also be attacked by a range of other fungal (e.g. *Phomosis*, Black Rot, Grape Anthracnose), bacterial (e.g. Pierce's disease, crown gall), and viral (e.g. Grape leafroll, corky bark) diseases and these can cause substantial commercial losses (Rombough, 2002). Bacterial and viral diseases cannot be controlled by chemical crop protection products, making preventative strategies vital for control. These can include (a) monitoring vineyards to allow early detection and then eradication of infected plants, (b) virus testing of cuttings used from existing vineyards to ensure that only clean material is used, (c) control of invertebrate virus vectors (e.g. aphids), and (d) use of resistant varieties (Rombough, 2002; Creasy and Creasy, 2009).

Invertebrate pests of grapevine

Grapevines are attacked by a range of invertebrate pests including Phylloxera, mealybugs, grape berry moth and leafhoppers which can cause substantial damage to shoots, roots, leaves and berry clusters (Creasy and Creasy, 2009; Goldammer, 2013) (Figure 1.8).

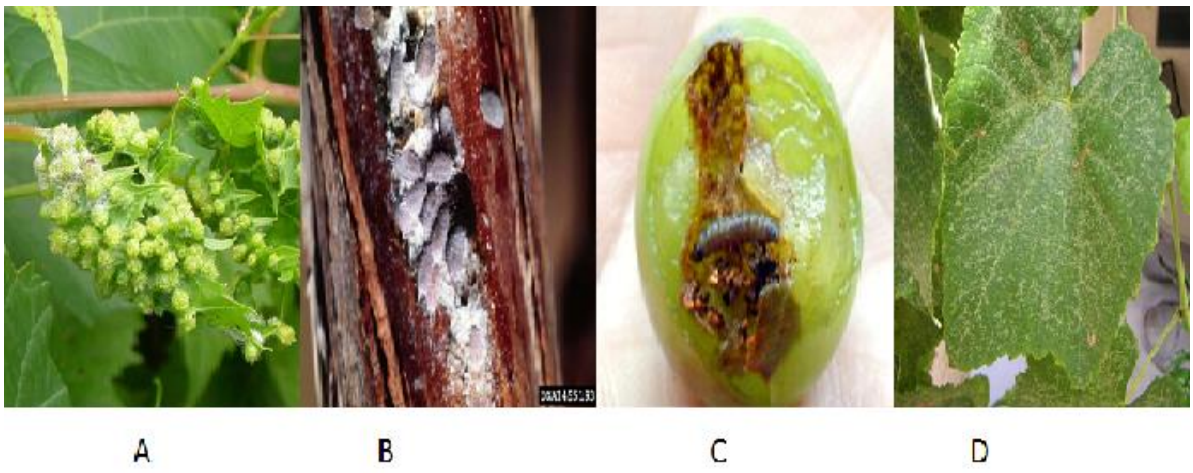


Figure 1.8 Invertebrate pests damages on grapevine: A- grape Phylloxera and B- mealybugs(Goldammer, 2013); C- grape berry moth (Isaacs, 2013); D- leafhopper (Morris, 2016)

Synthetic chemical pesticides are not permitted in organic grapevine production (Regulation, 1991). However, a range of alternative crop protection treatments are used for the control of invertebrate pests in organic grape production. These include some plant extract based treatments (e.g. neem, rotenone, pyrethrum), microbial fermentation based (e.g. spinosat) pesticides, oils and soaps, semi-chemical biologically derived pest control products (e.g. synthetic insecticides derived from *B. thuringiensis* bacteria) and behavioural control agents (e.g. pheromones mating disruption and mass trapping systems for control of lepidopteran pests) that are permitted for use in organic farming systems (Sams and Deyton, 2002; Ifoulis and Savopoulou-Soultani, 2004; Dufour, 2006). In addition, a resistant rootstock is widely used for control of soil pests (e.g. Phyloxera), but there are currently no varieties that are tolerant or resistant to above ground pests (Dufour, 2006; Creasy and Creasy, 2009).

1.4 Nutritional composition of grapes and wine

Grapes containing about 80% water, 18% carbohydrate, 0.7% protein, 0.9% fibre and 0.2% fat per 100 g (USDA, 2016) and have a low sodium content (Ferrari and Soares, 2003). Similar to other fruits, grapes are a rich source of phytochemicals with antioxidant activity, and especially polyphenols (Yang and Xiao, 2013; Georgiev *et al.*, 2016).

1.4.1 Polyphenols

Polyphenols one of the highly abundant group of secondary metabolites/phytochemicals, that are produced by plants during growth (Naczk and Shahidi, 2004; Ramos, 2007). They determine or are associated with functions/characteristics in plants including growth, pigmentation, reproduction and flavour. They are thought to be an important components of the plants protection mechanisms against both biotic and abiotic stress (e.g. pathogens, predators, UV radiation) (Bravo, 1998). The concentrations of polyphenolic compounds in plants are affected by a range of physiological and environmental factors, including ripeness of the fruit, plant variety, pedo-climatic conditions, and length of postharvest storage (Hans-Dieter Belitz, 2009).

Different genera/species of plants have contrasting polyphenol concentrations and profiles, and the classification of polyphenols found in grapes is shown in Figure 1.9 (Rasines-Perea and Teissedre, 2017).

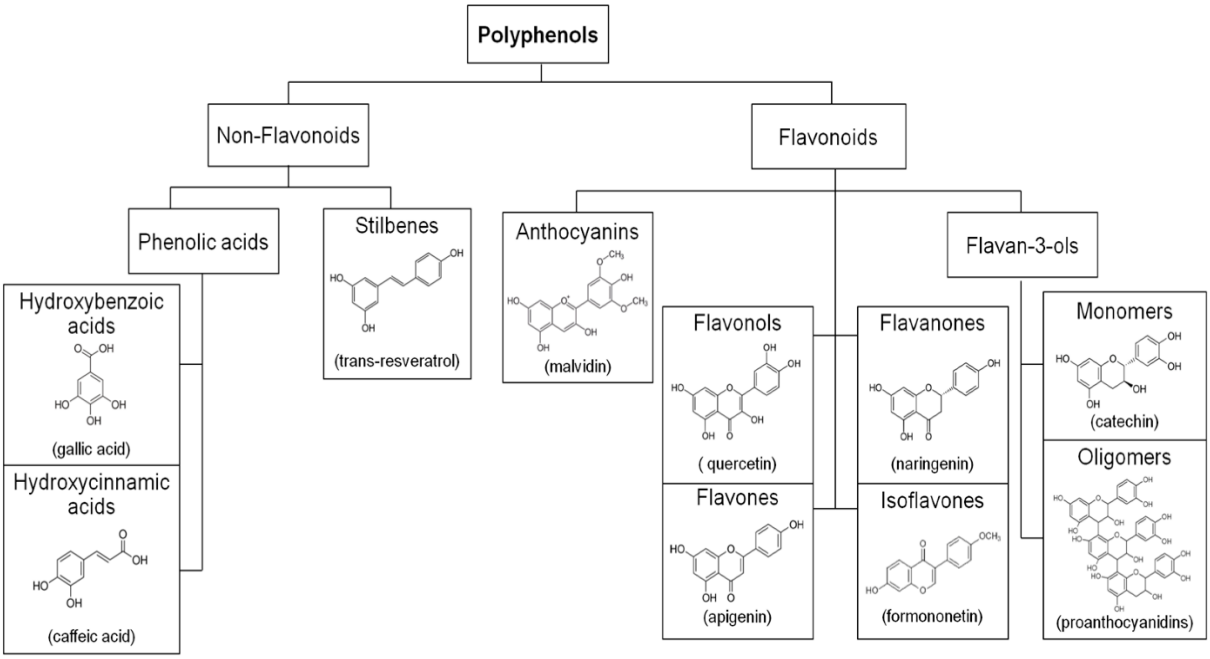


Figure 1.9 Classification of grape polyphenols

Phenolic acids

The most common phenolic acids that are found in grapes and wine (especially white varieties) are hydroxycinnamic acid derivatives (e.g. caffeic, p-coumaric, ferulic acids) (Pandey and Rizvi, 2009; Yang and Xiao, 2013). They tend to be the primary oxidized compounds, that cause browning in white wines (Waterhouse, 2002). The other main group of phenolic acids are hydroxybenzoic acids (e.g. gallic, vanillic, syringic acids), which usually appear as esters and are more common in red varieties than hyroxycinnamic acid derivatives (Waterhouse, 2002; Haminiuk *et al.*, 2012; Yang and Xiao, 2013).

Stilbenes

The presence and concentration of stilbenes in grapes are usually associated with biotic or abiotic stress, and have been linked to *Botrytis* and other fungal infections (Waterhouse, 2002; Stuart and Robb, 2013). Both *cis* and *trans* isomers of resveratrol are the main representative of the stilbenes found in grapes (mainly found in the skin) and have been found to be mainly produced in response to infection or injury/physical damage (Pandey and Rizvi, 2009; Yang and Xiao, 2013). Both resveratrol isomers are presents in wine, but only fresh grapes contain trans-resveratrol (Waterhouse, 2002). Resveratrol concentrations also depends on the variety of grape, and were reported to be higher in red rather than white grape varieties (Siemann and Creasy, 1992).

Anthocyanins

Anthocyanins are water-soluble flavonoid pigments, that are the main compounds responsible for the red and blue/black colours of grape skin (Waterhouse, 2002). Cyanidin, peonidin, delphinidin, petunidin and malvidin are the most commonly occurring anhocyanidins (sugar-free anthocyanins) in red/black grapes and red wine (Yang and Xiao, 2013). In the grape skin they appear as 3-*O*-glucosides (Wrolstad, 2000; Waterhouse, 2002). The total anthocyanin content and especially the profile of different anthocyanins have a substantial effect the on the quality of red wine. They are extracted from grapes and wine making residues for use as "natural" food colorants and nutraceuticals (Yang and Xiao, 2013)

Flavanols (flavan-3-ols)

In grapes, flavanols are abundant in both the seed and skin (Waterhouse, 2002) and exist as monomers (catechin, gallocatechin, epicatechin, epigallocatechin and epicatechingallate) (Yang and Xiao, 2013). Proanthocyanidins (condensed tannins) are an abundant form of

flavan-3-ols in grapes, that appear as oligomers (Gu *et al.*, 2003). This group of polyphenols are responsible for flavour features, such as astringency and bitterness in grapes and wine (Yang and Xiao, 2013).

Flavonols

Quercetin, myricetin (3'4'5' trihydroxy) and kaempferol (4' hydroxyl) are the main flavonols found in grape skin (Waterhouse, 2002). Besides these three major flavonols, isorhamnetin is also found in red varieties and in small amounts in white varieties (Makris *et al.*, 2006; Mattivi *et al.*, 2006).

The table below shows common phytochemicals found in grapes and grape-derived products, as described by Chia-Chi and Michael (2011) (Table 1.1).

Table 1.1 Phenolic phytochemicals occurring in grape and grape products

Resource	Phenolic phytochemicals	References
Whole grapes	Flavonol glycosides (quercetin, kaempferol, myricetin, laricitrin, isorhamnetin, syringetin), anthocyanins[a] (malvidin, peonidin, petunidin, cyanidin, delphinidin, pelargonidin), flavan-3-ols (catechin, epicatechin), phenolic acids (protocatechuic acid, gallic acid), hydroxycinnammates (caftaric acid, coutaric acid, fertaric acid), stilbenes (trans-resveratrol)	(Cantos *et al.*, 2002; Nicoletti *et al.*, 2008; Castillo-Muñoz *et al.*, 2009)
Grape skin	Flavonol glycosides (quercetin, kaempferol, myricetin, laricitrin, isorhamnetin, syringetin), anthocyanins[a] (malvidin, peonidin, petunidin, cyanidin, delphinidin, pelargonidin), flavan-3-ols (catechin, epicatechin, gallocatechin, procyanidin B1, B2, B4, other dimers, C1, other trimers, and tetramers), phenolic acids (protocatechuic acid, gallic acid), hydroxycinnammates (caftaric acid, coutaric acid, fertaric acid), stilbenes (trans-resveratrol, cis-resveratrol, resveratrol dimmers and tetramers), flavanonol glycosides (taxifolin)	(Cantos *et al.*, 2002; Pastrana-Bonilla *et al.*, 2003; Cavaliere *et al.*, 2008; Castillo-Muñoz *et al.*, 2009)
Red grape juice	Flavonols (quercetin glucoside, rutin, myricetin), anthocyanins[a] (malvidin, peonidin, petunidin, cyanidin, delphinidin), flavan-3-ols (catechin, procyanidin B2)	(Dávalos *et al.*, 2006)
Red wine	Flavonol glycosides (quercetin, kaempferol, myricetin, isorhamnetin), anthocyanins[a] (malvidin, peonidin, petunidin, cyanidin, delphinidin), flavan-3-ols (catechin, catechin gallate, epicatechin, epicatechin gallate, procyanidin B1, B2, B4, and trimers), phenolic acids (protocatechuic acid, gallic acid, ellagic acid, vanillic acid, syringic acid), hydroxycinnammates (caffeic acid, caftaric acid, coutaric acid, ferulic acid, fertaric acid, coumaric acid, sinapic acid), stilbenes (trans-resveratrol, trans-resveratrol glucoside)	(Preys *et al.*, 2006; García-Falcón *et al.*, 2007; Gómez-Alonso *et al.*, 2007; Pereira *et al.*, 2010)

[a]Anthocyanidins/anthocyanins are detected only in red grapes.

1.4.2 Health benefits of polyphenols in grapes and their positive link with organic production systems

Recent epidemiological studies have linked the consumption of grapes and grape products to a reduced risk of a range of chronic diseases including cardiovascular diseases, some cancers and neurodegenerative diseases. This was suggested to be at least partially due to grapes being a rich source of different phenolic compounds (Katiyar, 2008; Yadav *et al.*, 2009; Vislocky and Fernandez, 2010; Yu *et al.*, 2012; Nassiri-Asl and Hosseinzadeh, 2016). The antioxidant properties of these polyphenols are thought to protect against oxidative damage and reduce inflammation, which are thought to be the cause of degenerative diseases (e.g. cancer, arteriosclerosis, and also aging process) (Cao *et al.*, 1998; Rizvi, 2006; Pandey *et al.*, 2009). There are now numerous studies, which reported a positive link between consumption of antioxidant/polyphenol-rich foods/drinks and lower risk of cardiovascular disease (Kondrashov *et al.*, 2009; Venturini *et al.*, 2010). Another large epidemiological study that monitored diet and health of human participants over 20 years has described consumption of grapes and in particular red wine as a possible explanation for the "French paradox". The fact that French consumers, despite consuming high fat/animal fat diets, have a very low rate of coronary heart diseases (Renaud and de Lorgeril, 1992; Vrček *et al.*, 2011). In general, grapes and grape products are a very rich source of polyphenols, such as flavonoids, tannins, resveratrol, anthocyanins which have high antioxidant activity (Li and Pu, 2011; Olmez and Ozyurt, 2012; Halliwell and Gutteridge, 2015).

Effect of genetic, climatic and agronomic factors on polyphenol concentrations

A range of factors may affect the concentrations and profiles of phenolic compounds in fruit, including variety choice, climatic conditions, farming system, soil type, geographical location, disease severity, pest damage and fruit maturity (Mulero *et al.*, 2010). Due to the slower release of the essential nutrients, the ripening period was reported to be longer in organically grown fruit and vegetables compared to conventional and this was suggested as a reason for higher concentration of phenolic compounds in organically produced fruit and vegetables (Vrček *et al.*, 2011).

Organic agriculture aims to **(a)** protect and utilise biodiversity and **(b)** improve soil biological activity , structural stability and fertility, and thereby also plant, animal and human health (Le Guillou and Shcarpé, 2001; Mulero *et al.*, 2010). In contrast, the intensive use of chemical fertilizers and pesticides is increasingly recognised to have substantial negative impacts on the environment and food quality (Bellaport Vilà, 1988). This has led to the rapid increase in

consumer demand for organic farming products, which consumers perceive as being healthier and safer (Brandt and Mølgaard, 2001; Moyano *et al.*, 2009).

The demand for organic table grapes and wine and the area of organic grape production has also increased rapidly in Europe and elsewhere (Marenghi, 2002; Granato *et al.*, 2015; Ifoam, 2016). However, compared to other crops there is limited published information on the effect of organic management practices on the concentrations/profiles of nutritionally-relevant phytochemicals and minerals in grapes and grape products (Marenghi, 2002; Dani *et al.*, 2007a).

Apart from organic fertilisers (e.g. animal manures, composts) and legumes (to supply N), organic producers use a range of mineral fertilisers including finely ground raw phosphate (P), potassium sulphate (K_2SO_4), lime and micro-nutrient fertilisers and both sulphur and copper pesticides/fungicides (Nelson and Janke, 2007; Regulation, 2009). There is evidence that, excessive use of fertilisation in vineyards can negatively affect anthocyanin concentration in plants and possibly also total antioxidant activity in grapes (Malusa *et al.*, 2002). This is consistent with studies, which reported higher concentrations of bioactive phytochemical compounds in organic compared to conventional plants (Asami *et al.*, 2003; Olsson *et al.*, 2006).

Although there is a range of publications available on composition differences between organic and conventional grapes and grape products, the evidence has not been synthesised using meta-analysis methods. Also, there are currently no studies, which investigate the relative importance of differences in agronomic practices and confounding factors such as geographic location and associated pedo-climatic conditions, and grape variety choice on the differences detected between organic and conventional grapes.

1.5 Research Objectives

The overall aim of this study was to investigate the effect of grapevine management practices and variety choice on fruit yield and quality of table grapes and wine. In order to achieve this aim the project had the following specific objectives:

1. To carry out a systematic literature review and meta-analysis of published data on composition differences between organic and conventional fresh grapes and grape products;
2. To carry out a retail survey in UK supermarkets to investigate potential confounding effects of grape variety and supermarket supply chains (via a comparison of grapes available in winter [produced mainly in Southern Africa] and summer [produced mainly in Mediterranean countries]) on nutritionally relevant quality parameters in organically and conventionally grown table grapes;
3. To carry out a farm survey in Crete (Greece) to investigate effects of agronomic practices and variety choice on yield and nutritionally relevant quality parameters in organically and conventionally grown local grape varieties;
4. To carry out a wine survey in Crete (Greece) to identify quality differences between wines made from organically or conventionally grown local grape varieties;

CHAPTER 2. Materials and Methods

2.1 Systematic literature review and meta-analysis

Nowadays, organic farming practices have been drawn more attention by promoting biodiversity and prohibiting synthetic crop protection products and fertilisers. Moreover, recent comparative studies, which indicate beneficial aspects of organically grown crops in terms of nutrition, have become another contributor for this interest. One of the best tools to assess magnitude of difference between organic and conventional practices is a meta-analysis. It has many advantages over narrative literature review allowing the precision of evidence (including variation within and between studies) and quality of primary studies to be evaluated. Bias assessment of selective evidence and statistical measurement of effect size within confidence intervals are among the most important evaluations in meta-analyses.
A systematic literature review and meta-analysis of available publications were conducted in order to identify nutritionally relevant composition differences between organic and conventional grapes and grape products. In addition to this primary objective, the analyses also tried to clarify if composition differences were affected by (a) pedo-climatic differences between countries, (b) cultivation year, (c) study type (retail survey, farm survey or controlled experiment) and (d) data management (e.g. inclusion criteria, meta-analysis method) as a secondary objective.

2.1.1 Criteria for including and excluding studies

Types of study designs

This review has included all studies comparing compositional difference of organically and conventionally cultivated grapes and grape products. Eligible studies contained data derived from (a) farm surveys in which samples were collected from conventional and organic farms located in the same country or region, (b) field experiments with randomised block designs where conventional and organic samples were grown on the same farm, and (c) retail surveys/basket studies in which conventional and organic samples were purchased on the market in the same country or region. The number of replicate farms in farm surveys, replicate plots used in field experiments, and number of brands respectively were considered as units of replication to derive sample sizes.

Types of population

This study has included data on composition of fresh red and white table grapes and grape products, specifically grape juice, grape must, grape pomace, wine and wine vinegar.

Types of interventions

Because of the review's comparative nature, only studies, which compared conventionally cultivated grapes and grape products with organically cultivated grapes and grape products were included. Accordingly, studies were considered as eligible only if authors stated that the organic comparator was from (a) farms that were certified to organic farming standards, (b) farms or experimental plots that were managed according to organic farming standards, or (c) grape products labelled as having been produced from organically produced grapes.

Types of outcome measures

The total concentrations of polyphenols and the content of individual polyphenols measured in the given unit per weight or volume of the sample were considered as a primary outcome. The data on antioxidant activity of grapes and grape products were selected as a secondary outcome in this study. Other parameters such as pH level, percentage of moisture content and sugar content were included in exploratory analyses because they are also indicatives of differences in nutritional composition.

2.1.2 Search Strategy

The literature search strategy was based on previously published protocols by Brandt *et al.* (2013) and Barański *et al.* (2014). For relevant publications different online sources including Web of Science (all collections), Scopus (all collections), Ovid (CAB abstracts, Embase, EBM Review Full Text-Cochrane DSR, APC Journal Club and Dare) and EBSCO (GreenFile, MLA databases) were used. The search included studies published in the years 1992-2016, because 1992 was the year when legally binding organic farming regulation was first introduced in the EU. The search phrase contained tree groups of terms combined with Boolean logic operators ("OR", "AND") and with truncation (*,?) in order to find all contrasting interventions and participants for selected outcomes:

a) (organic* OR biologic* OR e?ologic* OR biodynamic*) AND
b) (conventional* OR integrated*) AND
c) (grape OR grape juice OR grapevine OR wine OR wine vinegar OR grape vinegar)

There were no language restrictions, and if needed, translation of the papers was done by external researchers.

2.1.3 Details of study coding categories

Screening and data extraction

The screening process and data extraction was conducted by two reviewers separately. All discrepancies and disagreements were discussed and resolved by the whole team.

Publications obtained from each search were merged into one list, from which any duplicates were removed, including reports that originated from the same study/data-set. The first stage of screening involved the evaluation of titles and abstracts. In the second stage, the full publication text was collected and read/evaluated to create a list of all potentially eligible studies. From each paper, information relevant for the review was extracted into an electronic database.

Authors of papers for which only published abstracts were found, were subsequently contacted by email and asked for full texts or relevant data. The reference lists of all papers were then checked for more potentially eligible publications. Moreover, authors of all collected papers were asked to supply any other publications that could be added to this review. The PRISMA flow diagram (Moher *et al.*, 2009) was presented as a summary of the screening process, showing the number of papers found, included in the meta-analysis and excluded with the reasons for exclusion.

Dealing with missing data

As a first step the authors of the study was contacted in attempt to obtain all data identified as missing. If this was unsuccessful, additional data available in the paper was used to calculate effect size or variability values based on an established method (Lajeunesse, 2013). Studies for which the calculation was not possible were excluded from the meta-analysis; the approach taken for dealing with missing data is explained in detail in Chapter 3, Results section (see below).

Assessment of risk of bias and strength of evidence

Studies included in the review were critically appraised and evaluated for potential source of bias associated with study design, analytical methods, selective outcome reporting and conflicts of interest (Baranski et al., 2017). The assessments included a form of statements for

which one of three responses were possible: 'Yes' when the statement reflected the content of the paper, 'No' when there was no information in the paper described by the statement or 'Unclear' when information provided did not reflect the statement. The last point on the checklist was the final rating of the overall methodological quality of the study.

An overall estimation of the strength of the findings was taken into account during the evidence synthesis as part of the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system report (Guyatt *et al.*, 2008; Meader *et al.*, 2014), which included information about overall risk of bias for all studies included in the meta-analysis, as well as inconsistency, indirectness and imprecision of the results, and publication bias.

2.1.4 Statistical procedures and conventions

Data synthesis

The summary of study characteristics was presented as descriptive results for the review. Data points that were included in meta-analysis were analysed separately as individual parameters (e.g. p-coumaric acid, syringic acid, resveratrol), a group of individual parameters as presented in the paper (e.g. anthocyanins, stilbenes, flavonoids) and a total parameter (e.g. total polyphenols, total anthocyanins, total antioxidant activity). Both weighted and unweighted meta-analyses methods were employed (Barański *et al.*, 2014).

The weighted analysis was conducted using the 'metafor' package (Viechtbauer, 2010) in the R statistical environment (R-Project). The effect size was calculated as standardised mean difference (SMD) as advised for studies in which data obtained by measuring the same parameters on different scales were included (Stewart, 2010; Koricheva and Gurevitch, 2013):

$$SMD = \frac{\bar{X}_O - \bar{X}_C}{S_{within}} \times J$$

where $\bar{X}o$ is the mean value for the experimental group (organic), $\bar{X}_C$ is the mean value for the control group (conventional), S_{within} is the pooled standard deviation of the two groups, and J is a factor used to correct for small sample size. J was calculated as:

$$J = 1 - \frac{3}{4 \times (n_C + n_O - 2) - 1}$$

where n_O and n_C are organic and conventional sample sizes.

S_{within} was calculated as:

$$S_{within} = \sqrt{\frac{(n_O - 1)S_O^2 + (n_C - 1)S_C^2}{n_O + n_C - 2}}$$

where S_O and S_C are the standard deviations in individual systems (organic and conventional) respectively.

The pooled SMD (SMD_{tot}) across all studies was calculated as:

$$SMD_{tot} = \frac{\sum_{i=1}^{n}(\frac{1}{v_i} \times SMD_i)}{\sum_{i=1}^{n}(\frac{1}{v_i})}$$

Where v_i is a sampling variance estimated as:

$$v_i = \frac{n_C + n_O}{n_C \times n_O} + \frac{SMD^2}{2 \times (n_C + n_O)}$$

The pooled or summary effect (SMD_{tot}) was calculated for all primary and secondary parameters reported in a minimum of 3 studies (Lipsey and Wilson, 2001).

A positive SMD value indicated a greater mean concentration of the observed compound in organic grape or grape product samples. Corresponding 95% confidence intervals for each SMD was also calculated within the 'metafor' package.

Random effects models were applied and meta-regression / subgroup analysis was used in order to assess variability between study designs, agricultural system, geographical location and others that is mentioned in review objectives (Barański *et al.*, 2017).

Data from publications containing only the mean values with measures of variability and/or sample size unavailable were only included in the unweighted meta-analysis. The effect size was calculated as an ln-transformed ratio of organic means: conventional means ($\bar{X}_O$ /$\bar{X}_C$) were expressed as a percentage. The significance was evaluated comparing the arithmetic average of the result with ln(100) using a resampling method (Gurevitch and Hedges, 1999). P values were derived from Fisher's one-sample randomisation test (Manly, 1997) and a $P>0.05$ was considered statistically significant.

In order to facilitate value judgements regarding the nutritional importance of the relative effect magnitudes, and to compare these between weighted and unweighted meta-analysis protocols, the mean percentage difference (MPD) was also calculated for all outcomes for which significant effects were detected (Barański *et al.*, 2014). The MPD was expressed as

"% higher" in conventional or organic grapes or grape products, and it provided an estimate for the magnitude of composition differences that was easier to correlate with available information on the possible health impacts of varying dietary intake levels for individual or groups of compounds than the SMD values. For each data-pair (where mean value for organic system samples equal $\bar{X}_O$ and mean value for conventional system samples equal $\bar{X}_C$) used for SMD calculations the MPD was calculated as:

$$+[(\bar{X}_O \times 100/\bar{X}_C) - 100] \text{ for data sets where } \bar{X}_O > \bar{X}_C, \text{ or}$$

$$-[(\bar{X}_C \times 100/\bar{X}_O) - 100] \text{ for data sets where } \bar{X}_O < \bar{X}_C$$

The 95% CI for the MPD was determined using a standard method (Hedges *et al.*, 1999).

Assessment of heterogeneity

Assessments of heterogeneity for all estimated effect sizes in the weighted meta-analysis were calculated as the weighted sum of squared differences between individual study effects and the pooled effect across studies, carried out using Q statistics and I^2 statistics inside the 'metafor' package (Higgins and Thompson, 2002). A significant heterogeneity was assumed if the I^2 was more than 25% and the *P* value for the Q statistics was greater than 0.01.

Sensitivity analysis

Sensitivity analyses were carried out to identify the effect of using different data handling and inclusion criteria on the results of the meta-analyses. These analyses were conducted by treating (a) data reported for each cultivar/variety of grape separately or averaged and (b) data reported for different years in the same publication as separate or averaged events in the weighted and unweighted meta-analyses (Barański *et al.*, 2014) (Table 2.1).

Table 2.1 Summary of inclusion criteria used for the standard weighted/unweighted meta-analysis and for the 3 sensitivity analyses

Analysis No	Cultivar or variety of the crop		Experimental years	
	Cultivar/variety averaged*	Each cultivar/variety as separate data point†	Experimental years averaged‡	Individual year as separate data point§
standard	+		+	
1		+	+	+
2	+			+
3		+		+

*If data from more than one variety were presented separately in the paper, the average was calculated and included in the analysis; †If data from more than one variety were presented separately in the paper, they were analysed separately, as individual data points; ‡If data from more than one experimental years were presented separately in the paper, the average was calculated and included in the analysis; §If data from more than one experimental years were presented separately in the paper, they were analysed separately, as individual data points.

2.2 Experimental design of retail (grapes - wine) and grape farm surveys

Both the retail survey of table grapes and the farm survey of table/wine grapes were conducted over two successive years. In contrast, the wine survey was only carried out in one year, but collected wines were from different production years. Dry matter, sugar content, total phenolic content, total antioxidant activity and total anthocyanin content were measured using colorimetric assays. High-performance liquid chromatography (HPLC) was used to analyse concentrations of individual anthocyanins. All assessments were carried out in the human nutrition laboratories at the School of Agriculture, Food and Rural Development at Newcastle University.

2.2.1 Retail Survey

The table grape retail survey was conducted in two seasons (winter and summer) of two years (2015 and 2016). Grapes were collected in three UK supermarkets (Tesco, Sainsbury's and Waitrose) and two branches of each retailer located in different parts of NE postcode areas (Kinston Park, Gateshead, Team Valley, Gosforth, Eldon Square and Hexham) were used as replicates. Some supermarkets had no replicate stores in 2016, because one of the stores did not sell organic grapes. Table 2.2 shows a detailed structure of the grapes retail survey.

Table 2.2 Retail survey design

2015				**2016**			
Winter (Feb-May)		**Summer (Jul-Nov)**		**Winter (Feb-May)**		**Summer (Jul-Nov)**	
replicant1	**replicant2**	**replicant1**	**replicant2**	**replicant1**	**replicant2**	**replicant1**	**replicant2**
Tesco (Kinston Park)	Tesco (Gateshead)	Tesco (Kinston Park)	Tesco (Gateshead)	Tesco (Kinston Park)	Tesco (Gateshead)	Tesco (Kinston Park)	Tesco (Gateshead)
Sainsbury's (Team Valley)	Sainsbury's (Gosforth)	Sainsbury's (Team Valley)		Sainsbury's (Team Valley)		Sainsbury's (Team Valley)	
Waitrose (Eldon Square)	Waitrose (Hexham)	Waitrose (Hexham)		Waitrose (Hexham)		Waitrose (Hexham)	

The winter season (when grapes were imported from South Africa) lasted from February until May, while the summer season (when grapes were imported mainly from Mediterranean countries such as Egypt, Morocco, Greece, Italy and Spain) was from July until November each year.

The collection of matching organic and conventional grape samples was carried out every Friday of each sampling week. Whenever possible organic and conventional samples were matched for the grape varieties and location. If this was not possible, grapes from either the a) same location and different varieties, b) different location and same variety or c) different location and different variety of organic and conventional grapes were collected. On each sampling date two replicates (plastic boxes) of organic and conventional grapes were collected. Depending on the season different colour grapes were available in the shop with black varieties only available during the summer season. All fresh grape samples were transferred to Newcastle University and kept frozen (-20°C) until sample preparation.

After defrosting for 2 hours, about 40-50 whole grape berries were randomly selected from each sample bunch, weighted (approximately 250-350 g), cut in half in order to allow removal of seeds and then crushed/homogenised (skin and pulp) for 30-120 seconds in a homogenizer (multipurpose food blender) to prepare grape juice. After homogenising, 5 aliquots of juice (for each sample) were labelled (date, management, supermarket, production country and cultivar name) and stored at -80°C until further analyses.

2.2.2 Farm Survey

The farm survey was designed as a comparison of farms (vineyards) in the Heraklion region of Crete, Greece. The survey was repeated for two years (2014 and 2015) between mid-August and mid-September each year. The samples were collected just before the farmers started harvesting. The three main local organically and conventionally grown grape varieties (Kotsifali, Vidiano and Vilana) were chosen for the farm survey. These varieties are mainly used for wine production, but also consumed as fresh fruit. In the first year 22 vineyards and in the second year 26 vineyards were used for sample collection. More detailed information on the vineyards used and the cultivars they produced can be found in Table 2.3.

Climatic conditions varied substantially between the two years included in the survey. In 2014 weather conditions were dry and hot with almost no rain before sample collection (July and August). In contrast, 2015 was wet with unusually heavy rainfall followed by very hot temperatures. Those conditions caused a substantial reduction in crop yields, especially in organic sites (e.g. Garakis, Koukis, Stilianou), due to foliar disease severity (powdery and downy mildew and *Botrytis*).

Table 2.3 Farm survey design

		2014 (***n=22***)	**2015** (***n=26***)
Vineyard	**Management**	**Cultivar**	**Cultivar**
Garakis (a)	Organic	Kotsifali (red)	Kotsifali (red)
Garakis (b)	Organic	Kotsifali (red)	Kotsifali (red)
Stilianou	Organic	Kotsifali (red)	Kotsifali (red)
Gavalas	Organic	Kotsifali (red)	Kotsifali (red)
Daskalakis	Organic		Kotsifali (red)
Lyrarakis	Conventional	Kotsifali (red)	Kotsifali (red)
Vrahassotakis	Conventional	Kotsifali (red)	Kotsifali (red)
Antonopoulos	Conventional	Kotsifali (red)	Kotsifali (red)
Tamiolakis	Conventional	Kotsifali (red)	Kotsifali (red)
Verigos	Conventional		Kotsifali (red)
Garakis	Organic	Vilana (white)	Vilana (white)
Stilianou	Organic	Vilana (white)	Vilana (white)
Gavalas	Organic	Vilana (white)	Vilana (white)
Koukis	Organic	Vilana (white)	Vilana (white)
Lyrarakis	Conventional	Vilana (white)	Vilana (white)
Antonopoulos (a)	Conventional	Vilana (white)	Vilana (white)
Antonopoulos (b)	Conventional	Vilana (white)	Vilana (white)
Tamiolakis	Conventional	Vilana (white)	Vilana (white)
Koukis	Organic	Vidiano (white)	Vidiano (white)
Stilianou	Organic	Vidiano (white)	Vidiano (white)
Korpis	Organic	Vidiano (white)	Vidiano (white)
Gavalas	Organic		Vidiano (white)
Lyrarakis	Conventional	Vidiano (white)	Vidiano (white)
Tsorsulaki	Conventional	Vidiano (white)	Vidiano (white)
Fragoulakis	Conventional	Vidiano (white)	Vidiano (white)
Tamiolakis	Conventional		Vidiano (white)

From each vineyard, 10 bunches of grapes were collected randomly by walking in a zig-zag pattern through the field to acquire samples covering the variation within the whole vineyard. They were placed into polyethylene cool boxes and transferred to the Livadopa experimental station (Sivas, Festos, Crete) by car, where they were prepared for longer storage. Ten individual healthy grape berries were cut from each bunch using scissors. Care was taken to leave a short 0.5-1 cm stem on each grape berry, to prevent wounding-related stress responses (e.g. induction of phenolic synthesis) and nutrient degradation in the berry. Between 50 and100 berries from 10 different bunches were then placed into labelled (date, management, vineyard name and cultivar) plastic bags and stored in a -20°C freezer. The same procedure was repeated for the back-up samples. After the survey was finished all samples were transported (on dry ice) to the School of Agriculture, Food and Rural Development at Newcastle University and then kept in a -20°C until sample preparation.

For sample preparation grape berries in separate plastic bags were left to thaw for 1-2 hours at ambient temperature. Each bag was then emptied into an aluminium tray, weighed (about 150-200 g), followed by the removal of the short stem. Each berry was then cut in half to allow removal of all seeds and then homogenised (only pulp and skin) for 30-120 seconds to make juice. Five aliquots of juice samples were labelled (date, management, vineyard name and cultivar) and transferred into -80°C freezer until further analyses.

2.2.3 Wine Survey

Different vintages (years of production), of both red and white organic and conventional wines were collected from wineries in the Heraklion region of Crete, Greece. Most of the wines were made from the same local varieties (Kotsifali and Vidiano), however wines made from non-local varieties were included in analyses if a common local variety was accounted for a high percentage of the grapes used (e.g. Kotsifali 70%/ Syrah 30% or Vidiano 70%/ Plyto 30%). Bottles of wine were collected from a range of different wineries and then transported to Newcastle University and stored in cool place (4° fridge). Later, 5 aliquots of wine was obtained from each wine bottle, labelled (vintage, management, wine name and cultivar) and kept at -80°C until further analyses.

2.2.4 ***Assessments***

2.2.4.1 Sugar Content (SC)

An OPTi Brix 54 Handheld Digital Refractometer (Bellingham + Stanley Ltd., Kent, U.K.) with a 0-54 ° Brix range and 0.1 ° Brix resolution was used for the determination of sugar content of individual berries and homogenized samples (juice). The sugar content of berries was determined by squeezing pulp on the prism (in triplicate). To assess sugar content in juice, a drop of the homogenized sample was placed on the prism. The sugar content reading on the LCD display of the device was recorded and the prism was carefully cleaned after every reading, and device was re-calibrated after each sample.

2.2.4.2 Dry Matter Content (DM)

Dry matter content was determined by oven drying at 80°C for 24 hours. Four to five grape berries from each sample were cut in half, to remove seeds, placed into labelled, pre-weighed aluminium trays (only tray-W_1) and then weighted (tray+berries-W_2). Trays were placed in a pre-heated oven (80°C) and left to dry for 24 hr until constant mass was obtained. After oven drying all the trays were placed into a desiccator for 1.5 hr, in order to avoid moisture being re-absorbed during cooling. Cooled trays with dried grapes (W_3) were then weighted again for fresh grape weight (W_4) calculation:

$$W_4 = W_2 - W_1$$

Dry Matter was calculated as:

$$\% \text{ DM} = \frac{W_3 - W_1}{W_4} \times 100$$

Water content was calculated as:

$$\% \text{ Water Content} = 100 - \% \text{ DM}$$

2.2.4.3 Total Phenolic Content (TPC)

Total phenolic content was determined by the Folin-Ciocalteau colorimetric assay method (Singleton *et al.*, 1999) and grape samples were extracted according to Tassoni *et al.* (2013).

Chemicals

Folin-Ciocalteau phenol reagent and gallic acid were obtained from Sigma-Aldrich. Sodium carbonate, methanol and hydrochloric acid (12N) were supplied by Fisher Scientific.

Sample extraction

Homogenized grape samples were thoroughly thawed for two hours under dim light at room temperature. After thawing, 0.5 g of sample was weighed in a screw top glass tube and 4 ml of MeOH:HCl (98:2) added. All samples then were placed into a rotary shaker to be extracted overnight under dim light. Extracted samples then were centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was then transferred to another glass tube for further dilution. For red samples, the dilution factor (DF) was set at 10, whereas for white samples DF was set at 5. Therefore, 1 ml of extracted white juice sample was diluted with 4 ml of the extraction solution (2% acidified methanol) and 1 ml of extracted red juice sample was diluted with 9 ml of the extraction solution (2% acidified methanol).
Wine samples did not require extraction and were diluted with 10% ethanol water solution.

Preparation of Folin-Ciocalteu reagents

Folin-Ciocalteu (FC) is a heteropoly phosphotungstate-molybdate reagent, which reacts with phenolic compounds by forming chromogens that can be detected by a spectrophotometer. The FC reagent solution was prepared fresh each time, by diluting the FC reagent with de-ionised water at a 1:10 ratio. It was then kept at 4°C until it was used again the same day.

Sodium Carbonate solution

Sodium carbonate is usually used as a buffer solution, in order to keep constant pH levels. A 7.5% sodium carbonate anhydrous solution was made by dissolving 7.5g sodium carbonate in 100 ml de-ionised water. The solution was kept at room temperature until used.

Gallic acid standard

Gallic acid (GA) is one of the major phenolic acids that presents in many plant parts (e.g. fruit, leaves) and the most commonly used standard for total phenolic content determination. In order to make 100 ml of GA standard stock solution, 10 mg dry GA was dissolved in 100 ml de-ionised water. For calibration curves, serial dilution of GA stock solution was made to achieve solutions with concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.57 and 0.78 μg /ml gallic acid equivalents (GAE).

Preparation of samples

For cuvette preparation, 20 μl of the sample, 1.58 ml de-ionised water and 100 μl FC solution was pipetted into cuvettes (BRAND® standard disposable cuvettes; pathlength 10mm, PMMA (semi-micro) and mixed well. After 5 min 300 μl of SC solution was added, covered

with parafilm and left for 2 hour at room temperature. Absorbance of samples was recorded at 765 nm wavelengths using a UV-VIS spectrophotometer (UV mini-1240, Shimadzu). Standards and blank sample with solely 2% acidified methanol were treated identically to samples. Samples were prepared in triplicate whereas blanks and standards were prepared in duplicates.

Calculations

A linear calibration curve was plotted from the absorbance values of diluted standards using Excel 2013 software. Concentration of samples were calculated in µg/ml according to the squares regression line equation and was expressed as mg GAE/ kg of sample's fresh weight (FW) of each sample.

2.2.4.4 *Total Antioxidant Activity (TAA)*

Trolox equivalent antioxidant capacity (TEAC) method was used for total antioxidant activity determination. In addition to this method DPPH (Thaipong *et al.*, 2006) and ABTS (Re *et al.*, 1999) assays were also performed with the same sample extraction as in the TPC analyses (Tassoni *et al.*, 2013).

Chemicals

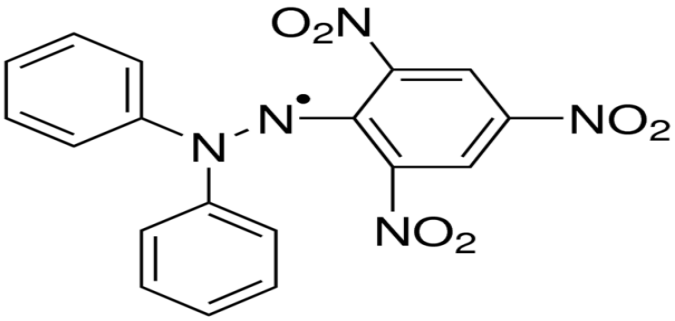
Potassium persulfate and radical scavenging assay reagents: 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), 2, 2- Diphenyl- 1- picrylhydrazyl (DPPH), 2, 2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Sodium chloride, sodium dihydrogen phosphate, sodium hydrogen phosphate, methanol and hydrochloric acid (12N) were supplied by Fisher Scientific.

TEAC standard solution

Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) is an antioxidant analogue to vitamin *E* and used as a standard for the determination of total antioxidant activity. In order to make 503.4 µM (250.3 µg/ ml) TEAC standard stock solution 6.3 mg Trolox was dissolved in 50 ml of 50% methanol/water solvent. This stock solution was then diluted to achieve 251.7, 125.85, 62.925 and 31.4625 µM Trolox using a 50% methanol/water solvent.

DPPH

2, 2- Diphenyl- 1- picrylhydrazyl (DPPH) is a free radical scavenger which basically operates via an unpaired valence electron at one of the nitrogen bridges (Sharma and Bhat, 2009).

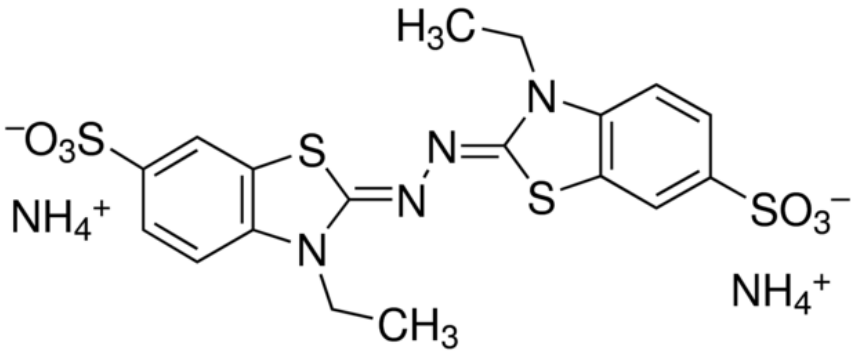


2, 2- Diphenyl- 1- picrylhydrazyl (DPPH) molecular structure (SigmaAldrich, 2017a)

In order to prepare the DPPH stock solution 24 mg DPPH reagent was dissolved in 100 ml methanol and kept in the dark at 4°C overnight. Stock solution was then used to prepare the DPPH working solution, by mixing 30 ml of stock solution and 80 ml of methanol. The working solution was also kept in the dark at 4°C until use (fresh solution was prepared every two days).

ABTS

ABTS (2, 2′-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid) is a free radical, which is generated by the reaction of a strong oxidizing agent (e.g. potassium persulfate) and ABTS peroxidase substrate (Shalaby and Shanab, 2013).



2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) (SigmaAldrich, 2017b)

The 7mM ABTS stock solution was prepared by dissolving 384.09 mg of ABTS in 100 ml of 18.2MΩ water. Meanwhile, 66.2 mg potassium persulfate ($K_2S_2O_8$) was dissolved in 100 ml

18.2MΩ water in order to make 2.45mM potassium persulfate stock solution. The above solutions were then mixed to make 100 ml ABTS working solution (90 ml of $ABTS^+$ stock solution and 10 ml of potassium persulfate stock solution) and kept in the dark, at room temperature overnight (approximately 16 hours) to complete the reaction that generates the radical cation ($ABTS^+$).

The 5 mM phosphate buffer solution (PBS) (pH 7.4) was prepared by mixing 4.5 g of sodium chloride (NaCl), 0.1839 g of sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) and 0.3677 g of sodium hydrogen phosphate ($Na_2HPO_4.12H_2O$) in a final volume of 500 mL 18.2MΩ water. The pH was adjusted by using a small quantity of hydrochloric acid (HCl).

Colorimetric reaction process

The colorimetric reaction was performed in a 96 well microplate (standard, flat bottom) (Greiner Bio-One Ltd., Stonehouse, UK) and analysed/read by a SpectraMax®Plus 384 Microplate Reader (VWR International Ltd.,UK).

For the DPPH reaction, 15 µl of sample (the blank (water), diluted standards (duplicate) and extracted plant samples (triplicate)) and 285 µl of DPPH working solution were pipetted accordingly into wells and incubated at 30^0C in the dark for 30 minutes. After incubation absorbance was read at 517 nm and values were recorded in an Excel sheet.

For $ABTS^+$ measurement, 10 µl of sample (the blank (water), diluted standards (duplicate) and extracted plant samples (triplicate)) and 290 µl of ABTS working solution was added into each well and incubated in the dark at 37^0C for 6 minutes. The absorbance of the ABTS working solution was adjusted to 0.7 at the wavelength of 734 nm by 5 mM phosphate buffer solution, before adding into each well. After incubation absorbance was read at 734 nm and values were recorded in an Excel sheet.

Calculation

Blank absorbance values were subtracted from sample absorbance values for both assays. For each assay, linear calibration curves were plotted from the absorbance values of diluted standards using Excel 2013 software. The antioxidant concentrations of samples were calculated according to the squares regression line equation and the results were expressed as mM Trolox Equivalent (TE).

2.2.4.5 *Total Anthocyanin Content (TAC)*

The extraction methods of total anthocyanin content from grapes described by Tassoni *et al.* (2013) and Chiou *et al.* (2014) was used with slight modifications. Total anthocyanin content was measured/determined using the pH differential method (Lee *et al.*, 2005).

Chemicals

Potassium chloride, sodium acetate, methanol and hydrochloric acid (12N) were supplied by Fisher Scientific.

Sample extraction

Half a gram (0.5 g (FW)) of grape juice was mixed with 4 ml of 0.1% acidified methanol solution and incubated in a water bath at 65°C for 2 hours, under dim light. After incubation samples were centrifuged at 4000 rpm for 10 min, at 25°C. Supernatants were then transferred into another glass tube for further dilution with pH buffers.

Wine samples did not require extraction. The dilution factor was the same for all analyses.

Buffer solutions

In order to prepare 0.025 M potassium chloride buffer (pH 1.0) 1.86 g of KCl was dissolved in 985-990 ml of 18.2MΩ water and the pH was adjusted to 1.0 (±0.05) by adding a few ml of HCl.

For 0.4 M sodium acetate buffer (pH 4.5) 54.43 g $CH_3CO_2Na{\cdot}3H_2O$ was dissolved in 985-990 ml of 18.2MΩ water and pH was adjusted to 4.5 (±0.05) by adding a few ml of HCl.

All extracted samples were then diluted by adding both buffers separately.

Preparation of samples

BRAND® standard disposable cuvettes (path length 10mm; PMMA (semi-micro)) were filled with 2-2.5 ml of pH diluted plant samples and the absorbance was measured by UV-VIS spectrophotometer (UV mini-1240, Shimadzu) at 520 nm (A_{250}) and 700 nm (A_{700}). Samples were prepared in triplicate and water was used as a blank. All the measurements were made and recorded within 20-30 minutes of preparation.

Calculations

The absorbance value (A) was calculated as:

$$A = (A_{520nm}-A_{700nm})\ pH1.0 - (A_{520nm}-A_{700nm})\ pH4.5$$

The total anthocyanin concentration of the sample was then calculated as:

$$\text{mg/l} = \frac{A \; x \; MW \; x \; DF \; x \; 10^3}{\varepsilon \; x \; l}$$

MW: molecular weight of chosen pigment (cyd-3-glu: 449.2 g/mol; mal-3-O-glu: 493.4374 g/mol)

DF: dilution factor

10^3: factor to convert from g to mg

ε: molar extinction coefficient in L x mol^{-1} x cm^{-1} (cyd-3-glu: 26900; mal-3-O-glu: 28000)

l: cuvette's path length (1 cm)

Two of the most common anthocyanin pigments (cyaniding 3-glucoside and malvidin 3-glucoside) were used in the calculation as an equivalent. Results were expressed in mg/kg of sample fresh weight (FW).

2.2.4.6 High-performance liquid chromatography (HPLC)

Concentrations of individual anthocyanins in grape and wine samples were detected and quantified according to Kammerer *et al.* (2004) with slight modifications.

Chemicals

Formic acid, acetonitrile, methanol (for HPLC grade) and hydrochloric acid (12N) were obtained from Fisher Scientific.

Sample extraction

Aliquots of 0.5g of grape juice sample were weighed into labelled 2ml centrifuge tubes, mixed with 1.5ml of 0.1% acidified methanol and vortexed for 2 hours for complete extraction. Vortex tubes were then centrifuged at 10600 rpm for 5 min and the supernatant was transferred into a second tube. The extraction was repeated adding 0.5 ml of 0.1% acidified methanol into the remaining residue and vortexed for another 15 min. The extracts were centrifuged and the supernatants were combined and centrifuged again. After centrifugation, extracts were passed through filters (Dutscher Scientific UK, Ltd, Syringe Filter Nylon, non-sterile (0.45 µm, 25 mm) and transferred to clean centrifuge tubes. Aliquots were transferred into HPLC amber vials and stored at -80°C until they were directly injected into the HPLC.

Wine samples only needed to be centrifuged, filtered and stored at -80°C until needed when they were directly injected into the HPLC.

HPLC analysis of anthocyanins

Analyses and separation of individual anthocyanin components were performed using a Phenomenex, Synergi™ 4 µm Hydro-RP 80Å (C18 phase, 250 x 4.6 mm) column, fitted with a C18 guard column (3.2-8.0 mm internal diameters) at a temperature of 25°C. The HPLC system (Shimadzu Corporation, Japan) was equipped with LabSolution software, a DGU-20A3R degasser, 2 LC-20AD pump, a SIL-20AC HT autosampler, a SPD- M20A diode array detector and a CTO- 20AC column oven. The detector was set to an acquisition range of 190-700nm.

Water/formic acid/acetonitrile (A) (87:10:3) and water/formic acid/acetonitrile (B) (40:10:50) were used as a mobile phase with a flow rate of 0.8 ml/min. The gradient programme for the mobile phases (A:B) was at 0.02 min (10:90), 5 min (10:90), 15 min (25:75), 20 min (31:69), 25 min (40:60), 35 min (50:50), 45 min (100:0), 50 min (10:90) and 55 min (10:90). The injection volume was 50 µl for all samples and quantification was performed at 520 nm.

Identification and quantification of individual compounds

Identification was based on peak relative retention times and elution order of chromatograms obtained by Kammerer *et al.* (2004). Individual anthocyanins were quantified using a calibration curve of malvidin-3-O-glucoside in the range of 50 to 0.05µg/ml.

LC-MS analysis was performed separately by Newcastle University Protein and Proteome Analysis (NUPPA) laboratory team to confirm the identity of the peaks based on m/z identified by HPLC analysis.

LC-MS method specifications

Anthocyanin extracts from grape were provided in a neat and 1/10 dilution. Samples were acidified with Trifluroacetic Acid (TFA) to a final concentration of 0.1% (v/v). Each sample was analysed with an individual LCMS experiment using a Thermo RSLC Nano LC coupled to a Sciex 6600 mass spectrometer. Mobile phases were made as follows; loading buffer 4% (v/v) acetonitrile with 0.1% (v/v) TFA, buffer A 4% acetonitrile 0.1% Formic Acid (FA), buffer B 80% acetonitrile 0.1% FA. Separation was carried out using a linear gradient from 4-80% Buffer B over 40 min. This followed a 10 min wash at 90% Buffer B, then a column equilibration at 4% Buffer B to return the column to original stating conditions. 5 µL sample (1/10 dilution) were loaded onto the 300 µm C18 trap column for desalting before being

resolved on a 23 cm 75 µm ID home packed analytical column containing Dr Maisch 3 µm particle size stationary phase. Analytes were injected online into the mass spectrometer, which acquired data in a data dependant format. Survey scans were performed over an m/z range of 400-1200. From each survey, the 30 most intense ions selected for MSMS, charge state +1 to +5 were considered for MSMS. Precursors were fragmented with a rolling collision energy, based on the charge state of the peptide ion. Total cycle time was 1.7 sec.

Data were visualised using Analyst v2.2 (Sciex). Extracted ion chromatograms were made using previously published anthocyanin m/z values. The MSMS spectra for relevant m/z were exported and compared to previously published data.

2.3 Statistical analyses

Nonlinear mixed-effects models (Pinheiro and Bates, 2000) were used to analyse the data in a series of analyses to produce ANOVA *p*-values for main effects and all interactions using the nlme (non-linear mixed effects) package in R software (R-Project).

For the retail surveys, three-factor ANOVA with year, management system and variety choice as fixed effects was carried out. In addition data from individual years were used in two-factor ANOVA with management system and variety choice as fixed effects. The hierarchical nature of the experimental design was reflected in the random error structures that were specified as supermarket/ year/ management system. Where analysis at a given level of a factor was carried out, that factor was removed from the random error term.

For the farm surveys, three-factor ANOVA with year, management system and variety choice as fixed effects was carried out. In addition data from individual years were used in a two factor ANOVA model with management system and variety choice as fixed effects. The hierarchical nature of the experimental design was reflected in the random error structures that were specified as farm/ year/ management system. Where analysis at a given level of a factor was carried out, that factor was removed from the random error term.

The normality of the residuals of all models was tested using QQ-plots. Differences between the varieties and interactions between factors were tested using Tukey contrasts in the general linear hypothesis testing (glht) function of the multcomp package in R software (R-Project). A linear mixed effects model was used for the Tukey contrasts, containing a treatment main effect, with three levels, with the random error term specified as described above.

The relationships between environmental, as well as agronomic factors on grape quality were investigated using redundancy analysis (RDA) for the farms survey data. Redundancy

analysis is a constrained ordination process that seeks combinations of explanatory variables (in this case environmental, agronomic and/or quality traits) that best explains variations in the dependent variables (e.g. phenolic concentrations). In all cases, the RDAs were carried out using the CANOCO package (Ter Braak and Šmilauer, 2012). Automatic forward selection of the environmental and agronomic or phenolic factors within the RDAs was used and their significance in explaining additional variance calculated using Monte Carlo permutation tests.

CHAPTER 3. Systematic literature review and meta-analysis of data on difference in antioxidants concentration between organic and conventional grape and grape products

3.1. Results

A total of 2284 publications were identified in the initial literature search, of which 2145 were excluded after thoroughly examining abstracts, as they did not match the composition comparison strategy of the protocol. The remaining 139 papers were read and papers that did not report suitable data (e.g. composition parameters) were rejected. Overall, 35 peer-reviewed publications fulfilled the criteria of the meta-analysis protocol. The flow diagram below indicates detailed information of the online search process and results according to the methodology protocol (Figure 3.1).

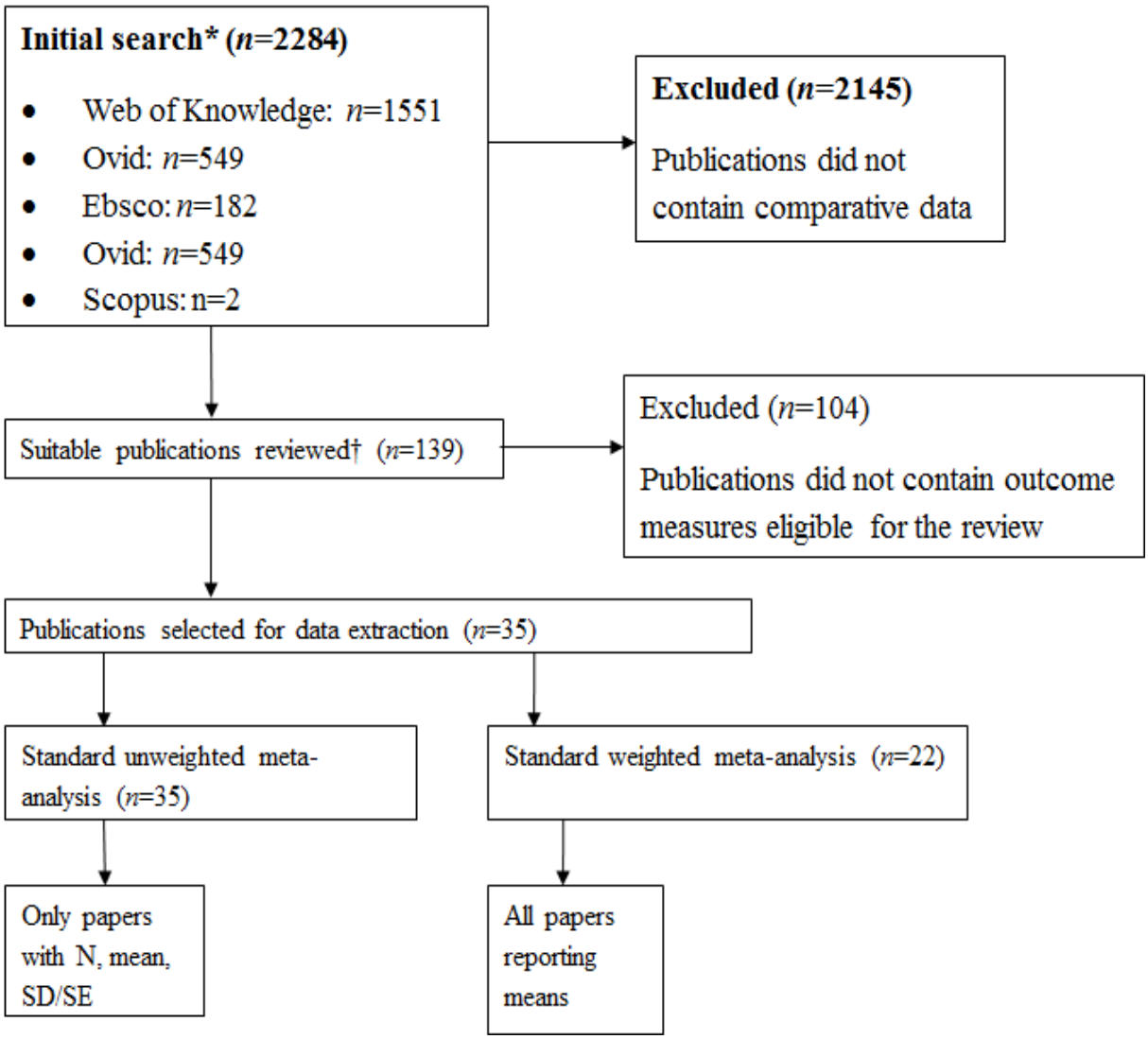


Figure 3.1 Summary of the online search results and selection strategy to identify papers included in the meta-analyses. *Review carried out by one reviewer; †Data extraction carried out by two reviewers.

Detailed information about the selected publications which met the inclusion criteria are listed in Table 3.1. Although most of the studies were from Europe (e.g. Romania, Italy, Germany, Spain), Brazil had the majority of publications (n=13) as a single country. There was only one paper which originated from the USA. The study type of the publications was mainly market surveys or basket studies (n=17), followed by comparison of farms (n=11) and field experiments (n=7). The majority of data points were on red grape juice and wine, together with red and white fresh fruit, skin and vinegar samples (Table 3.1).

Table 3.1 Reference, study type, location of study, crop/product and colour of product information of the comparison publications that were included in the meta-analysis.

Literature	ST	Location	Crop/Product	Colour
Akçay *et al.* (2004)	BS	Turkey	Grape (wine)	red
Artem *et al.* (2015)	EX	Romania	Grape (skin)	red
Tobolková *et al.* (2014)	BS	Slovak Republic	Grape (wine)	white
Buchner *et al.* (2014)	BS	Brazil	Grape (juice)	red
Hodor and Ciobanu (2012)	EX	Romania	Grape (skin)	red/white
Bunea *et al.* (2012)	EX	Romania	Grape (skin)	red/white
Burin *et al.* (2010)	BS	Brazil	Grape (juice)	red
Cardozo *et al.* (2013)	BS	Brazil	Grape (juice)	red
Corrales *et al.* (2010)	CF	Germany	Grape (juice)	white
da Silva Haas *et al.* (2016)	BS	Brazil	Grape (juice)	red
Dani *et al.* (2007a)	BS	Brazil	Grape (juice)	red/white
Di Renzo *et al.* (2007)	BS	Italy	Grape (wine)	red
de Freitas *et al.* (2010)	BS	Brazil	Grape (juice)	red
Garaguso and Nardini (2015)	BS	Italy	Grape (wine)	red
Granato *et al.* (2015)	BS	Brazil	Grape (juice)	red
Laureati *et al.* (2014)	CF	Italy	Grape (wine)	red
Levite *et al.* (2000)	CF	Switzerland	Grape (juice)	red/white
Machado *et al.* (2011)	BS	Brazil	Grape (juice/vinegar)	red
Malusa *et al.* (2002)	CF/EX	Italy	Grape (skin)	red
Margraf *et al.* (2016)	BS	Brazil	Grape (juice)	red
Martin and Rasmussen (2011)	CF	USA	Grape (wine)	red
Miceli *et al.* (2003)	CF/BS	Italy	Grape (wine)	red
Mulero *et al.* (2010)	CF	Spain	Grape (skin)	red
Mulero *et al.* (2009)	CF	Spain	Grape (skin)	red
Ongaratti *et al.* (2014)	BS	Brazil	Grape (juice)	white
Rodrigues *et al.* (2013)	BS	Brazil	Grape (juice)	red
Rodrigues *et al.* (2012)	BS	Brazil	Grape (juice)	red
Tassoni *et al.* (2013)	CF	Italy	Grape (fruit/wine)	red/white
Tassoni *et al.* (2014)	CF	Italy	Grape (fruit/wine)	red/white
Tinttunen and Lehtonen (2001)	BS	Finland	Grape (wine)	red/white
Toaldo *et al.* (2015)	EX	Brazil	Grape (juice)	red
Vian *et al.* (2006)	EX	France	Grape (skin)	red
Vrček *et al.* (2011)	CF	Croatia	Grape (wine)	red/white
Yıldırım *et al.* (2004)	EX	Turkey	Grape (wine)	red/white
Zafrilla *et al.* (2003a)	CF	Spain	Grape (wine)	red/white

ST, Study type (CF – Comparison of Farms, BS – Basket Study, EX – Controlled Experiment);

Among reviewed papers some did not provide enough information (e.g. measures of variance, replication number, parameter units), thus authors (n=13) were contacted and asked to provide missing information. Only seven authors provided the information that was requested. In addition, one of the publications (Burin *et al.*, 2010) had a possible mistake in the presented data-set: the mean value presented in Table 2 for the total phenolic (TP) parameter was 21374.56 mg/L. After unsuccessful contact with the author, the assumption of the correct value was made (new value was 2137.4 mg/L) on the basis of values for the same parameter presented in other publications and the data point was finally added to meta-analyses. There were also 15 data points (e.g. epigallocathecin, *trans*-resveratroloside, epigallocathecin-gallate) that were excluded from the meta-analysis because of the unrealistically low standard errors from the paper by Tassoni *et al.* (2013) (data from Fig. 2 and 4) and by Tassoni *et al.* (2014) (data from Fig.1 and 3).

A total of four meta-analyses were carried out, including one standard analysis and three sensitivity analyses. A significant difference between organically and conventionally cultivated grapes/grape products was detected for anthocyanins (group), total flavonoids and three individual phenolic acids (p-coumaric acid, syringic acid and ferulic acid) by both weighted and unweighted standard meta-analysis (Table 3.2). Significant difference or trends towards significant difference was detected for other parameters, such as stilbenes (group), tannins, piceatannol, caffeic acid, cis-piceid, epicatechin and resveratrol separately by unweighted or weighted meta-analyses (Table 3.2).
Anthocyanins (group) and total flavonoids concentrations were significantly higher in organic, while concentrations of the tree phenolic acids (p-coumaric acid, syringic acid and ferulic acid) were higher in conventional grapes/grape products (Table 3.2). The mean percentage difference (which was calculated to estimate the magnitude of difference between organic and conventional grapes/grape products), based on data from the weighted meta-analyses suggests that organic grapes/grape products had an 87% higher anthocyanins and 12% higher total flavonoid concentrations, while conventional grapes/grape products had an 113% higher p-coumaric acid, 43% higher syringic acid and 17% higher ferulic acid concentrations. Heterogeneity between studies and data-points was 82 and 54% detected by weighted meta-analyses, indicating higher and medium variation between estimates, however no heterogeneity was detected for three individual phenolic acids (p-coumaric acid, syringic acid and ferulic acid) (Table 3.2). The unweighted meta-analyses also identified significantly higher tannins and resveratrol (a stilbene) concentrations in organic, higher epicatechin (e flavanols) concentrations in conventional grapes/grape products (Table 3.2).

Table 3.2 Meta-analysis results of grape and its products' composition parameters for which significant differences were detected by the standard weighted and unweighted meta-analysis protocols

	Unweighted meta-analysis					Weighted meta-analysis						
Parameter	***n***	**Ln(R)**	***P*†**	**MPD‡**	**95%CI**	***n***	**SMD**	**95%CI**	***P*†**	**Heterogeneity§**	**MPD‡**	**95%CI**
anthocyanins *	29	4.87	0.024	42.12	-9.3, 93.54	10	4.71	2.14, 7.29	0.0003	Yes (82%)	86.57	47.85, 125.28
flavonoids**	6	4.72	0.017	12.01	5.49, 18.52	6	0.97	0.28, 1.66	0.006	Yes (54%)	12.01	5.49, 18.52
p-coumaric acid	7	4.15	0.016	-76.56	-153.96, 0.84	4	-0.66	-1.08, -0.25	0.002	No	-112.70	-239.03, 13.62
syringic acid	6	4.33	0.030	-37.02	-71.93, -2.1	5	-0.76	-1.16, -0.36	0.000	No	-42.45	-83.17, -1.73
ferulic acid	4	4.45	0.062	-17.01	-25.45, -8.57	4	-0.79	-1.37, -0.21	0.007	No	-17.01	-25.45, -8.57
stilbenes *	48	4.73	0.151	63.73	-17.47, 144.94	32	-2.05	-4.08, -0.02	0.048	Yes (96%)	49.64	-61.84, 161.12
tannins	5	4.70	0.030	10.51	1.07, 19.95	5	1.35	-0.2, 2.91	0.088	Yes (89%)	10.51	1.07, 19.95
piceatannol	5	4.10	0.059	-73.50	-121.77, -25.21	4	-2.46	-5.3, 0.38	0.090	Yes (89%)	-66.87	-126.9, -6.84
caffeic acid	9	4.58	0.465	-15.27	-89.23, 58.69	5	1.29	-0.11, 2.68	0.070	Yes (86%)	26.63	-6.93, 60.2
cis-piceid	4	4.30	0.063	-38.57	-68.08, -9.05	4	-5.87	-13.28, 1.54	0.120	Yes (97%)	-38.57	-68.08, -9.05
epicatechin	14	4.23	0.043	-69.43	-134.82, -4.03	10	-3.08	-13.25, 7.09	0.553	Yes (99%)	-90.03	-171.81, -8.25
resveratrol	13	5.39	0.001	239.39	-24.3, 503.08	9	0.43	-0.17, 1.02	0.157	Yes (21%)	266.82	-115.32, 648.96

n, number of data points included in the comparison; MPD, mean percentage difference; SMD, standardised mean difference of fixed-effect model. Ln(R) = Ln (ORG/CONV × 100%); †*P* value <0.05 indicates significance of the difference in composition between organic and conventional crop/crop based food; ‡Magnitude of difference between organic (ORG) and conventional (CONV) samples (value <0 indicate higher concentration in CONV, value >0 indicate higher concentration in ORG); §Heterogeneity and the I^2 Statistic; *group of particular parameters; **total of particular parameters

The results of the GRADE (Grading of Recommendations, Assessments, Development and Evaluation) assessment for composition parameters for which the weighted meta-analysis identified significant difference or trends toward significant difference are shown in Table 3.3. Examination of funnel plots identified no publication bias for anthocyanins, while strong or medium publication bias was detected for all other parameters.

The overall reliability of evidence gathered in the standard weighted meta-analysis were assessed as moderate or low for the majority of parameters, whereas for tannins a very low overall strength of evidence was identified (Table 3.3).

Table 3.3 GRADE (Grading of Recommendations, Assessments, Development and Evaluation) assessment of the strength of evidence for standard weighted meta-analysis for parameters that have significant difference and trends toward significant difference.

Parameter	**Effect magnitude†**	**Precision‡**	**InconsistencyŁ**	**Publication bias§**	**Overall reliability‖**
anthocyanins *	moderate	high	moderate	not detected	moderate
stilbenes *	small	high	high	suspected	low
flavonoids**	small	medium	low	strongly suspected	low
p-coumaric acid	small	high	low	suspected	moderate
syringic acid	small	medium	low	suspected	moderate
ferulic acid	small	low	low	suspected	low
tannins	small	low	low	strongly suspected	very low
piceatannol	small	medium	low	strongly suspected	low
caffeic acid	small	high	low	suspected	moderate

*-group of particular parameters

**-total of particular parameters

Standardised mean difference values (SMD) and 95% confidence intervals

† Study quality was considered low because of high risks of bias and potential for confounding. However, we considered large effects to mitigate this sensu GRADE; large effects were defined as 20 %, moderate effects as 10–20% and small as 10 %.

‡ Precision was based on the width of the pooled effect CI and the extent of overlap in the substantive interpretation of effect magnitude sensu GRADE.

ŁInconsistency was based on the measure of heterogeneity and the consistency of effect direction sensu GRADE

§Publication bias was assessed using visual inspection of funnel plots, Egger tests, two fail-safe number tests, and trim and fill. Overall publication bias was considered strong when indicated by two or more methods, moderate when indicated by one method, and low when indicated by none of the methods.

‖The overall quality of evidence was then assessed across domains as in standard GRADE appraisal.

Figures 3.2 to 3.6 show forest plots with SMDs and corresponding 95% CI (confidence interval) for the parameters for which significant difference were detected by weighted meta-analyses.

The forest plots for parameters, which had only a trend toward significant difference between agronomic practices (stilbenes, tannins, piceatannol and caffeic acid) and results for the three sensitivity analyses, which had the same/similar results as the standard meta-analyses (due to the same data points) is presented in Appendix 1 (Figures A1-A4 and Tables A1-A3).

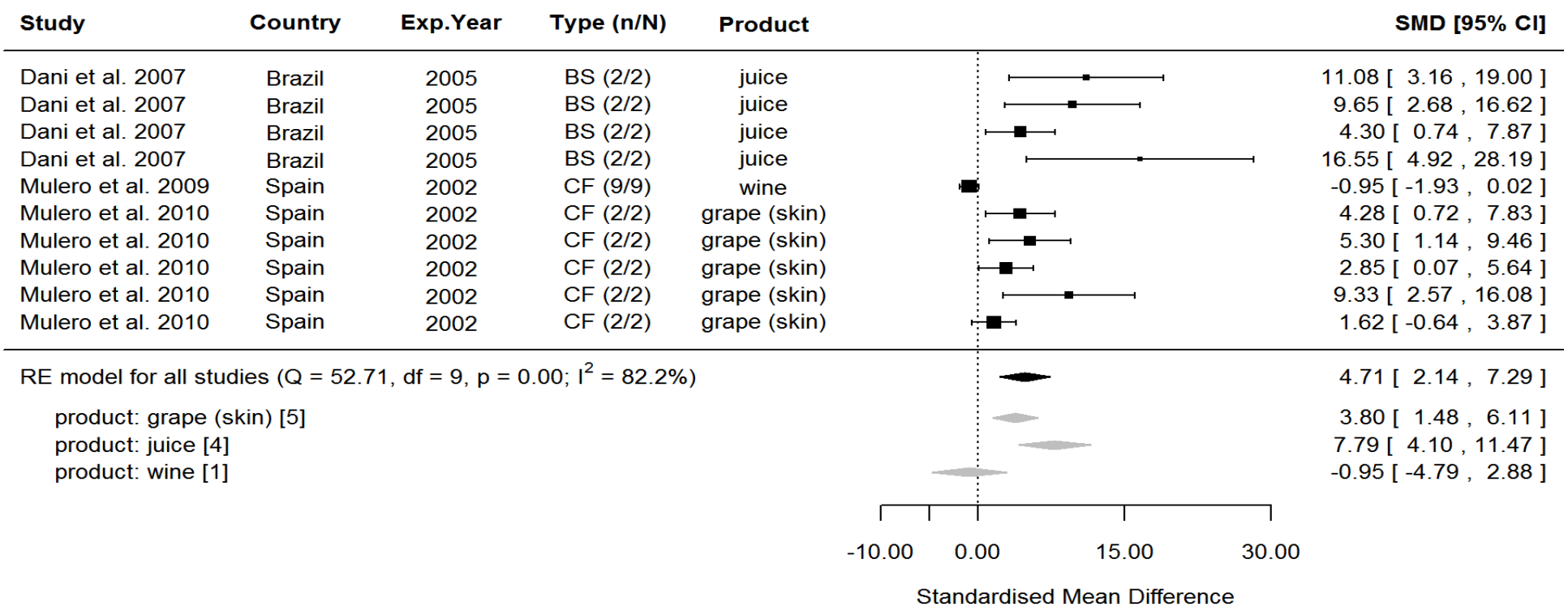


Figure 3.2 Forest plot showing the results of the comparison of anthocyanins content between organic and conventional grapes and grape products using standardised mean differences (SMD) with 95% confidence intervals (95% CI), for studies included in the standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods. BS- basket study; CF-comparison of farms; EX-controlled experiment

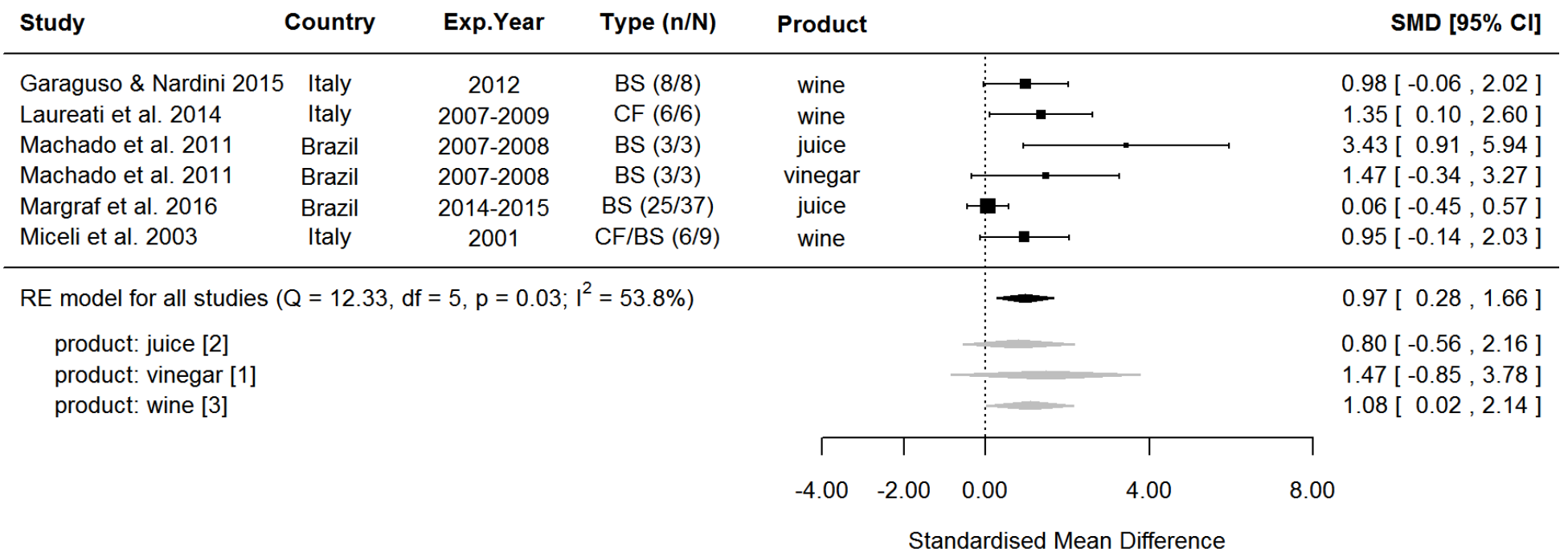


Figure 3.3 Forest plot showing the results of the comparison of flavonoids (totals) between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n- number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analyzed parameter is higher (+) or lower (-) in organic foods. BS- basket study; CF-comparison of farms; EX-controlled experiment

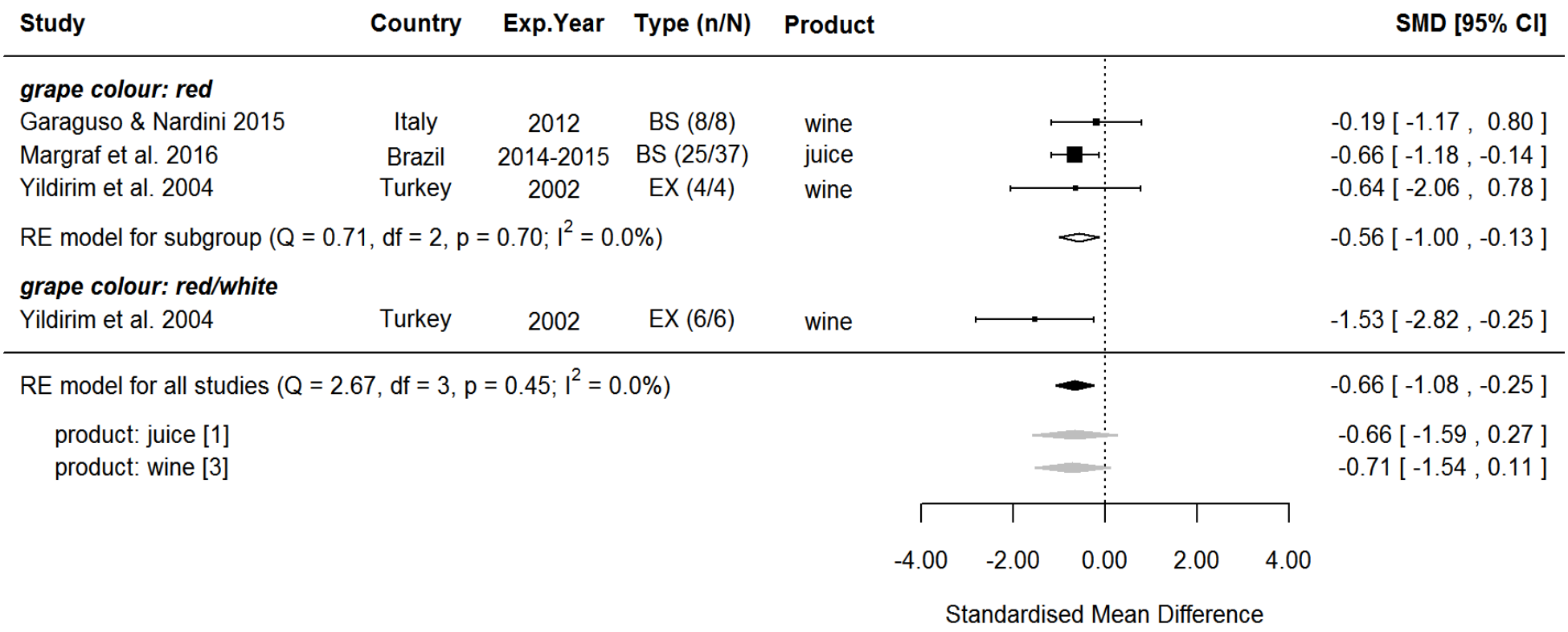


Figure 3.4 Forest plot showing the results of the comparison of p-coumaric acid between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods. BS- basket study; CF-comparison of farms; EX-controlled experiment

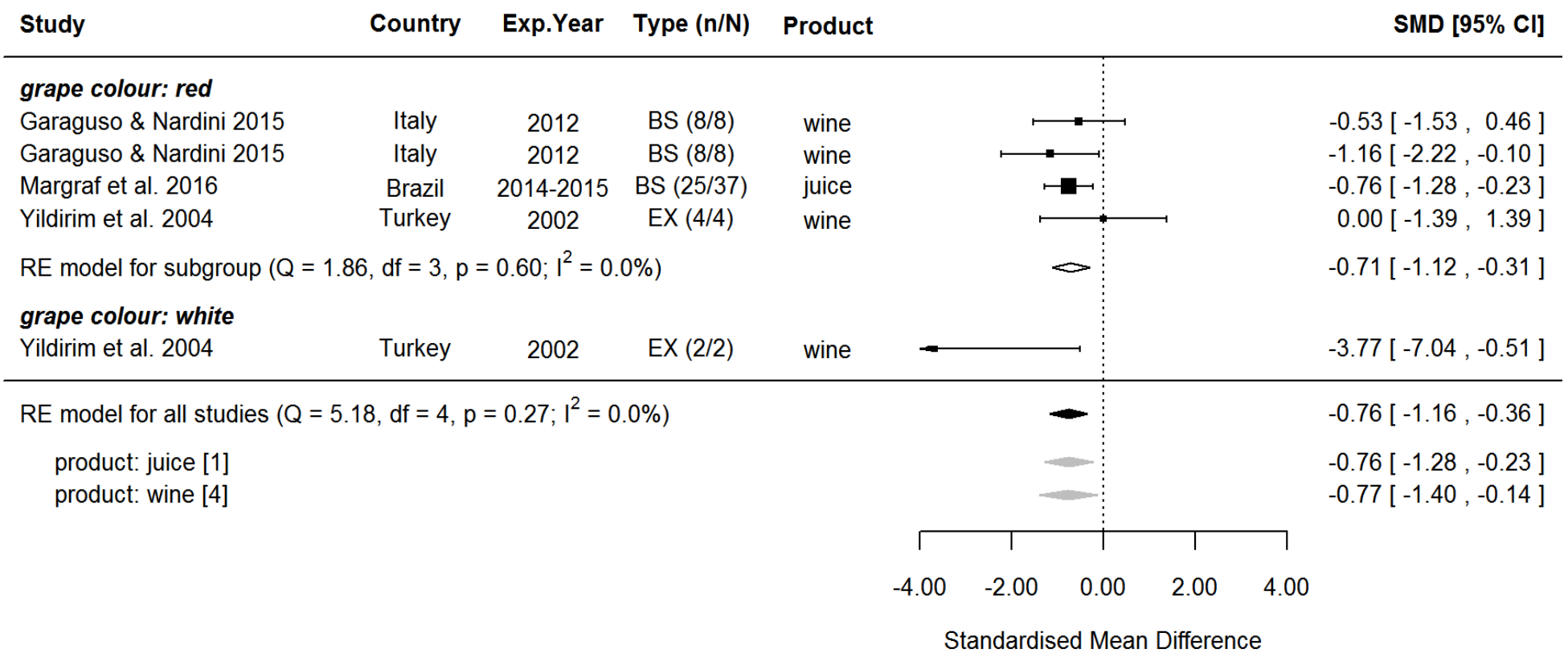


Figure 3.5 Forest plot showing the results of the comparison of syringic acid between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods. BS- basket study; CF-comparison of farms; EX-controlled experiment

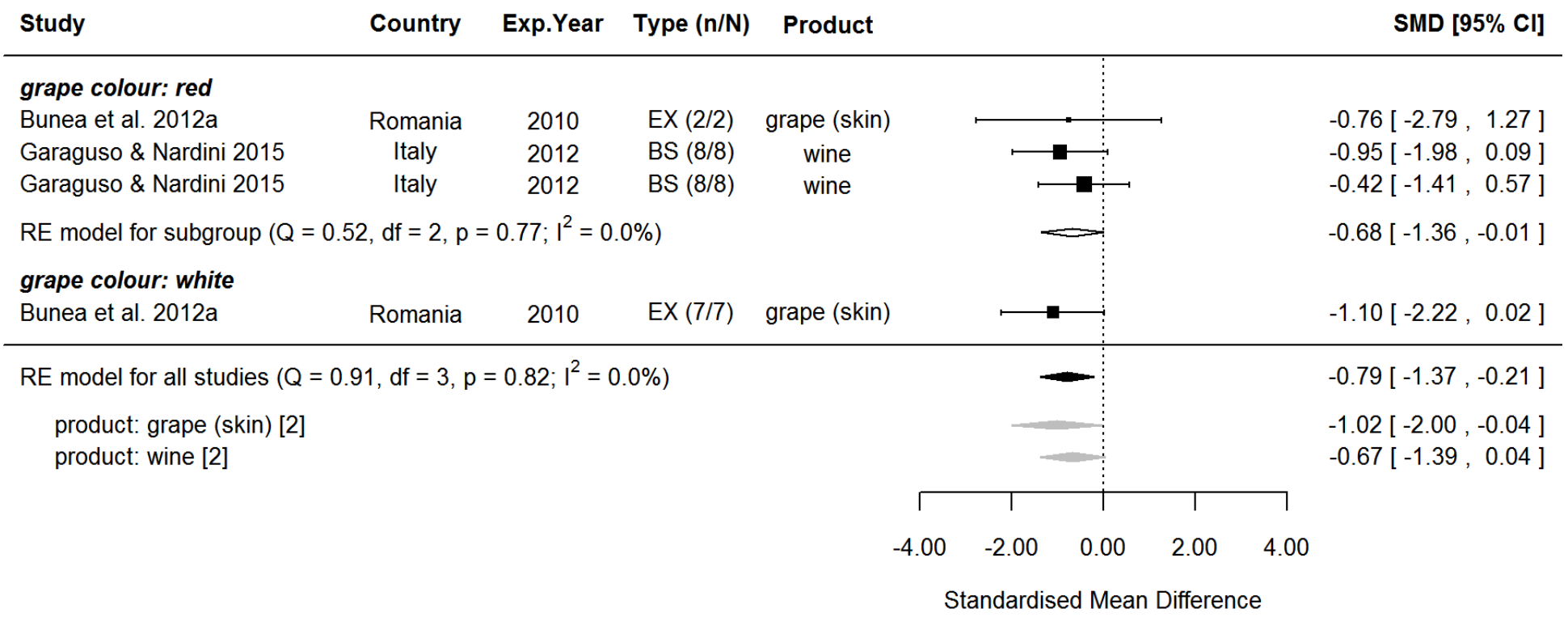


Figure 3.6 Forest plot showing the results of the comparison of ferulic acid between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods. BS- basket study; CF-comparison of farms; EX-controlled experiment

3.2 Discussion

The results of the meta-analyses were based on a relatively small number of 35 peer-reviewed publications, which resulted in less than 10 data points being available for most specific composition parameters. Furthermore, the heterogeneity of composition data collected via three different experimental approaches (retail and farm surveys and controlled experiments), in contrasting agronomic and pedo-climatic backgrounds would be expected to be relatively high. Despite the small sample size for most individual composition parameters, significant differences were detected for some nutritionally desirable compounds found in grapes/grape products with the organic systems having a higher concentration of anthocyanins and total flavonoids. These results correspond with the meta-analyses carried out by Brandt *et al.* (2011) and Barański *et al.* (2014) which were based on data for all crops (fruit and vegetables) and contradict overall outcome of systematic literature reviews/meta-analyses by Dangour *et al.* (2009) and Smith-Spangler *et al.* (2012), which indicated no significant difference in composition of organic and conventional crops. Weighted meta-analyses based on standardised mean difference (SMD) were used in order to combine studies which measured the same parameter differently (Brandt *et al.*, 2013) in the presented review. This allowed not to miss out any relevant data that can contribute to the detection of significant composition difference. However, this approach was not used by the contrasting and contradicting systematic literature review by Dangour *et al.* (2009).

There are a range of publications which indicate negative association between the fertilisation regime and phenolic concentration, as higher N fertiliser inputs tend to cause reduction of phenolic concentration (Sander and Heitefuss, 1998; Rühmann *et al.*, 2002; Brandt *et al.*, 2011; Almuayrifi, 2013). According to Hilbert *et al.* (2015) a higher supply of N can decrease anthocyanin concentration in grape berry skin during maturation. The negative effect of high N fertiliser inputs on wine quality (by reducing anthocyanin content, which causes color loss) was also detected by Keller and Hrazdina (1998). This fertiliser effect could explain the significantly higher levels of anthocyanins by 87 % and total flavonoids by 12% in organic grape/grape products that were found in the current meta-analyses (Table 3.2).

Higher concentrations of antioxidant activity in organic grapes/grape products was detected by unweighted meta-anlyses only in two sensitivity analyses (Appendix A: Table A1,A3), where cultivars were treated seperately. As there were no significant results from polyphenol content in order to be correlated with antioxidant activity, it can be assumed that anthocyanins and total flavonoids of separate cultivars have a more impact on antioxidant activity than averaged cultivars. This is confirmed by the studies of Orak (2007) and Wang *et al.* (1997),

which indicate that in different cultivars, higher antioxidant activity can be related to either total or individual anthocyanin contents.

Higher concentrations of polyphenols in organic crops can be explained as a protective or resistance response to many abiotic (e.g. climatic, water and nutrients stress) and biotic (e.g. pests, diseases) factors (Nicholson and Hammerschmidt, 1992; Bennett and Wallsgrove, 1994; Almuayrifi, 2013). However, as mentioned before, the differences between cultivars also have to be considered as a confounding factor when comparing levels of secondary metobolites, thus difference in the level of secondary metobolites can be much bigger between cultivars rather than between the same cultivar grown under different agricultural practices (Brandt and Mølgaard, 2001). For example, the concentration of anthocyanins in the samples of Cabarnet Sauvignon grape cultivar was 1078.6 mg/l and 1938.6 mg/l in two different years according to González-Neves *et al.* (2004), however other red cultivars indicated lower concentrations of anthocyanin, such as 783.2 mg/kg in Carignan and 58.6 mg/kg in Gewürztraminer according to Orak (2007). Another confounding factor in the meta-analyses was likely to have been contrasting climatic conditions in different years, since it is well known that phytochemical concentrations in grapes vary greatly between years (Conradie *et al.*, 2002; González-Neves *et al.*, 2004; van Leeuwen *et al.*, 2004).

Thus, considering that both grape cultivar and season were likely confounding factors and the samples size of available studies for the meta-analyses was lower reported results here need to be interpreted with caution and that a substantial number of additional primary studies needs to be carried out to estimate whether or not and to what extent production systems affect grape phytochemical concentrations.

There are several studies that reported a positive link between the consumption of antioxidant/polyphenol-rich foods/drinks and lower risk of cardiovascular disease (Kondrashov *et al.*, 2009; Venturini *et al.*, 2010; Wang *et al.*, 2014), also some comparative intervention studies that assessed the health impact of organic food consumption by having higher antioxidant concentration (Grinder-Pedersen *et al.*, 2003; Søltoft *et al.*, 2011; Smith-Spangler *et al.*, 2012). However, there is still a lack of knowledge about intake levels of antioxidant compounds and exact identification of individual antioxidant compounds to be more responsible for protection from particular diseases.

Overall, the result that organic grapes/grape products contain around 40 to 80% higher levels of anthocyanins (as suggested by the results of both the weighted and unweighted meta-analysis) from this study could have substantial positive impacts on human health, if it is confirmed in future studies. However, cohort/epidemiological studies investigating health impacts of consumption of foods with a high anthocyanin contents reported variable

outcomes, with some studies reporting anticancer, antitumor, anti-inflammatory and anti-oxidative effect of anthocyanins (Meyer *et al.*, 1997; Burns *et al.*, 2000; de Pascual-Teresa *et al.*, 2010), while others showed no significant effect of increasing anthocyanin intake (Orak, 2007; Yang *et al.*, 2009).

Cohort studies investigating the impact of increased total flavonoid intake showed more consistent results and linked higher intakes to a reduction in cardiovascular diseases (Reed, 2002; Georgiev *et al.*, 2014; Wang *et al.*, 2014). However, the difference for total flavonoids detected in this study was much smaller (around 12% higher in organic grapes) and is therefore less likely to have a substantial health effect.

Based on the evidence available, it is therefore currently not possible to estimate potential health impacts of switching to organic grape consumption and give clear guidance to consumers as to the health benefits this may deliver.

CHAPTER 4. Retail survey; nutritional composition of organic and conventional table grapes available in UK supermarkets in the winter and summer seasons 2015 and 2016

4.1. Results

A retail survey was conducted to investigate the effect of year, production season (winter vs summer), management systems (organic vs conventional) and variety choice, on the nutritional composition of grapes. Table grape samples collected in different UK supermarkets were analysed for **(a)** dry matter (DM), **(b)** sugar content in pulp (SC_p) and juice (SC_j) (BRIX°), **(c)** total antioxidant activity (TAA; estimated based on DPPH and TEAC assays) and **(d)** concentrations of nutritionally-relevant antioxidants (total phenolic (TPC) and anthocyanin (TAC) content). Since variety is known to affect grape quality parameters, only variety-matched pairs of grapes from organic and conventional production were included in the analyses (samples where the same variety was available from organic and conventional production in supermarkets on a given date).

Separate 3-factor ANOVAs (with year, management system and variety as factors) were carried out for white, red and black grape varieties.

White varieties

Highly significant main effects ($p<0.0001$) of variety were detected by ANOVA for five composition parameters (dry matter content, sugar content in grape pulp and juice, total phenolic content and total antioxidant activity) (Table 4.1). There was also a highly significant main effect ($p=0.0007$) of year for total antioxidant activity (TEAC). There was no significant main effect of management systems for any of the composition parameters assessed.

The mean dry matter (DM) content for different varieties ranged from 16.9% to 22.3%. The DM content in the varieties Timpson and Thompson were significantly higher than the other three varieties (Early Sweet, Sugraone and Superior), which had similar DM contents.

The sugar content in both grape pulp and juice was highest in Timpson (19.8 and 20.5 respectively) and lowest in Sugraone (15.7 and 16.0 respectively).

Total phenolic content ranged between 998 and 1500 mg GAE/kg, with the highest concentration found in Sugraone and the lowest in Thompson (Table 4.1).

The total antioxidant activity (DPPH) in grapes from different varieties ranged between 5.6 and 11.3 mM TE/g and was highest in the variety Superior and lowest in Timpson (Table 4.1).

Interaction between factors

Significant 2-way interactions between management and variety were identified for sugar content (both pulp and juice) and total antioxidant activity (DPPH) (Table 4.1). There was also a 2-way interaction between variety and year for total antioxidant activity (TEAC) (Table 4.1). A 3-way interaction ($p=0.04$) was detected for pulp sugar content only (Table 4.1).

The sugar content of pulp and juice was significantly higher in organically grown grapes of the variety Timpson; in all other grape varieties the sugar concentrations in organically and conventionally grown grapes were not significantly different (Figure 4.1).

Total antioxidant activity (DPPH) was significantly higher in conventionally grown grapes of the variety Superior; in all other grape varieties the antioxidant activity (DPPH) in organically and conventionally grown grapes was not significantly different (Figure 4.2).

Total antioxidant activity (TEAC) was significantly higher in 2015 than 2016 in the variety Sugraone, while there was no significant difference between years for all other varieties (Figure 4.3). Although there was no statistical significance, large numerical differences were also detected for other varieties, with some having higher antioxidant activity in 2015 (Sugraone, Timpson) and others having higher activity in 2016 (Early Sweet, Thompson) (Figure 4.3).

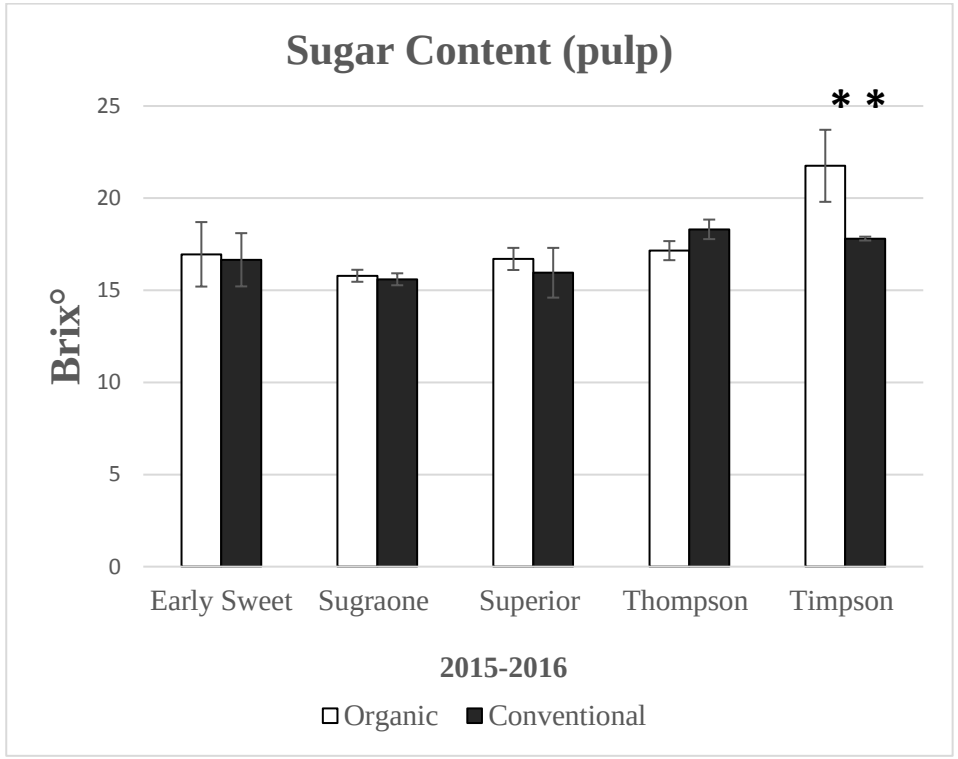
A significant 3-way interaction between management system, variety and year was detected only for the sugar content of pulp only (Table 4.1). Significant differences between production systems were detected only for the varieties Thompson and Timpson produced in 2016, when conventionally grown Thompson, but organically grown Timpson grapes had higher sugar content (Figure 4.4). However, it should be pointed out that for certain varieties and year only one or a very small number of sample pairs (organic vs conventional) were available, so these data should be reviewed with care.

Table 4.1 Effect of, and interaction between, management, variety and year on the dry matter content (DM), sugar content (SC) of pulp/juice, total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /ABTS assays and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) of white grape varieties from UK supermarkets in 2015-2016 summer (s) and winter (w) seasons (3-way ANOVA)

	DM %	**SC pulp (Brix°)**	**SC juice (Brix°)**	**TPC mg GAE/ kg**	**TAA (DPPH) mM TE/g**	**TAA (TEAC) mM TE/g**	**TAC mg cyan/ kg**	**TAC mg mal/ kg**
Year (yr)								
2015 ***(n=40)***	18.2±0.3	16.8±0.3	17±0.3	1267.9±72.8	7.7±0.3	0.9±0.1	12.4±1.3	13.1±1.4
2016 ***(n=40)***	17.7±0.4	16.4±0.3	16.5±0.3	1332.7±48.7	7±0.4	0.6±0.1	13.7±1.3	14.4±1.3
Management (man)								
ORG ***(n=40)***	18.2±0.4	16.6±0.3	16.5±0.3	1288.5±60.8	7.3±0.3	0.7±0.1	13.5±1.1	14.2±1.1
CONV ***(n=40)***	17.7±0.3	16.6±0.3	16.9±0.3	1312.1±63.4	7.3±0.4	0.8±0.1	12.6±1.4	13.3±1.5
Variety (var)								
Early Sweet (w) ***(n=4)***	17.3±0.6**c**	16.8±0.9**bc**	16.8±0.8**bc**	1219.9±102.7**ab**	6.4±1.1**bc**	0.7±0.3	12.1±4.4	12.8±4.7
Sugraone (s) ***(n=44)***	17.2±0.3**c**	15.7±0.2**c**	16±0.2**c**	1500.5±53.1**a**	7.8±0.3**b**	0.7±0.1	14.7±1.3	15.5±1.3
Superior (s) ***(n=4)***	16.9±0.5**c**	16.3±0.6**bc**	16.4±0.3**bc**	1042.1±164.7**b**	11.3±2.2**a**	0.9±0.3	11.6±3.2	12.2±3.4
Thompson (w) ***(n=24)***	19±0.4**b**	17.7±0.4**b**	17.5±0.4**b**	998.2±58.1**b**	6.2±0.2**c**	0.7±0.1	10.6±1.6	11.2±1.7
Timpson (s)***(n=4)***	22.3±1.9**a**	19.8±1.4**a**	20.5±1.3**a**	1249.7±102.8**ab**	5.6±0.5**c**	0.6±0.2	12±0.7	12.6±0.7
ANOVA (P values)								
Man	NS	NS	NS	NS	NS	NS	NS	NS
Var	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	NS	NS	NS
Yr	NS	NS	NS	NS	T	**0.0007**	NS	NS
2-way Interactions								
man:var	T	**0.0205**	**0.0203**	NS	**0.0072**	NS	NS	NS
man:yr	NS	T	NS	NS	NS	T	NS	NS
var:yr	NS	NS	NS	NS	NS	**0.0017**	NS	NS
3-way Interaction								
man:var:yr	NS	**0.037**	NS	NS	NS	T	T	T

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

A)



B)

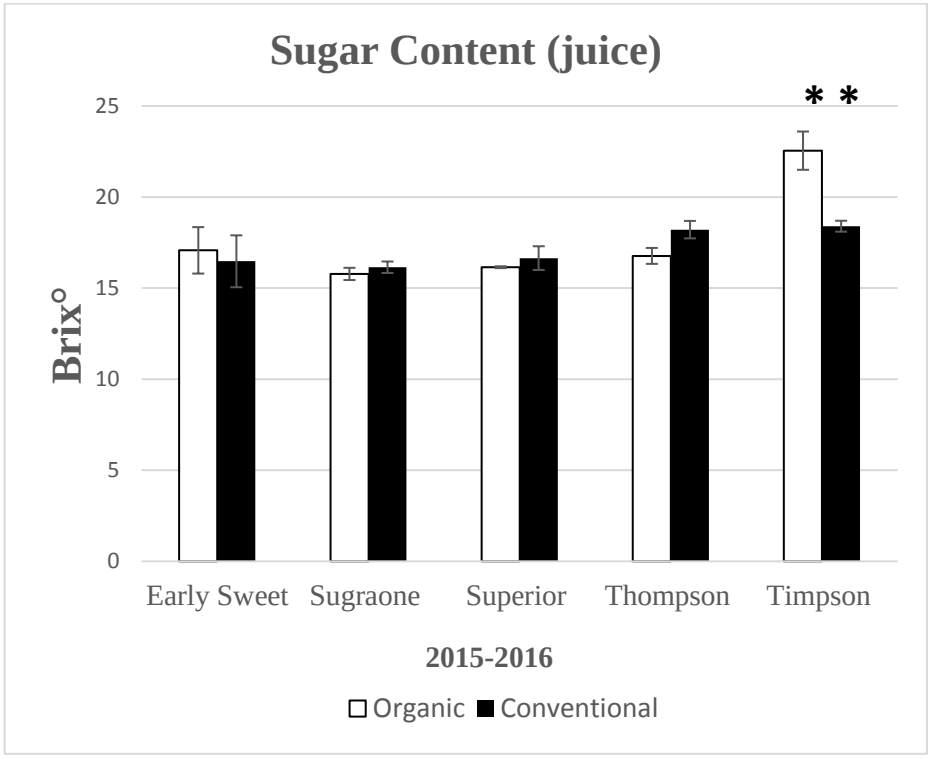


Figure 4.1 Level of sugar content of pulp (A) and juice (B) in white grape varieties; **-indicates significant interaction (management system and variety) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

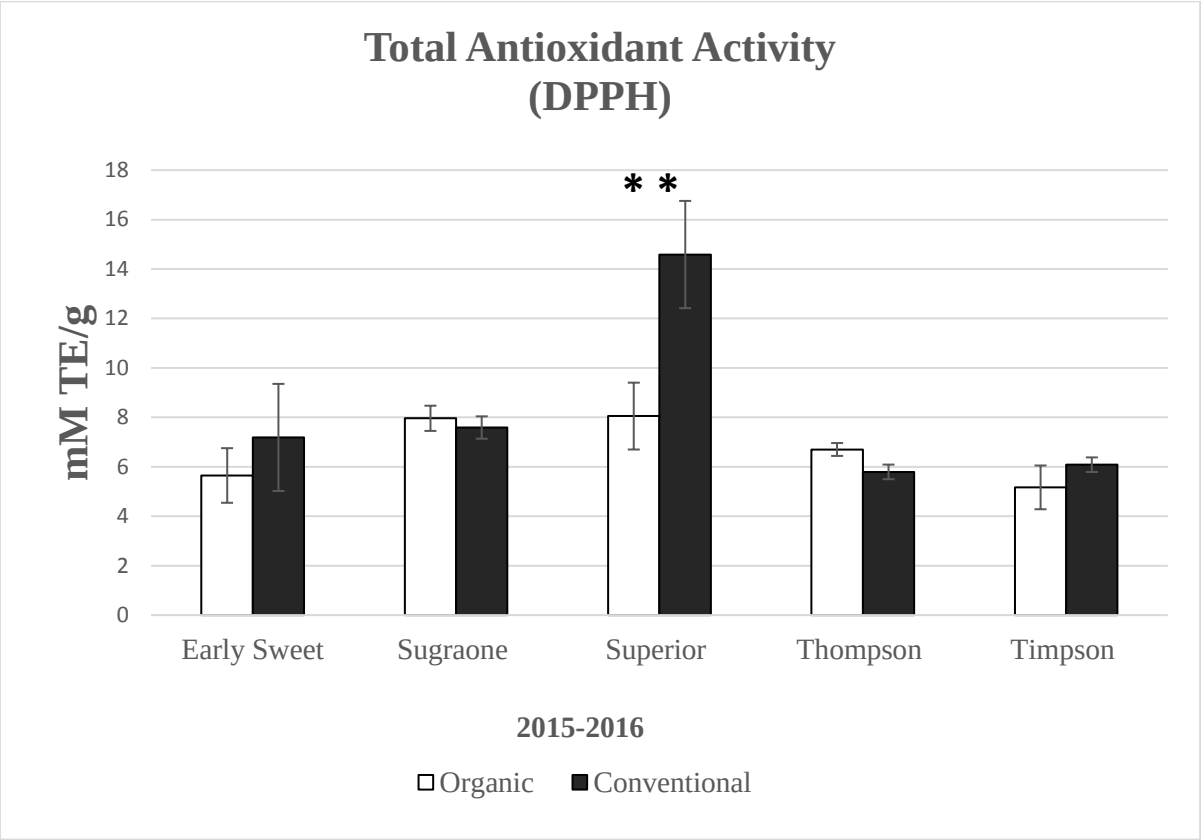


Figure 4.2 Level of total antioxidant activity by DPPH assay in white grape varieties; **-indicates significant interaction (management system and variety) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

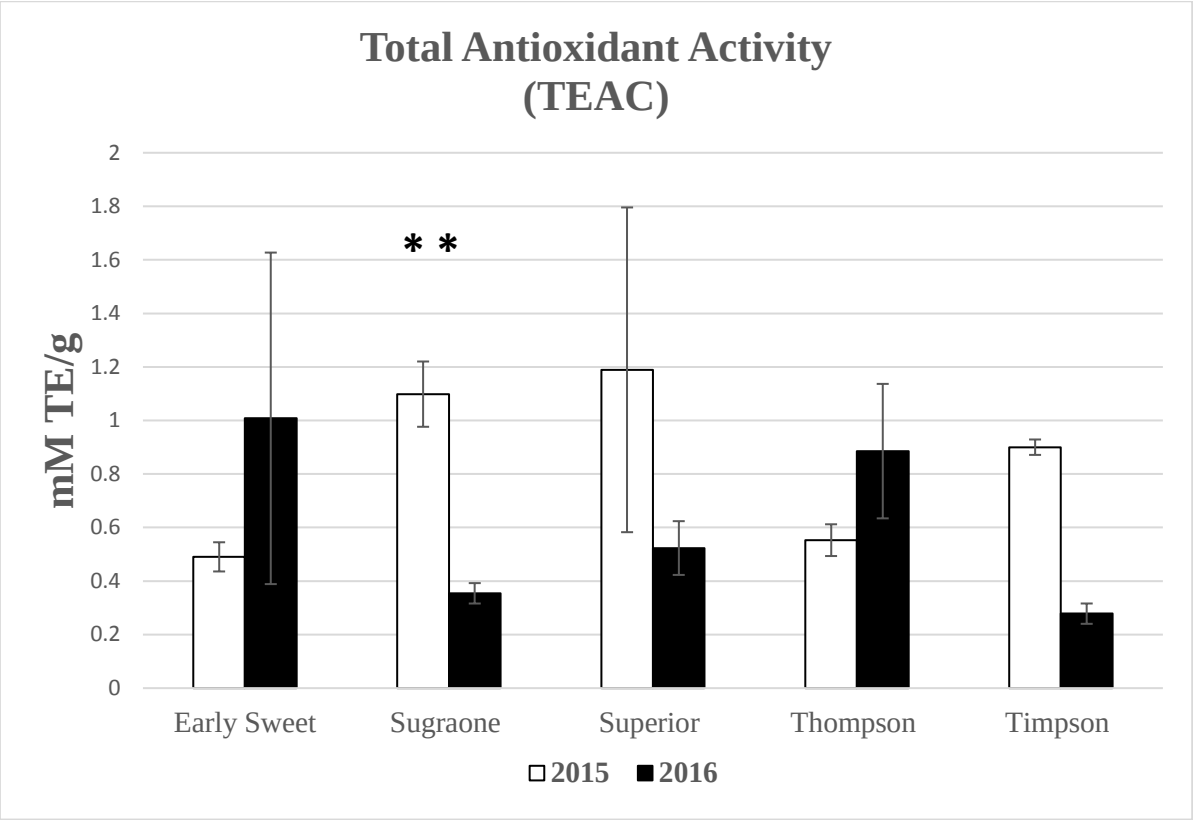


Figure 4.3 Level of total antioxidant activity by ABTS assay in white grape varieties; **-indicates significant interaction (variety and year) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.01$

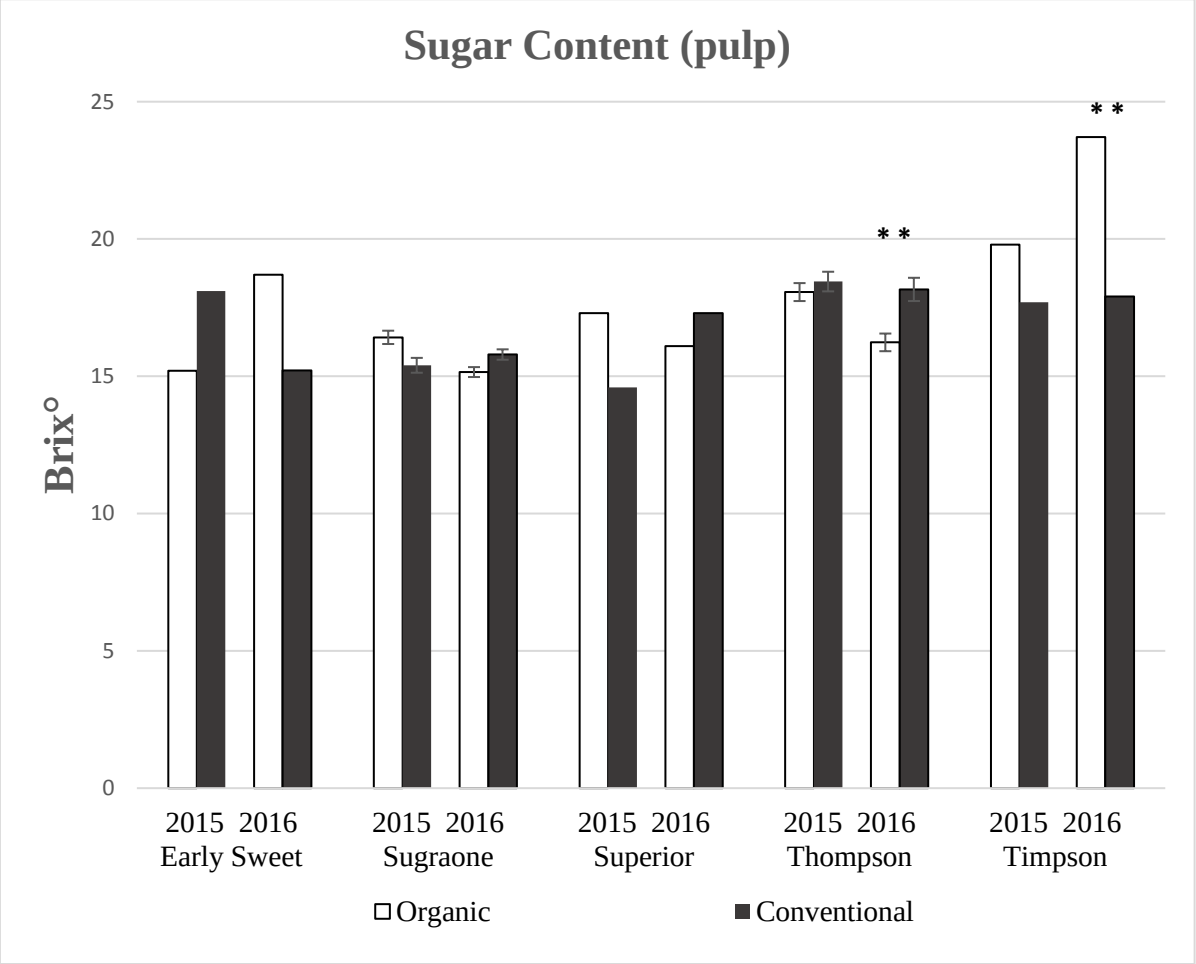


Figure 4.4 Level of Sugar Content (pulp) in white grape varieties over two years; **-indicates significant interaction (management system, variety and year) in each variety by 3-way ANOVA and Tukey post hoc test for $p<0.01$

Red varieties

A significant main effect of management system was detected for total anthocyanin content only, with concentrations found to be two times higher in conventional than organic grapes (Table 4.2).

A significant main effect of variety was only detected for total antioxidant activity (DPPH), with activity found to be significantly lower in the variety Flame than in the other 3 varieties which had similar levels of antioxidant activity (Table 4.2).

Significant main effects of year were detected for dry matter (higher in 2016), sugar content in juice (higher in 2016) and total antioxidant activity (DPPH, higher in 2016; TEAC, higher in 2015) (Table 4.2).

Interaction between factors

A significant 2-way interaction between variety and year and a weak 3-way interaction were detected for total antioxidant activity (DPPH) (Table 4.2). Compared to 2015, a significantly higher level of total antioxidant activity (DPPH) were detected in 2016, for the varieties Alison, Crimson and Flame, but not for Sweet Celebration (Figure 4.5).

A significant 3-way interaction was detected only for total antioxidant activity (DPPH) (Table 4.2). A significant effect of production system was only observed for the variety Sweet Celebration variety in 2016, when conventionally grown fruit had higher antioxidant activity than organic fruit (Figure 4.6). However, it should be pointed out that for certain varieties and year only one or a very small number of sample pairs (organic vs conventional) were available, so this data should be reviewed with care.

Table 4.2 Effect of, and interaction between, management, variety and year for the dry matter content (DM), sugar content (SC) of pulp/juice, total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /ABTS assays and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) of red grape varieties from UK supermarkets in 2015-2016 summer (s) and winter (w) seasons (3-way ANOVA)

	DM %	**SC pulp (Brix°)**	**SC juice (Brix°)**	**TPC mg GAE/ kg**	**TAA (DPPH) mM TE/g**	**TAA (TEAC) mM TE/g**	**TAC mg cyan/ kg**	**TAC mg mal/ kg**
Year (yr)								
2015 ***(n=16)***	19.3±0.5	17.6±0.4	18±0.4	1885.8±125.6	11.3±0.6	0.9±0.1	96.9±19.1	102.3±20.1
2016 ***(n=16)***	21.7±0.6	18.9±0.5	19.2±0.4	2105.1±93.1	16±0.4	0.6±0	118.3±27.5	124.9±29
Management (man)								
ORG ***(n=16)***	20.4±0.6	18.4±0.5	18.7±0.4	2018.1±112.6	13.7±0.8	0.8±0.1	70.2±10.2	74.1±10.8
CONV ***(n=16)***	20.6±0.6	18.1±0.5	18.5±0.4	1972.9±115.4	13.6±0.8	0.7±0.1	145±29.1	153.1±30.7
Variety (var)								
Allison (s) ***(n=8)***	20.8±0.6	18.1±0.5	18.9±0.4	1952.8±153.4	14.3±1**a**	0.9±0.1	106.8±29.6	112.7±31.2
Crimson (s) ***(n=16)***	20.3±0.7	18±0.6	18.3±0.5	1957.5±114.1	14.3±0.7**a**	0.8±0.1	98±18.9	103.4±19.9
Flame (w) ***(n=4)***	20.2±0.8	18.3±0.2	18.5±0.3	2169.6±298.8	9.1±2**b**	0.4±0.1	89.1±40.6	94±42.9
Sweet Celebration (w) ***(n=4)***	21±1.4	19.3±1.1	19.4±1.2	2058.7±217.2	13.9±1.2**a**	0.9±0.2	166.4±91.9	175.6±97
ANOVA (P values)								
Man	NS	NS	NS	NS	NS	NS	**0.014**	**0.014**
Var	NS	NS	NS	NS	**<.0001**	T	NS	NS
Yr	**0.0013**	T	**0.0151**	NS	**<.0001**	**0.008**	NS	NS
2-way Interactions								
man:var	**0.0317**	NS	NS	NS	NS	NS	NS	NS
man:yr	NS	NS	NS	T	NS	T	NS	NS
var:yr	NS	NS	NS	NS	**0.0037**	NS	T	T
3-way Interaction								
man:var:yr	NS	NS	NS	NS	**0.0417**	NS	NS	NS

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

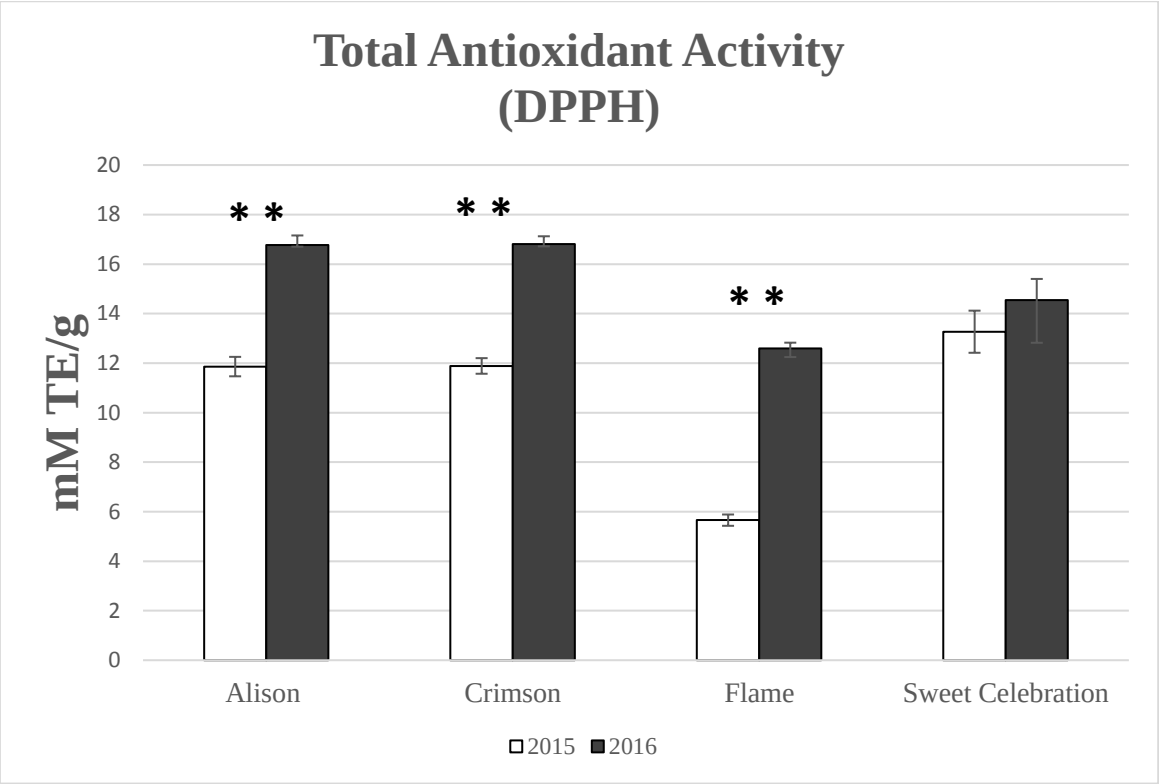


Figure 4.5 Level of total antioxidant activity by DPPH assay in red grape varieties; **-indicates significant interaction (variety and year) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.01$;

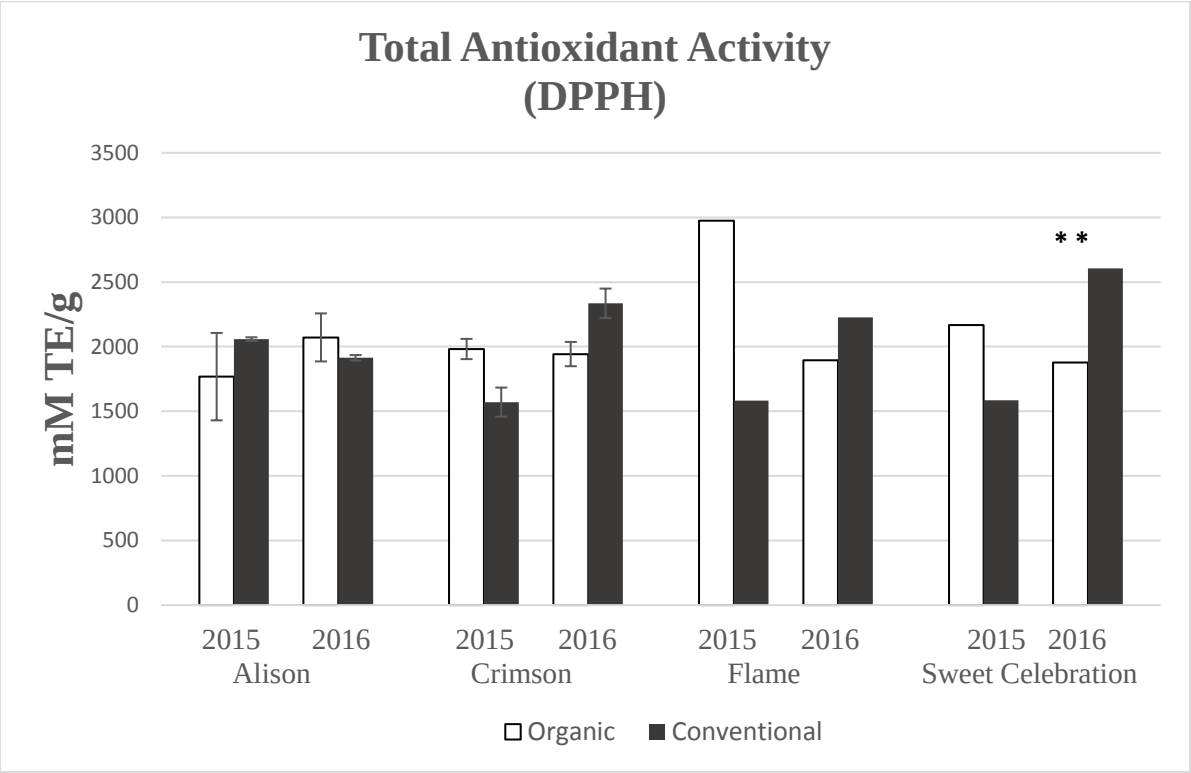


Figure 4.6 Level of total antioxidant activity by DPPH assay in red grape varieties; **-indicates significant interaction (management system, variety and year) in each variety by 3-way ANOVA and Tukey post hoc test for $p<0.01$;

Black varieties

Significant main effects of management were detected for sugar content (juice) and total anthocyanin content, with concentrations of both found to be higher in organic grapes (Table 4.3).

Significant main effects of variety were detected for total antioxidant activity (DPPH) and anthocyanin content; both were significantly higher in Midnight Beauty than the other two varieties (Summer Royal and Autumn Royal) (Table 4.3).

Significant main effects of year were detected for total phenolic content and total antioxidant activity (TEAC); both were higher in 2015 than 2016 (Table 4.3).

Interaction between factors

Significant 2-way interactions between management and year were detected for total phenolic content and total antioxidant activity (TEAC) (Table 4.3). Both parameters were significantly higher in organically grown grapes than conventionally grown in 2015, while there was no significant difference between production systems in 2016 (Figure 4.7).

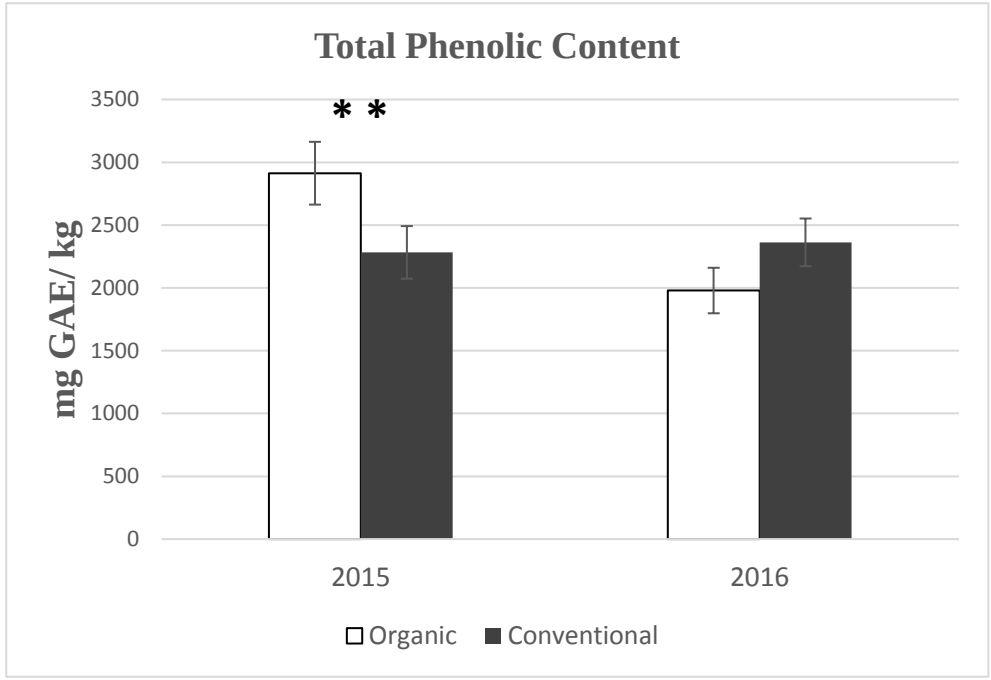
A significant 2-way interaction between management and variety was detected for total anthocyanin content (Table 4.3). For the variety Midnight Beauty organically grown grapes had significantly higher concentration of total anthocyanin content than in conventionally grown grapes (Figure 4.8). However, there were no significant differences in total anthocyanin content between organically and conventionally grown grapes for the varieties Autumn Royal and Summer Royal.

Table 4.3 Effect of, and interaction between, management, variety and year for the dry matter content (DM), sugar content (SC) of pulp/juice, total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /ABTS assays and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) of black grape varieties from UK supermarkets in 2015-2016 summer (s) and winter (w) seasons (3-way ANOVA)

	DM %	**SC pulp (Brix°)**	**SC juice (Brix°)**	**TPC mg GAE/ kg**	**TAA (DPPH) mM TE/g**	**TAA (TEAC) mM TE/g**	**TAC mg cyan/ kg**	**TAC mg mal/ kg**
Year (yr)								
2015 ***(n=14)***	18.7±0.7	17.3±0.5	17.7±0.5	2598±179.4	16.3±1.1	2.5±0.3	564.8±84.8	596.1±89.5
2016 ***(n=14)***	18.2±0.8	16.9±0.5	17.1±0.5	2170.9±136.8	15.1±0.6	0.6±0.1	667.5±75.7	704.5±79.9
Management (man)								
ORG ***(n=14)***	19±0.8	17.7±0.4	18.3±0.5	2446.1±196.9	16.2±0.9	1.7±0.4	713.6±91.8	753.2±96.9
CONV ***(n=14)***	17.9±0.6	16.4±0.5	16.5±0.4	2322.7±136.3	15.2±0.8	1.3±0.2	518.6±58.6	547.4±61.9
Variety (var)								
Autumn Royal (s) ***(n=4)***	17.3±0.5	16.2±0.4	16.5±0.2	2010±265.4	13.8±1**b**	1±0.2	239.5±91.2**c**	252.8±96.2**c**
Midnight Beauty (s) ***(n=20)***	19±0.6	17.4±0.4	17.8±0.4	2494.6±146.9	16.8±0.7**a**	1.8±0.3	721.1±55.1**a**	761±58.2**a**
Summer Royal (s) ***(n=4)***	16.8±1.4	16.2±0.9	16.4±0.7	2208±217.7	12±1.1**b**	1±0.4	468.1±157.4**b**	494±166.2**b**
ANOVA (P values)								
Man	NS	T	**0.0184**	NS	NS	NS	**0.0202**	**0.0202**
Var	NS	NS	NS	NS	**0.0147**	T	**0.0013**	**0.0013**
Yr	NS	NS	NS	**0.0338**	NS	**<.0001**	NS	NS
2-way Interactions								
man:var	NS	NS	NS	NS	NS	NS	**0.0434**	**0.0434**
man:yr	NS	NS	NS	**0.0214**	NS	**0.0338**	NS	NS
var:yr	NS	NS	NS	NS	NS	T	NS	NS
3-way Interaction								
man:var:yr	NS	NS	NS	NS	NS	NS	NS	NS

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

A)



B)

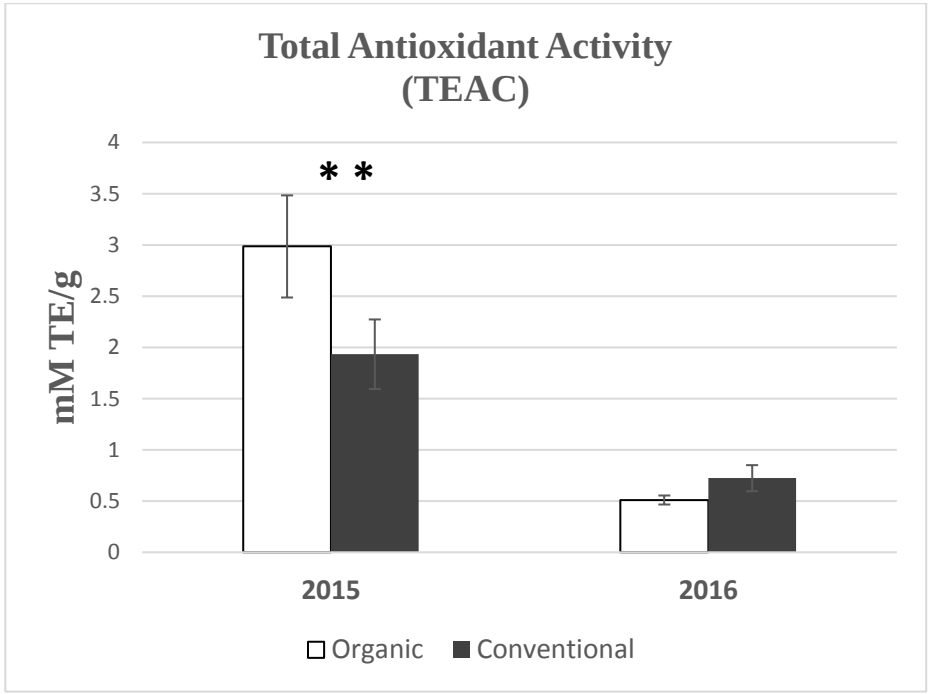


Figure 4.7 A) Level of total phenolic content; B) Level of total antioxidant activity by ABTS assay in black varieties;

**-indicates significant interaction (management system and year) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

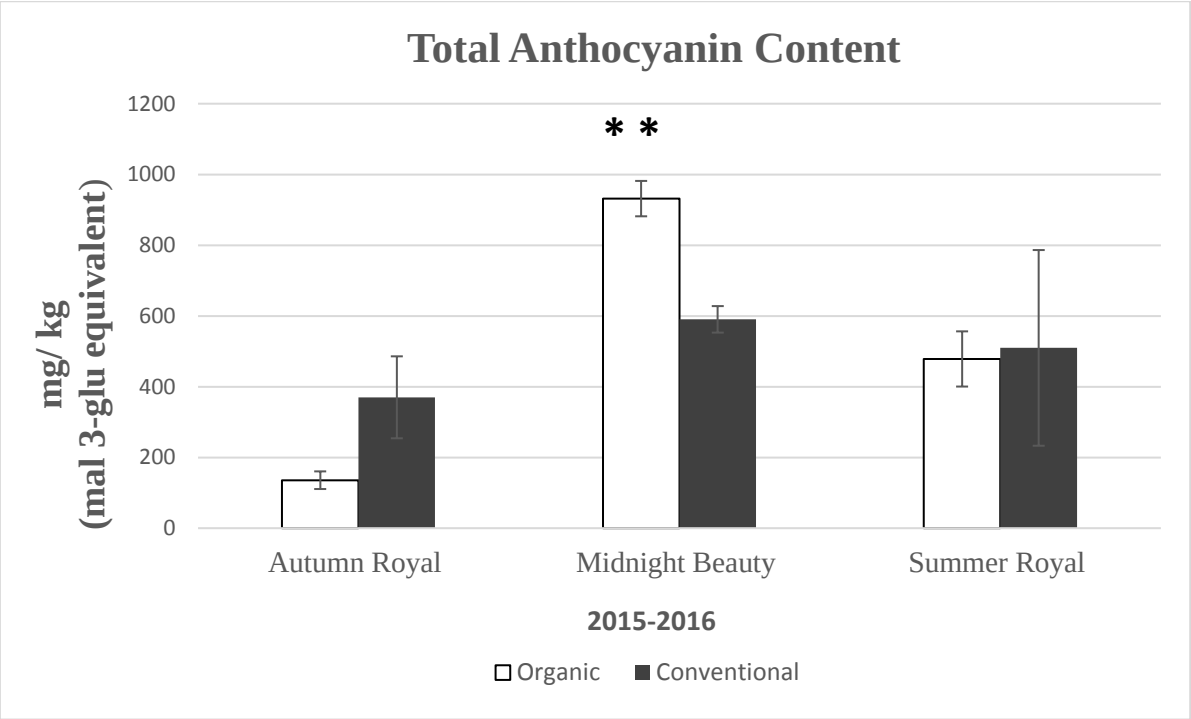


Figure 4.8 Level of total anthocyanin content in black grape varieties;
**-indicates significant interaction (management system and variety) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$

Anthocyanins profiles in red and black grape varieties (2015-2016)

HPLC analyses of anthocyanin profiles were only carried out for selected red and black grape varieties (Allison, Crimson and Midnight Beauty) and for 2 sampling dates (one in the summer and on in the winter season) in each of the 2 years.

Significant main effects of management system were detected for 2 individual anthocyanins (cyanidin 3-O-glucoside and peonidin 3-O-glucoside), with both being detected in significantly higher concentrations in conventional grapes (Table 4.4).

Significant main effects of variety were detected for all individual anthocyanins. There were large differences in anthocyanin profiles between the red (Allison and Crimson) and the black grape varieties (Midnight Beauty). Midnight Beauty contained significantly higher concentrations of 4 of the 7 anthocyanin compounds (Delphinidin 3-O-glucoside, Petunidin 3-O-glucoside, Malvidin 3-O-glucoside, Malvidin 3-O-p-coumaroylglucoside) than the 2 red varieties. In contrast Midnight Beauty contained lower concentrations of 2 of the 7 anthocyanin compounds (cyanidin 3-O-glucoside, peonidin 3-O-glucoside) than the two red varieties, but only the difference between Midnight Beauty and Allison was significant (Table 4.4). There were also significant differences in the anthocyanin profiles between the two red varieties (Table 4.4). Fruit of the variety Allison had significantly higher concentrations of cyanidin 3-O-glucoside and peonidin 3-O-p-coumaroylglucoside than fruit of the variety Crimson (Table 4.4).

Interaction between factors:

Significant 2-way interactions between management and variety were detected for 3 (cyanidin 3-O-glucoside; peonidin 3-O-glucoside, malvidin 3-O-glucoside) of the 7 individual anthocyanins (Table 4.4).

The concentration of cyanidin 3-O-glucoside was higher in conventionally grown red grape varieties (Allison and Crimson) and higher in the organically grown black variety (Midnight Beauty). However, a significant effect of management systems was detected only for the variety Allison (red variety) (Figure 4.9 (A)).

The concentration of peonidin 3-O-glucoside was higher in conventionally grown fruit of the red varieties (Allison and Crimson) and higher in organically grown fruit of the black variety (Midnight Beauty), but differences between production systems were only significant for the red varieties (Figure 4.9 (B)).

The concentration of malvidin 3-O-glucoside was higher in conventionally grown fruit of the red varieties (Allison and Crimson) and higher in organically grown fruit of the black variety

(Midnight Beauty), but a significant difference was only detected for the black variety (Figure 4.9 (C)).

A significant interaction between management and year was only detected for malvidin 3-O-p-coumaoylglucoside (Table 4.4). Concentration of malvidin 3-O-p-coumaoylglucoside were two times higher in conventional grapes in 2015, but two times higher in organic grapes in 2016 (Figure 4.10).

Significant 2-way interactions between variety and year were detected for 3 (cyanidin 3-O-glucoside, malvidin 3-O-glucoside, peonidin 3-O-p-coumaroylglucoside) of the 7 individual anthocyanins (Table 4.4).

Concentrations of cyanidin 3-O-glucoside were higher in 2015 for all three varieties (Allison, Crimson and Midnight Beauty), however a significant difference between years was detected only for the red variety Allison (Figure 4.11 (A)).

A significantly higher concentration of malvidin 3-O-glucoside in 2016 compared to 2015 was detected only for the black variety (Midnight Beauty). For the red varieties (Allison and Crimson) concentrations of malvidin 3-O-glucoside were numerically higher in 2015 than 2016, but the difference was not statistically significant (Figure 4.11 (B)).

Peonidin 3-O-p-coumaroylglucoside concentrations were higher in 2015 for all three varieties, however the difference between years was significant only for the red variety Allison (Figure 4.11 C).

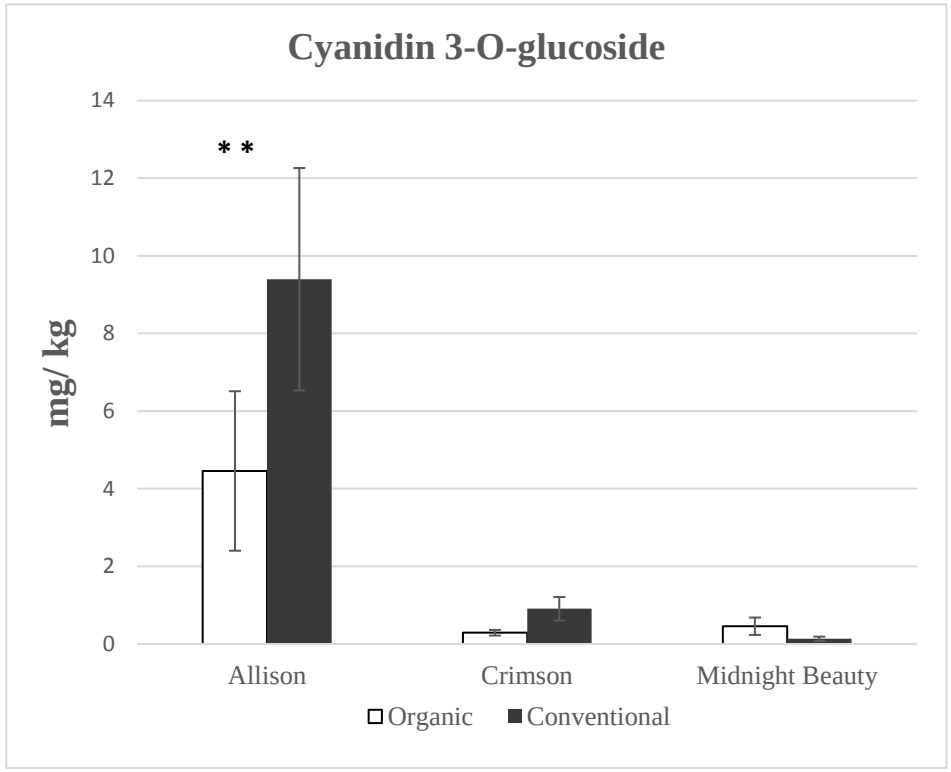
A significant 3-way interaction was only detected for malvidin 3-O-p-coumarylglucoside (Table 4.4). A significant difference was observed for the black variety Midnight Beauty; higher concentrations of malvidin 3-O-p-coumaroylglucoside were detected in organically produce grapes compared to conventional ones in both years (2015 and 2016) (Figure 4.12). However, it should be pointed out that for certain varieties and year only one or a very small number of sample pairs (organic vs conventional) were available, so data should be reviewed with care.

Table 4.4 Effect of, and interaction between, management system and year for individual anthocyanins (*Delphinidin 3-O-glucoside, Cyanidin 3-O-glucoside, Petunidin 3-O-glucoside, Peonidin 3-O-glucoside, Malvidin 3-O-glucoside, Peonidin 3-O-p-coumaroylglucoside, Malvidin 3-O-p-coumaroylglucoside)* of red and black varieties in 2015-2016 summer (s) and winter (w) seasons (3-way ANOVA)

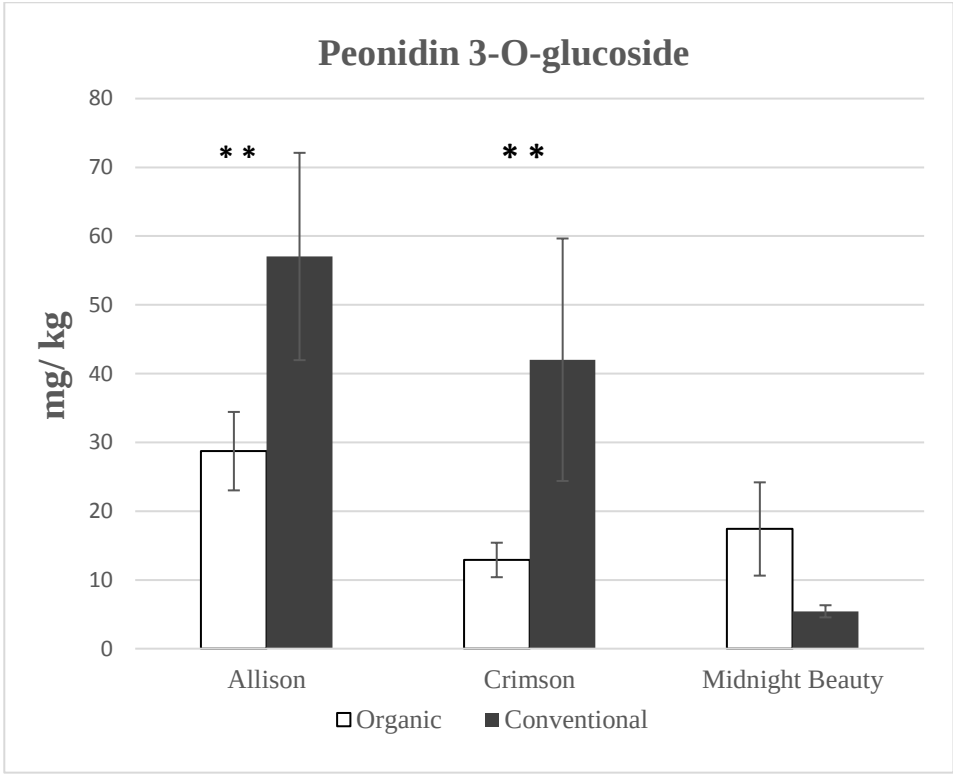
	Delphinidin 3-O-glucoside	**Cyanidin 3-O-glucoside**	**Petunidin 3-O-glucoside**	**Peonidin 3-O-glucoside**	**Malvidin 3-O-glucoside**	**Peonidin 3-O-p-coumaroylglucoside**	**Malvidin 3-O-p-coumaroylglucoside**
Year (yr)							
2015 ***(n=12)***	1.5±0.6	4±1.6	3.1±1.2	37±9.6	33.3±12.9	4±1	19.6±10.4
2016 ***(n=12)***	1.4±0.7	1.3±0.5	3.3±1.5	17.6±2.4	49±20.7	2.1±0.6	20.2±9.3
Management (man)							
ORG ***(n=12)***	1.6±0.8	1.7±0.9	3.6±1.7	19.7±3.4	45.7±20.3	3.2±0.9	19.5±9.5
CONV ***(n=12)***	1.3±0.5	3.5±1.5	2.8±1.1	34.8±9.6	36.6±13.9	2.9±0.8	20.2±10.2
Variety (var)							
Allison (w) (red) ***(n=8)***	0.6±0.3 **b**	6.9±1.9 **a**	0.8±0.4 **b**	42.9±9.2 **a**	5.6±2.2 **b**	4.7±1 **a**	0.5±0.1 **b**
Crimson (s) (red) ***(n=8)***	0.04±0.02 **b**	0.6±0.2 **b**	0.1±0.01 **b**	27.5±9.9 **ab**	2.8±1.1 **b**	0.5±0.3 **b**	0.1±0.1 **b**
Midnight Beauty (s) (black) ***(n=8)***	3.8±0.8 **a**	0.3±0.1 **b**	8.7±1.6 **a**	11.4±3.9 **b**	115±16 **a**	3.9±0.9 **a**	59±11.4 **a**
ANOVA (P values)							
Man	NS	**0.0158**	NS	**0.0223**	*T*	NS	NS
Var	**0.0009**	**<.0001**	**0.0001**	**0.0039**	**<.0001**	**0.0032**	**<.0001**
Yr	NS	**0.0013**	NS	**0.0064**	**0.028**	**0.03**	NS
2-way Interaction							
man:var	NS	**0.0117**	NS	**0.0213**	**0.0364**	NS	NS
man:yr	NS	NS	NS	T	NS	NS	**0.0069**
var:yr	NS	**0.0006**	NS	NS	**0.0193**	T	NS
3-way Interaction							
man:var:yr	NS	NS	NS	NS	NS	NS	**0.0021**

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*; Mean values are expressed as mg/ kg

A)


Cyanidin 3-O-glucoside
mg/ kg
14
12
10
8
6
4
2
0
* *
Allison
Crimson
Midnight Beauty
Organic
Conventional

B)


Peonidin 3-O-glucoside
mg/ kg
80
70
60
50
40
30
20
10
0
* *
* *
Allison
Crimson
Midnight Beauty
Organic
Conventional

C)

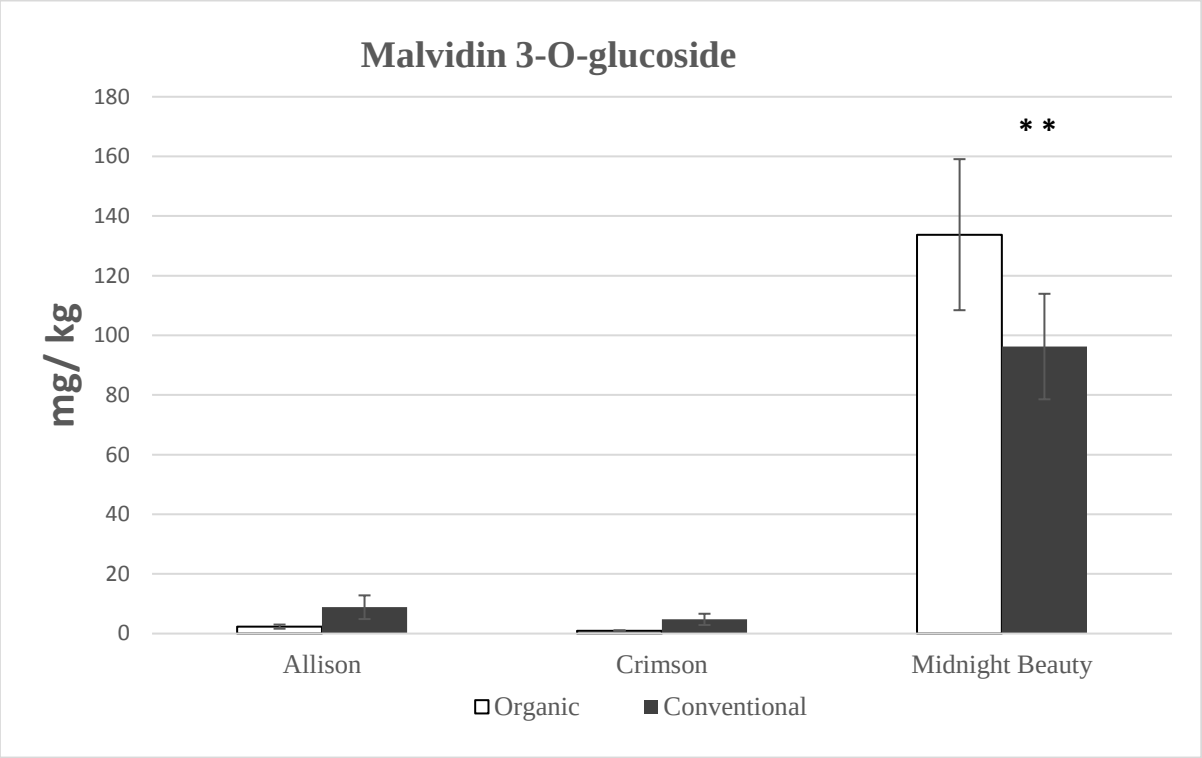


Figure 4.9 A) Level of cyaniding 3-O-glucoside; B) Level of peonidin 3-O-glucoside; C) Level of malvidin 3-O-glucoside; **-indicates significant interaction (management system and variety) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

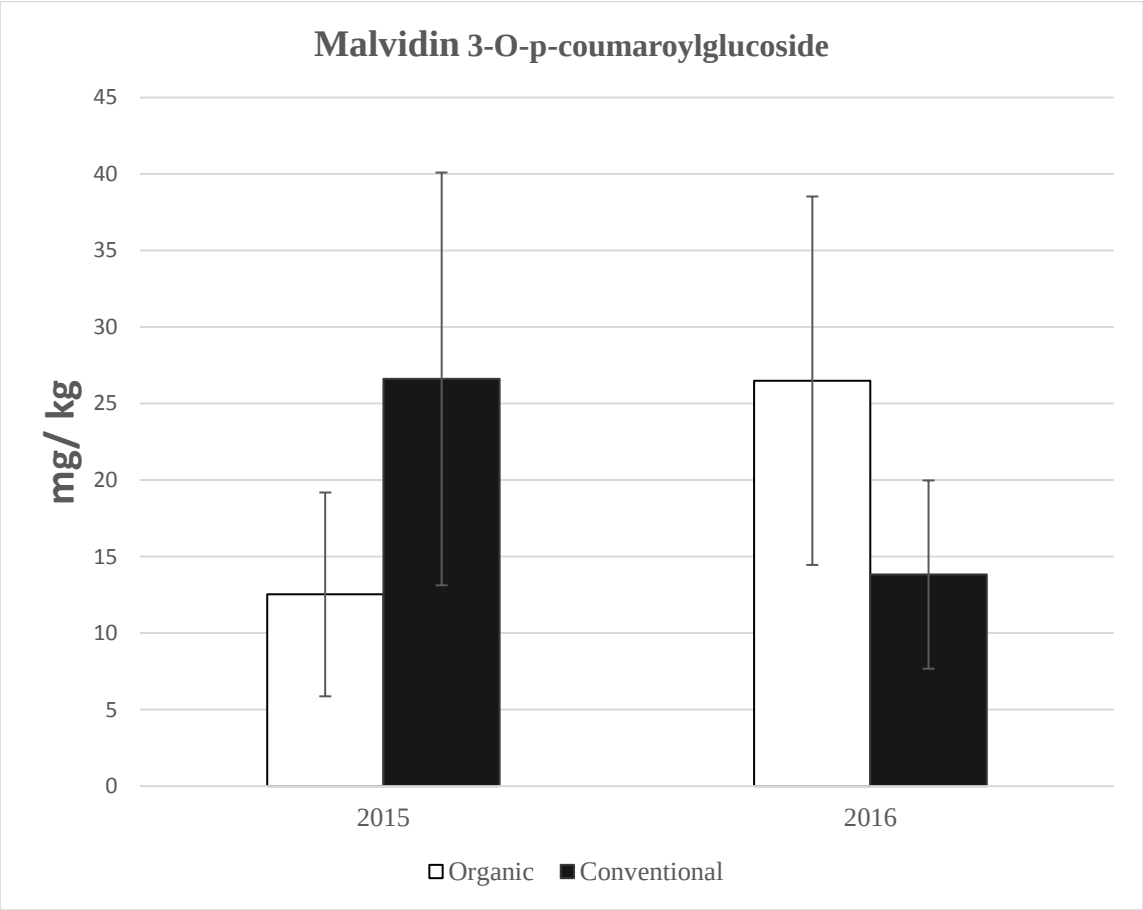
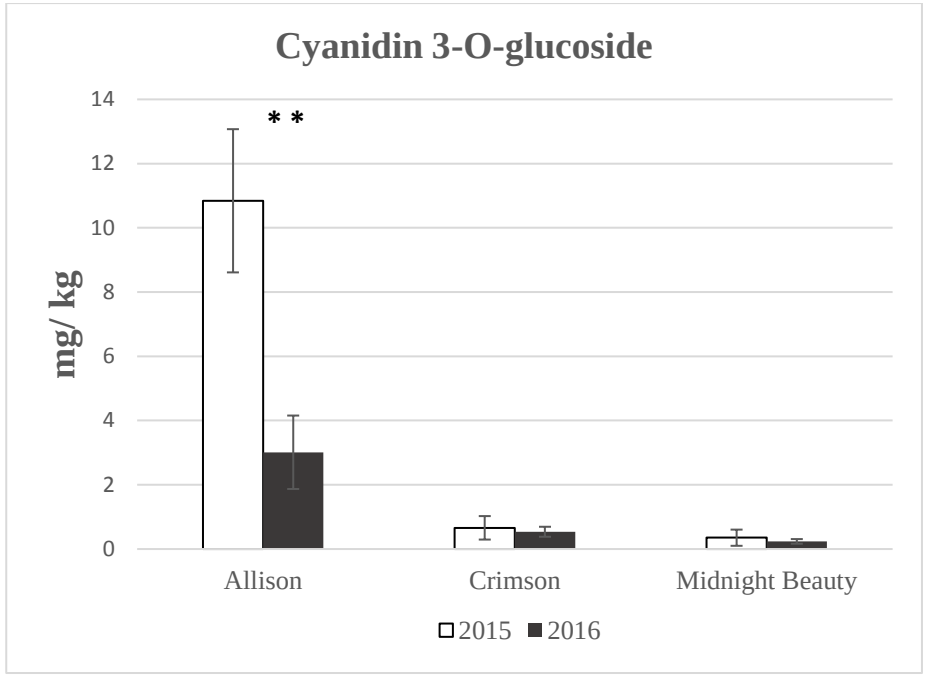
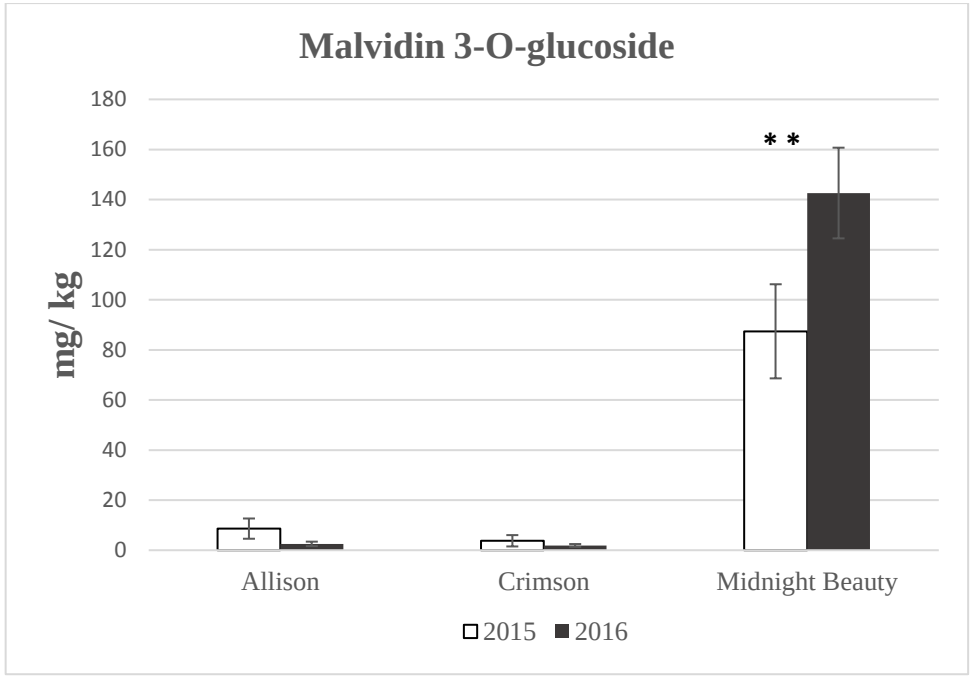


Figure 4.10 Level of malvidin 3-O-p-coumaroylglucoside; **-indicates significant interaction (management system and production year) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

A)


Cyanidin 3-O-glucoside
mg/ kg
14
12
10
8
6
4
2
0
* *
Allison
Crimson
Midnight Beauty
2015
2016

B)


Malvidin 3-O-glucoside
mg/ kg
180
160
140
120
100
80
60
40
20
0
* *
Allison
Crimson
Midnight Beauty
2015
2016

C)

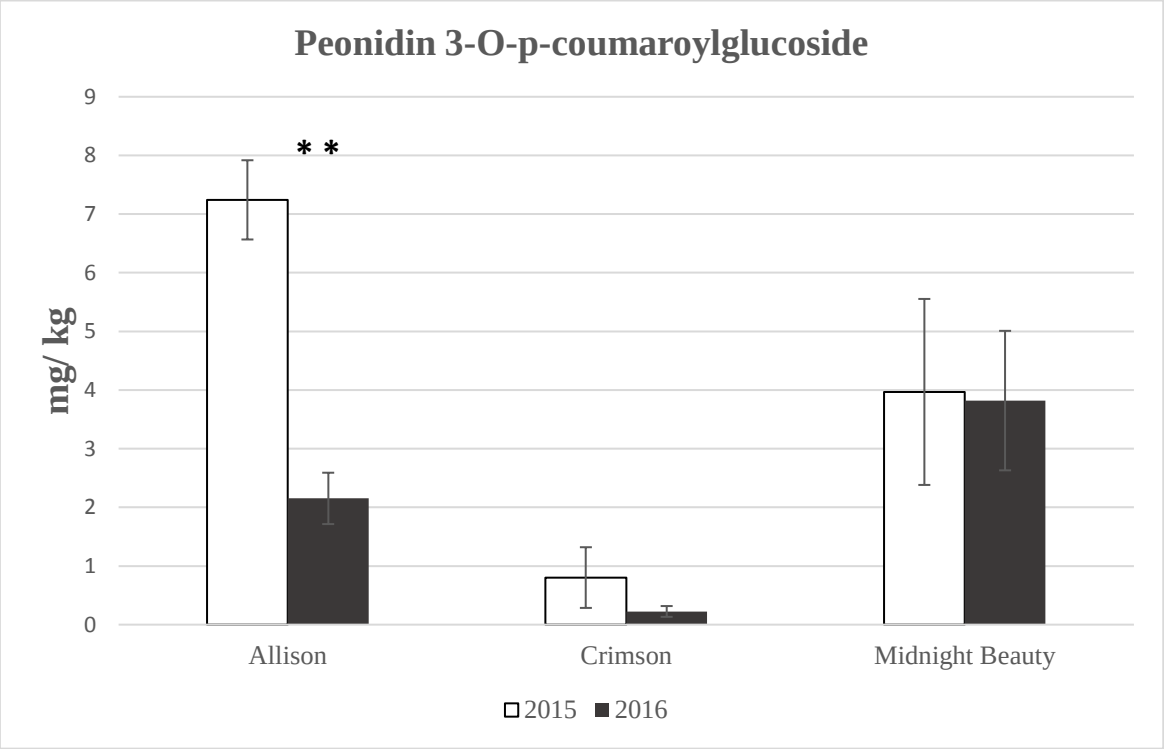


Figure 4.11 A) Level of cyaniding 3-O-glucoside; B) Level of malvidin 3-O-glucoside; C) Level of peonidin 3-O-p-coumaroylglucoside; **-indicates significant interaction (variety and production year) in each variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

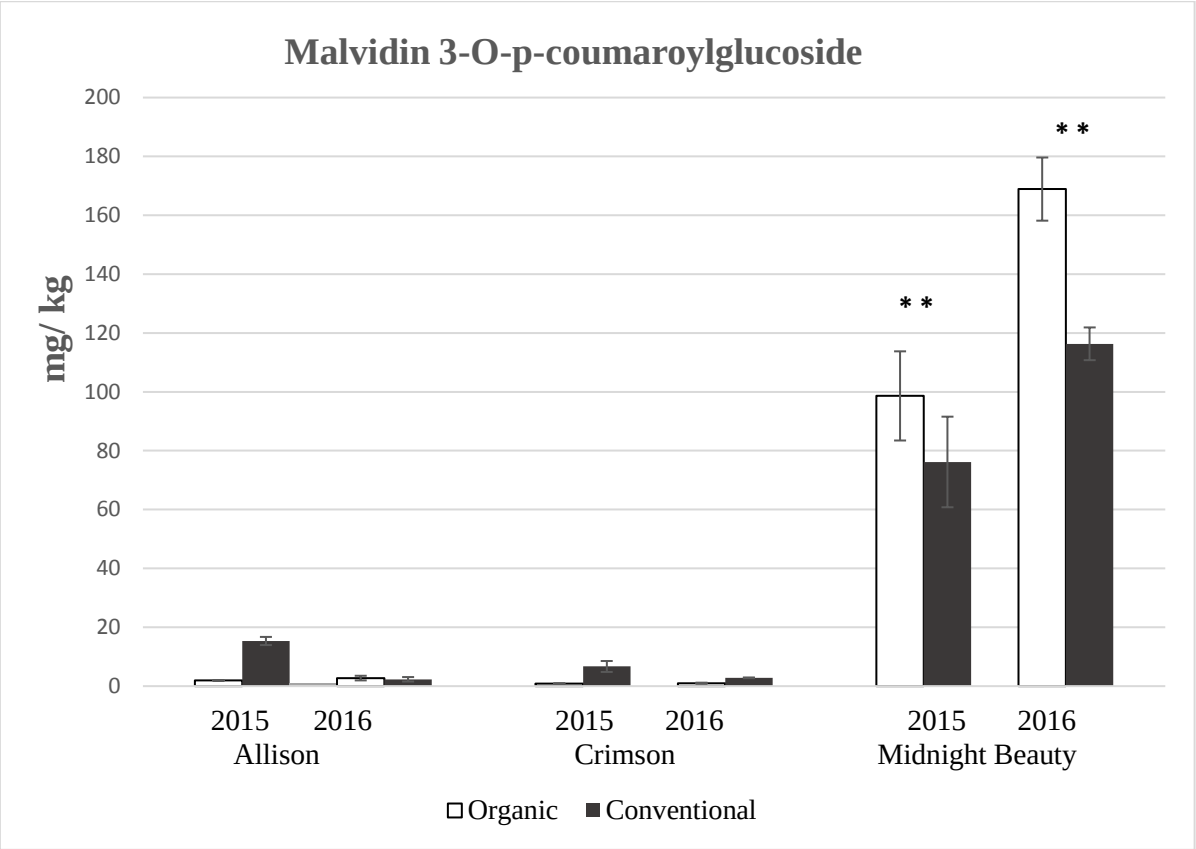


Figure 4.12 Level of malvidin 3-O-p-coumaroylglucoside; **-indicates significant interaction (management system, variety and production year) in each variety by 3-way ANOVA and Tukey post hoc test for $p<0.05$;

4.2 Discussion

Overall the retail survey, in which organic and conventional samples of the same grape varieties were compared, did not detect consistent composition differences between organically and conventionally produced grapes, but identified substantial effects of variety choice and to a lesser extent year/production season. It was not possible to get information on the exact production protocols and pedo-climatic conditions on the farms that were used to produce the grapes purchased in supermarkets. It was therefore not possible to identify potential confounding effects of specific agronomic/management (e.g. fertilisation, crop protection, tillage, irrigation, ripening stage at harvest), soil and climatic drivers on grape composition here.

Sugar concentrations in both organic and conventional grape samples used in this study were between 15.7 to 20.5 Brix°, which is consistent with sugar levels reported in previous studies (Meyer *et al.*, 1997; Soyer *et al.*, 2003; Jensen *et al.*, 2008). The finding of significant differences in sugar content between grape cultivars, is also consistent with previously listed studies.

The finding that the sugar content of one white grape variety (Timpson) was significantly higher in organically produced grapes, contradicts a wide range of other comparative studies which consistently found similar sugar contents in both organically and conventionally grown grape varieties (Basker, 1992; Malusa *et al.*, 2002; Burin *et al.*, 2010; Corrales *et al.*, 2010; Granato *et al.*, 2015; Toaldo *et al.*, 2015).

Similar to the results of the meta-analysis (Chapter 3) the retail survey detected no consistent effects of production system (organic versus conventional) on total antioxidant activity in grapes. However, with white grapes one variety had higher antioxidant activity in conventionally grown and with black grapes one variety had higher antioxidant activity in organically grown samples.

Phenolic concentrations in grape samples varied substantially (between 998 and 2494 mg GAE/kg) depending on the variety of grape. Concentrations were higher in black and red grape varieties than white grape varieties, which is consistent with previous studies by Katalinić *et al.* (2010), Dani *et al.* (2007b) and Toaldo *et al.* (2015). This and the finding of significant differences in phenolic concentrations between white varieties included in the survey, indicates that variety choice is a major confounding factor in studies focused on quantifying effects of production systems (e.g. organic vs conventional) on grape composition/quality. Since the grapes collected for the retail survey originated from different

countries and continents, it can be assumed that apart from variety choice, geographical location will have affected the concentration of total phenolic content, as reported previously (González-Neves *et al.*, 2004; Orak, 2007; Margraf *et al.*, 2016). For example, Margraf *et al.* (2016), reported table grape juice samples from different parts of Brazil had significantly different concentrations of total phenolic content, with samples from Parana having a 70% higher phenolic content (2712 mg GAE/l) than samples from Santa Catarina (1649) mg GAE/l). Similarly, concentrations of total phenolic in Cabernet-Sauvignon grapes from Uruguay (1509 mg/l) were substantially lower than in grapes of the same variety grown in Turkey (2348 mg/l) (González-Neves *et al.* (2004); Orak (2007)).
The interaction between year and production systems in black grapes (where phenolic content in organic grapes was significantly higher in 2015, but numerically lower in 2016 compared to conventional grapes) also suggests that there can be substantial confounding effects of environmental conditions in studies comparing the effects of production systems on grape composition/quality.

Total antioxidant activity (DPPH) also varied greatly depending on the grape variety; mean values were between 5.6-11.3 mM TE/g for white grape varieties and 9.1-14.3 mM TE/g for red/black grape varieties. However, there was no main effect of production system. These results are broadly consistent with total antioxidant activity levels reported in previous studies (Pastrana-Bonilla *et al.*, 2003; Burin *et al.*, 2010; Bunea *et al.*, 2012; Tassoni *et al.*, 2013; Toaldo *et al.*, 2015) and confirm previous studies which reported substantial differences in antioxidant activity between grape varieties but not management systems (Bunea *et al.*, 2012; Margraf *et al.*, 2016).
A significant main effect of year was also detected for many of the grape varieties; grape samples from 2015 had higher total antioxidant activity (TEAC) compared to 2016. However, for white (but not red or black) varieties, a significant interaction between variety and year was also detected, with three varieties (Sugraone, Superior and Timpson) showing higher antioxidant activity (TEAC) in 2015, while for other two varieties (Early Sweet and Thompson) activity was higher in 2016. Since the Sugraone, Superior, Timpson varieties were collected in the summer season (when grapes are from Mediterranean countries) and the Early Sweet, Thompson varieties were collected in the winter season (when grapes are from South Africa), this interaction may reflect contrasting climatic differences between years in these two regions (Conradie *et al.*, 2002; van Leeuwen *et al.*, 2004). The finding that some varieties had higher values in one year and some varieties in the other survey year is consistent with findings in several previous studies which reported that grape varieties show

contrasting responses to differences in climatic conditions between years (González-Neves *et al.*, 2004; Orak, 2007). Interactions between climate, cultivar and location with respect to vine development and berry composition were also identified in long term trials carried out by van Leeuwen *et al.* (2004) and Conradie *et al.* (2002).

There were no main effects of management systems for total antioxidant activity (DPPH) in any variety, but a significant interaction between management system and variety was detected when the white varieties were compared. One variety (Superior) showed significantly higher antioxidant activity under conventional compared to organic production methods, while antioxidant activity in the other 4 white varieties was not affected by production system. This result is consistent with those reported by (a) Dani *et al.* (2007a) who reported higher antioxidant activity in conventionally produced grapes of the variety Niagara than organic grapes of the same variety and (b) da Silva Haas *et al.* (2016), who reported higher antioxidant activity in conventional compared to organic grape juice. A study by Mulero *et al.* (2010) recorded significantly higher antioxidant activity in organically grown fruit during the early stage of fruit ripening, but similar levels in organic and conventional fruit at harvest time. The study by Mulero et al (2010) therefore also indicates that, apart from variety and pedo-climatic background conditions, the ripening stage at harvest may potentially be an additional confounding factor in studies comparing the quality of grape/products from organic and conventional production systems.

For black grape varieties, there was an interaction between year and production systems which showed a similar trend to the results found for total phenolic content, where organic black grapes had significantly higher antioxidant activity (TEAC) in 2015 but similar activity in 2016, when compared to conventional grapes. This again indicates a strong confounding influence of climatic conditions on the expression of grape composition differences associated with the use of contrasting production systems (organic vs conventional).

Phenolics are known to be a major group of phytochemicals contributing to total antioxidant activity and close positive correlation between total phenolic content and total antioxidant activity in black varieties have been reported by several previous studies (Orak, 2007; Mulero *et al.*, 2010; Toaldo *et al.*, 2015; Margraf *et al.*, 2016). For example, Margraf et al (2016) reported significantly higher concentration of total phenolic content and total antioxidant activity (TEAC) in organic Bordo, Isabella and Concord red grape varieties. However, results are different to those obtained by Corrales *et al.* (2010), who found higher antioxidant activity in fruit skin extracts of conventionally grown fruit and a higher total phenolic content in extracts from organically grown fruit.

Total anthocyanin concentrations were, as expected, substantially (8-40 times depending on the variety) higher in red and black than white varieties. Different to the other composition parameters assessed, there were significant but contrasting main effects of managements system on the anthocyanin concentrations in red and black grape varieties.
For the red varieties, significantly higher anthocyanin concentrations were detected in samples from conventional production. These results contradict the results of the meta-analyses which found higher concentrations of anthocyanins in organic grapes (Chapter 3), but are consistent with results from studies by Vian *et al.* (2006) and Tassoni *et al.* (2013), that both reported significantly higher levels of anthocyanin in conventional compared to organically grown red/black grapes of the varieties Syrah and Pignoletto.
For black varieties there were significant main effects of both management system and variety and a two-way interaction between these factors. Different to red grapes anthocyanin concentrations were overall higher in organic compared to conventional grapes, but a significant difference was only detected for the variety Midnight Beauty, which had the highest anthocyanin concentrations of the 3 varieties assessed in the survey. These results are consistent with the results of the meta-analysis (Chapter 3) and the majority, but not all, comparative studies carried out previously (Mulero *et al.*, 2010; Rodrigues *et al.*, 2013; Toaldo *et al.*, 2015). For example, the total anthocyanin contents of red grape juices reported by Toaldo *et al.* (2015) were 1592.5 mg/L for organic and 420.01 mg/L for conventional juice samples. Several studies reported that harvest time affects the relative difference between organic and conventional crops with the difference becoming smaller over time (Dani *et al.*, 2007b; Mulero *et al.*, 2010).

Anthocyanin profiling of red and black grape varieties focused on a range of major individual anthocyanin compounds that were previously identified and investigated in grapes (Kammerer *et al.*, 2004; Vian *et al.*, 2006; Dani *et al.*, 2007a; Mulero *et al.*, 2010). Concentrations of these compounds are known to vary depending on variety, climate, location and the maturity stage of grapes (Vian *et al.*, 2006). The finding that anthocyanin profiles differed considerably between varieties, years and production systems in the study reported here was therefore not surprising.
The finding of significant main effects of management system is also broadly consistent with previous studies, although previous studies used different varieties. For example, similar to the study reported here, Toaldo *et al.* (2015) reported higher concentrations of peonidin 3-O-glucoside in conventional (10.9 mg/l) than in organic (2.5 mg/l) red grape juice. Also, similar to the study reported here, a study by Dani *et al.* (2007a) reported a higher concentration of

malvidin 3-O-glucoside in organically produced grape juice made from the variety Bordo However, different to the study reported here Vian *et al.* (2006), Toaldo *et al.* (2015) and Mulero *et al.* (2010) found higher concentrations of malvidin 3-O-glucoside only in conventionally grown grape varieties.

The significant interaction between production system and variety detected for 3 of the 7 individual anthocyanin compounds quantified in this study may at least partially explain the inconsistency between studies with respect to the effect of management system on specific anthocyanin compounds. All previous studies were carried out with different red/black varieties than those used in the study reported here. Apart from variety, year (and associated differences in climatic conditions) and management system (organic vs conventional), a range of specific agronomic practices (e.g. use or non-use of irrigation) may also confound the effect of organic versus conventional management practice. For example, Esteban *et al.* (2001) showed that irrigation has a significant effect on anthocyanin concentrations and that there are interactions between irrigation (with and without) and year; anthocyanin concentration were higher in non-irrigated grapes in the first year but higher in irrigated grapes in the next year. This and other studies have concluded that soil type, irrigation and year/season specific climatic conditions are major drivers for anthocyanin content and profiles in grapes (Adams, 2006; Conde *et al.*, 2007).

Overall this chapter suggests that the sugar content, phytochemical concentrations (phenolics and anthocyanins) and profiles and total antioxidant activity in grapes may be determined by very complex interactions between variety choice and pedo- climatic conditions, and to a lesser extent, management system (organic vs conventional), and non-production system-specific management parameters (e.g. irrigation, tillage). Limitations for this experiment, such as no control of grapes age, stage of ripening at harvest, detailed information about irrigation, tillage, fertilization, soil properties, and climatic changes, should be considered for future investigation.

CHAPTER 5. Farm survey; nutritional composition of common local grape varieties collected from organic and conventional vineyards in Crete in the 2014 and 2015 growing seasons

5.1 Results

Farm surveys were carried out in two successive years (2014 and 2015) to identify the effect of production systems (organic vs conventional) and variety choice (three local varieties, Kotsifali (red), Villana (white) and Vidiano (white), that are widely grown in Crete), and potential interactions between these two factors, on grape yield and a range of composition parameters including **(a)** the proportion of dry matter (DM), **(b)** sugar content (SC) in pulp and juice (BRIX°), **(c)** total antioxidant activity (TAA) estimated by DPPH and TEAC assays and **(d)** concentrations of nutritionally-relevant antioxidants (total phenolic and anthocyanins content (TPC and TAC). The survey was carried out in the Heraklion prefecture region of Crete, which is the main wine growing area on the island.

Since anthocyanin concentrations are known to be more than ten times higher in red grape varieties, results for total anthocyanin content from white and red grape varieties were analysed separately (Table 5.1).

Yields were similar for all three varieties in both years and there was no significant differences in yield between organic and conventional production systems (Table 5.1). Although year/production season had no significant main effect on yield, substantial differences in fruit composition were detected in grapes harvested in the two different years. When results/data from both years were analysed by 3-way ANOVA, significant main effects of year were detected for all composition parameters that were assessed (except for the total anthocyanin content in white varieties (Table 5.1). Both grape yield and concentrations of all composition parameters were higher in 2014 than in 2015 (Table 5.1).

Significant main effects of variety were detected for the total phenolic content and total antioxidant activity (DPPH and TEAC) (Table 5.1). The polyphenol concentration and total antioxidant activity determined by TEAC assay were significantly higher in the varieties Kotsifali and Vidiano than Villana, however there was no significant difference between Kotsifali and Vidiano (Table 5.1).

Significantly higher total antioxidant activity (DPPH) was found in grapes of the red variety (Kotsifali), compared to the 2 white varieties (Villana and Vidiano) (Table 5.1). The red variety also had more than 10 times higher anthocyanin concentrations than the 2 white varieties.

A significant main effect of management was only detected for total antioxidant activity (TEAC), with higher activity found in organic compared to conventional grapes (Table 5.1).

Interaction between factors

Very few significant interactions were detected for composition parameters and none for grape yield.

A significant 2-way interaction between management and variety was detected only for total antioxidant activity (TEAC) (Table 5.1). Although no significant difference was detected for the varieties Kotsifali and Villana, organically produced variety Vidiano had a significantly higher antioxidant activity (TEAC) than conventionally produced grapes (Figure 5.1).

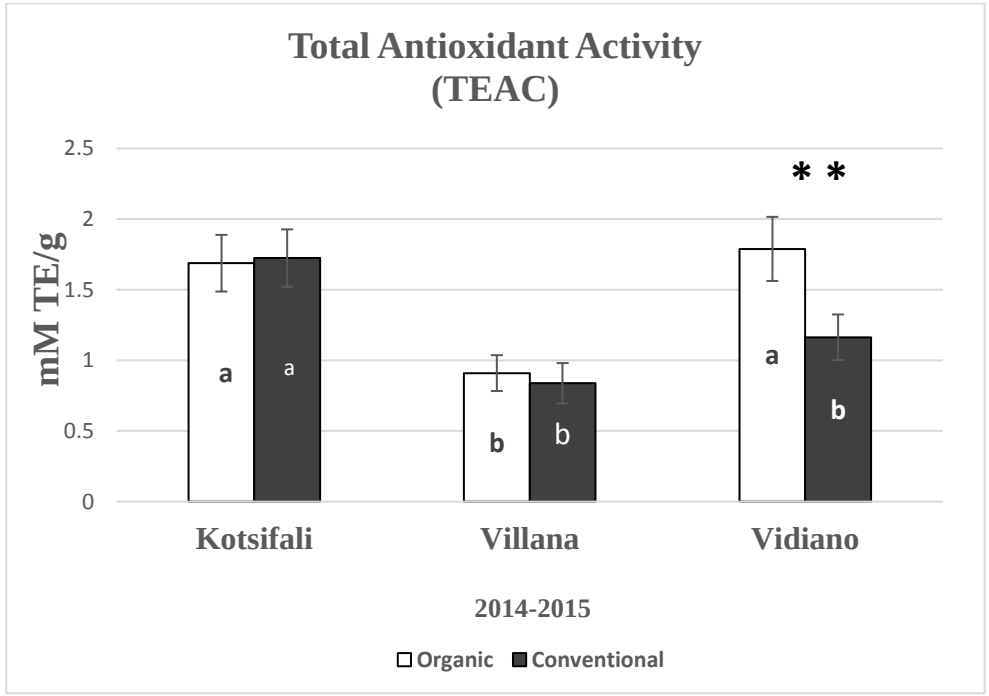
A significant 2-way interaction between variety and year was detected only for total antioxidant activity (DPPH) (Table 5.1). For all three varieties significantly higher antioxidant activity was detected in 2014 compared to 2015, but the relative difference in concentration differed between varieties (Figure 5.1).

Table 5.1 Effect of, and interaction between, management, variety and year for the yield, dry matter content (DM), sugar content (SC) of pulp/juice, total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /ABTS assays and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) of Kotsifali (red), Villana (white) and Vidiano (white) local grape varieties (3-way ANOVA)

	Yield t/ha	**DM %**	**SC pulp (Brix°)**	**SC juice (Brix°)**	**TPC mg GAE/ kg**	**TAA (DPPH) mM TE/g**	**TAA (TEAC) mM TE/g**	**TAC (red) mg cyan/ kg**	**TAC (red) mg mal/ kg**	**TAC (wh) mg cyan/ kg**	**TAC (wh) mg mal/ kg**
Year (yr)											
2014 ***(n=22)***	14.6 ± 1.1	23 ± 0.6	21.5 ± 0.7	21.5 ± 0.5	1797.5 ± 131.9	10 ± 0.8	1.8 ± 0.1	379.4 ± 41.4	400.4 ± 43.7	10 ± 2.9	21.1 ± 6.1
2015 ***(n=26)***	14.2 ± 1.1	21.3 ± 0.5	18.5 ± 0.4	19.1 ± 0.5	1265.9 ± 84	7 ± 0.5	1 ± 0.1	261.3 ± 20.5	275.8 ± 21.7	16.2 ± 2.9	17.1 ± 3
Management (man)											
ORG ***(n=24)***	14 ± 1.3	21.6 ± 0.6	19.2 ± 0.5	19.7 ± 0.6	1570.5 ± 122	8.3 ± 0.7	1.5 ± 0.1	300.7 ± 42.4	317.4 ± 44.8	11.2 ± 2.4	15.9 ± 3.6
CONV ***(n=24)***	14.8 ± 0.9	22.5 ± 0.6	20.5 ± 0.7	20.7 ± 0.5	1448.7 ± 117.2	8.4 ± 0.7	1.3 ± 0.1	326.9 ± 29.6	345 ± 31.2	15.4 ± 3.4	22 ± 5.5
Variety (var)											
Kotsifali ***(n=18)***	14.6 ± 1.3	23.5 ± 0.5	20.7 ± 0.6	21.6 ± 0.6	1698.7 ± 113.6**a**	12 ± 0.6**a**	1.7 ± 0.1**a**	313.8 ± 25.3	331.2 ± 26.7		
Villana ***(n=16)***	15.7 ± 7	21.1 ± 0.7	19.3 ± 0.8	19.2 ± 0.7	1079.1 ± 101.1**b**	6.3 ± 0.3**b**	0.9 ± 0.1**b**			8.3 ± 1.9	12.3 ± 3.6
Vidiano ***(n=14)***	12.5 ± 1	21.3 ± 0.6	19.4 ± 0.8	19.6 ± 0.6	1758.4 ± 167.5**a**	6 ± 0.3**b**	1.5 ± 0.2**a**			18.9 ± 3.4	26.5 ± 5.1
ANOVA (P values)											
Man	NS	NS	*T*	NS	NS	*T*	**0.0355**	NS	NS	NS	NS
Var	NS	*T*	NS	*T*	**0.0019**	**<.0001**	**<.0001**			NS	NS
Yr	NS	**0.0066**	**0.0002**	**0.0013**	**0.0001**	**<.0001**	**<.0001**	**0.0275**	**0.0275**	NS	NS
2-way interactions											
man:var	NS	NS	NS	NS	NS	NS	**0.0125**			NS	NS
man:yr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
var:yr	NS	*T*	NS	NS	NS	**<.0001**	NS			NS	NS
3-way interaction											
man:var:yr	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

A)



B)

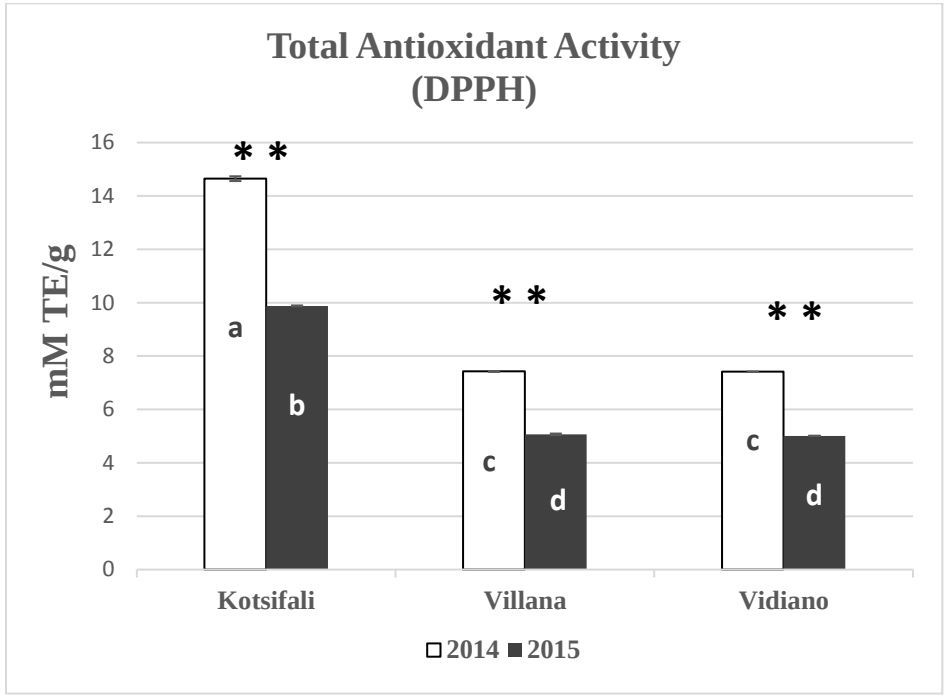


Figure 5.1 A) Level of total antioxidant activity by ABTS assay; B) Level of total antioxidant activity by DPPH assay; a, b, c, d correspond to significantly different mean values in different varieties and **-indicates significant interaction (management system and variety; variety and year) in one variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

Anthocyanins profiles in red grape variety Kotsifali (2014-2015)

No significant main effects of management system and year/production season were detected for individual anthocyanins quantified for Kotsifali grape samples by HPLC analysis (Table 5.2).

There were also no 2-way interactions for any of the individual anthocyanin components that could be detected.

Table 5.2 Effect of, and interaction between, management system and year for the individual anthocyanins (*Delphinidin 3-O-glucoside, Cyanidin 3-O-glucoside, Petunidin 3-O-glucoside, Peonidin 3-O-glucoside, Malvidin 3-O-glucoside, Peonidin 3-O-p-coumaroylglucoside, Malvidin 3-O-p-coumaroylglucoside)* of red variety Kotsifali (2-way ANOVA)

	Delphinidin 3-O-glucoside	**Cyanidin 3-O-glucoside**	**Petunidin 3-O-glucoside**	**Peonidin 3-O-glucoside**	**Malvidin 3-O-glucoside**	**Peonidin 3-O-p-coumaroylglucoside**	**Malvidin 3-O-p-coumaroylglucoside**
Management (man)							
ORG **(n=9)**	3.3 ± 1	11.9 ± 3.3	7.1 ± 1.4	76.0 ± 14.8	57.9 ± 6.4	3.6 ± 0.8	3.6 ± 1.6
CONV **(n=9)**	3.9 ± 1.1	10.1 ± 2.3	7.7 ± 1.4	60.6 ± 10.8	59.0 ± 5.9	5.0 ± 1.4	7.5 ± 3.5
Year (yr)							
2014 **(n=8)**	5.1 ± 1.4	12.7 ± 3	9.3 ± 1.8	69.3 ± 10.4	65.9 ± 7.9	5.1 ± 0.9	6 ± 1.7
2015 **(n=10)**	2.5 ± 0.4	9.6 ± 2.7	5.9 ± 0.7	67.5 ± 14.6	52.4 ± 3.7	3.7 ± 1.3	5.1 ± 3.3
ANOVA (P values)							
Man	NS	NS	NS	NS	NS	NS	NS
Yr	*T*	NS	NS	NS	NS	NS	NS
Interaction							
man x yr	NS	NS	NS	NS	NS	NS	NS

The values presented as means±SE; Mean values are expressed as mg/ kg

Association between agronomic, site and soil drivers and grape yield and quality parameters by multivariate analyses

The bi-plot in Figure 5.2 shows the results of a redundancy analysis examining associations between agronomic, site and soil drivers and grape yield and quality parameters.

Overall 23% of the variation in data was explained, with axis 1 accounting for 17% and axis 2 for a further 6% of total variation (Figure 5.2).

Distance between vines-plant rows (DISV; f-value = 2.8, p-value = 0.08) and irrigation (IRR (yes/no); f-value = 1.5, p-value = 0.20), were identified as the strongest drivers for both yield and quality parameters, with all other drivers accounting for smaller amounts of additional variation, although neither were statistically significant. Other drivers included soil type (ST) (clay loam [cl]; argil clay loam [acl], clay loam $CaCO_3$ [clcc]), orientation (ORI) of the orchard (rows from east to west [ew= west facing orchard]; rows from north to south [ns=south facing orchard]), slope (SLO), elevation (ELE), age of the vines (AGE) and distance between rows (DISR) (f-values < 2.1, p-values > 0.13) (Figure 5.2).

There were strong positive associations between yield and DISV (and to a lesser extent SLO, ELE, AGE and the clay soils [cl and acl]), but strong negative association with sandy loam (sl) and limestone (lim) soil types along axis 1 (Figure 5.2).

In contrast, there were negative associations between DISV (and to a lesser extent SLO, ELE, AGE and the soil types (cl and acl)) and all nutritional quality parameters assessed along axis 1, and these were strongest for total phenolic content (TPC) and total antioxidant activity measured using the ABTS assay (TAAB) (Figure 5.2).

Finally, there were positive associations between all nutritional quality parameters assessed (except for TPC), and lim, sl and clcc soils along the negative axis 2 (Figure 5.2).

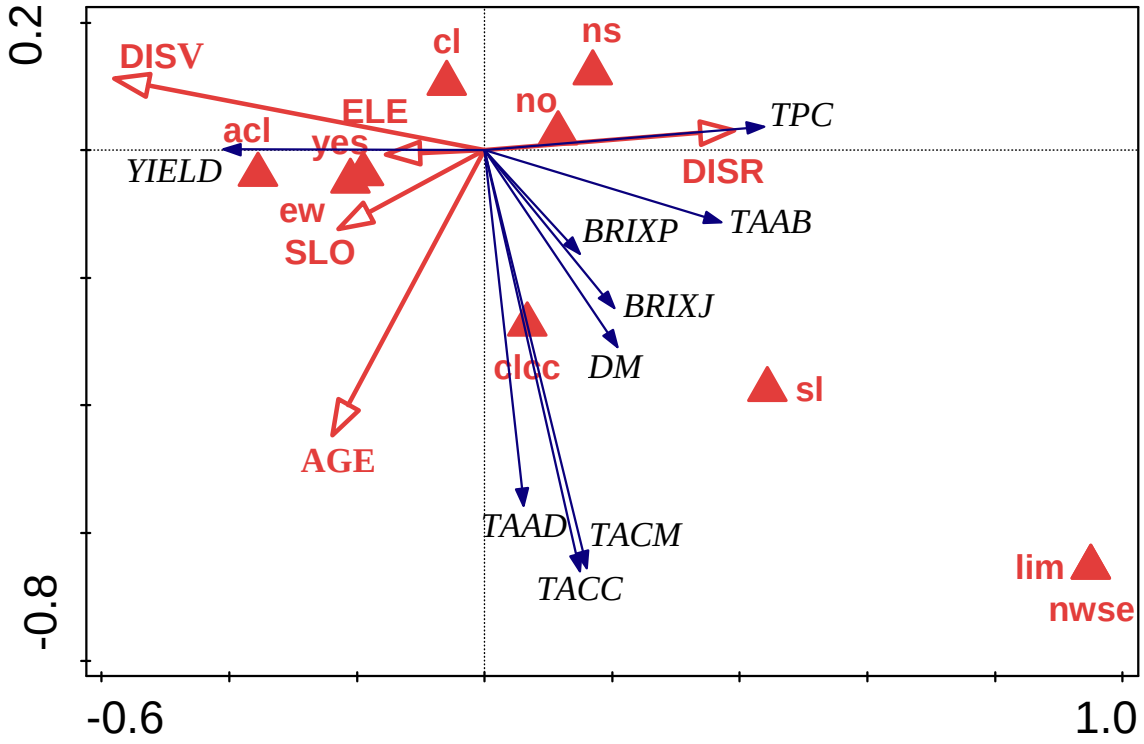


Figure 5.2 Association between agronomic, site, soil drivers (DISV-distance between vines with in rows; DISR-distance between rows; IRR-irrigation (yes/no); ST-soil type (clay loam [cl]; argil clay loam [acl], limestone [lim]; sandy loam [sl]; clay loam $CaCO_3$ [clcc]); ORI-orientation (east-west [ew]; north-south [ns]; northwest-southeast [nwse]); SLO-slope; ELE-elevation; AGE-age) and grape yield, quality parameters (YIELD-yield; TPC-total phenolic content; TAAB-total antioxidant activity (ABTS assay); TAAD-total antioxidant activity (DPPH assay); BRIXP-sugar content of pulp; BRIXJ-sugar content of juice; DM-dry matter; TACM-total anthocyanin content (malvidin equivalent); TACC-total anthocyanin content (cyaniding equivalent)) examined by redundancy analysis.

5.2 Discussion

The farm survey conducted in Crete, Greece did not detect differences in yield between organic and conventional production systems. This was unexpected since several previous studies and a recent meta-analysis of published yield comparisons between organic and conventional crops reported significantly higher yields in conventional compared to organic production (Malusa *et al.*, 2002; De Ponti *et al.*, 2012; Guesmi *et al.*, 2012; Seufert *et al.*, 2012). For example, Malusa *et al.* (2002) reported that the average yield of the variety Grignolino in organic production systems was 20% lower in a study carried out in Italy.

This may have been due to the semi-arid climate in Greece, where there is virtually no rainfall and low relative humidity between June and August/early September when grapes are harvested (Lotter *et al.*, 2009; De Ponti *et al.*, 2012). As a result downy mildew disease (caused by the oomycete fungus *Plasmopara viticola*) pressure is very low in most seasons (Francesca *et al.*, 2006; Williams *et al.*, 2007; Bois *et al.*, 2017). This view is supported by several previous studies (Gessler *et al.*, 2011; Liu *et al.*, 2015) carried out in regions with higher downy mildew disease pressure, which concluded that lower yields are at least partially due to higher downy mildew severity and grape losses in organic farming systems. In conventional grape production a range of synthetic as well as Cu-based fungicides are used to control the disease, while in organic farming only the relatively ineffective Cu-fungicides can be used, but only (a) if derogations are obtained from the organic certification body and (b) only up to a total input level of 6 kg Cu ha^{-1} $year^{-1}$ (Dufour, 2006; Nelson and Janke, 2007; Regulation, 2009)

Although significantly higher antioxidant activity (TEAC) was detected in organic compared to conventional grapes of the variety Vidiano, the farm survey did not detect consistent differences between organically and conventionally produced grapes for other analysed composition parameters. The finding of a significant interaction between variety and production systems, further supports the conclusion from the supermarket survey, that variety choice is an important cofounding factor in studies comparing the nutritional composition of organically and conventionally produced grapes.

As with the supermarket survey, the farm survey, also identified substantial effects of variety and production year/season. The lower antioxidant activity and phenolic acid concentrations in 2015 compared to 2014, were most likely due to the higher rainfall in late August in 2015, which resulted in damage to the grape skins. As a result grapes were harvested early to avoid severe yield losses due to fungal spoilage (Dr Nikos Volakakis and Mr Pakos Panagiotis,

personnel communication). Physical damage is known to increase phenolic concentrations and antioxidant activity in grapes (Nicholson and Hammerschmidt, 1992; Almuayrifi, 2013).

Effects of climatic conditions on fruit quality parameters were also reported in several previous studies (Esteban *et al.*, 2001; Conradie *et al.*, 2002; van Leeuwen *et al.*, 2004; Adams, 2006; Conde *et al.*, 2007; Xu *et al.*, 2010).

The **total phenolic concentrations** were significantly higher in both Kotsifali (a red variety) and Vidiano (a white variety) compared to Villana (the second white variety) and interestingly, numerically higher in the white variety Vidiano (1759 mg GAE/kg) compared to the red variety Kotsifali (1699 mg GAE/kg). These results contradict results from many other studies, which compared white with red or black grape varieties and (Yıldırım *et al.*, 2005; Dani *et al.*, 2007b; Katalinić *et al.*, 2010; Bunea *et al.*, 2012), which reported higher phenolic concentrations in red/black grape varieties. For example, Bunea *et al.* (2012) reported total phenolic concentrations of between 149-580 mg GAE/kg for a group of white but concentrations ranging between 953-1341 mg GAE/kg for a range of red/black varieties. Also, Yıldırım *et al.* (2005) reported higher polyphenolic concentration in red grapes (2850 mg/L) compared to white grapes (443 mg/L). Although, phenolic concentrations are known to vary depending on variety choice, geographical location, climatic conditions (González-Neves *et al.*, 2004; Orak, 2007; Margraf *et al.*, 2016), concentrations of phenolics in the local Cretan white grape varieties (and especially Vidiano) were still higher than those reported in the previous studies listed above.

Total antioxidant activity (DPPH) of grape varieties was also affected by variety choice, and as expected, concentrations were significantly higher in the red variety (Kotsifali) compared to the two white varieties (Vidiano and Villana). These results are consistent with those of several other studies (Bunea *et al.*, 2012; Tassoni *et al.*, 2013; Tassoni *et al.*, 2014; Toaldo *et al.*, 2015), which reported higher total antioxidant activity (DPPH) in red/black grape varieties compared to white varieties.

The differences between varieties in **total antioxidant activity (TEAC)** were similar to those obtained for total phenolic content and are consistent with the results of the supermarket survey reported in Chapter 4 and several previously published studies that all reported positive correlation between total phenolic content and total antioxidant activity (see discussion section of Chapter 4). However, it should be pointed out that total antioxidant activity (TEAC) in this study was relatively low (between 0.9-1.7 mM TE/g) compared to several previous studies (Pastrana-Bonilla *et al.*, 2003; Xu *et al.*, 2010; Toaldo *et al.*, 2015; da

Silva Haas *et al.*, 2016; Margraf *et al.*, 2016). For example, total antioxidant activity (TEAC) of red and white grape juice were reported as being 52 and 21 mmol TE/L respectively by Toaldo *et al.* (2015) and the lowest antioxidant activities were reported in a study by Pastrana-Bonilla *et al.* (2003), who found 17.6 μM/g for red and 13 μM/g for white grape varieties.

The finding that total antioxidant activity (TEAC) was higher in organic than conventional white grapes (varieties Villana and Vidiano) contradict findings by Corrales *et al.* (2010), who found higher antioxidant activity in conventionally grown white grapes compared to organically grown (Riesling variety) (27 mmol TE/g and 16 mmol TE/g respectively). However, Toaldo *et al.* (2015) reported similar results to the findings of the farm survey.

No significant main effect and interaction was detected for individual anthocyanin compounds. However, malvidin 3-glucoside and peonidin 3-glucoside had highest concentration among seven anthocyanin compounds, which is consistent with the results of several other studies (Kallithraka *et al.*, 2005; Vian *et al.*, 2006; Dani *et al.*, 2007b; Mulero *et al.*, 2010; Toaldo *et al.*, 2015).

In the farm survey, a range of agronomic and site specific parameters (e.g. fertilisation; irrigation; tillage; crop protection; pruning system; soil type, slope, elevation, row distance and orientation in the vineyard) was recorded to allow potential effects of these parameters on grape yield and quality to be determined by RDA. However, the soil, site and agronomic drivers included in **the multivariate analyses (RDA)** explained only a relatively small proportion of the variation. This was probably due to results from two growing seasons with very contrasting weather conditions being included in the analyses. However, data from appropriate local weather stations could not be obtained and included as drivers in the analyses. This limitation could be considered for future investigation.
There appeared to be trade-offs between yield and quality parameters (sugar and dry matter content, antioxidant concentrations and activity) with drivers associated with increased yield (e.g. irrigation, wide distances between rows, higher elevation, steeper slopes and older trees) also resulting in lower antioxidant concentrations and activity, and lower sugar dry matter anthocyanin and phenolic content. To our knowledge, there are no previous studies in which association between agronomic, site and soil drivers and grape yield and quality parameters was analysed. However, several studies analysed association between environmental and quality parameters (Son *et al.*, 2009), and between variety, region, vintage and quality parameters (Anastasiadi *et al.*, 2009; Godelmann *et al.*, 2013) have been previously reported.

Similar to the supermarket survey, results from the farm survey suggest that the phytochemical concentrations (total phenolics and anthocyanins), and total antioxidant activity in grapes are determined by very complex interactions between variety choice, and pedo- climatic conditions, and to a lesser extent, the management system (organic vs conventional), and non-production system-specific management parameters (e.g. irrigation, vineyard site).

CHAPTER 6. Wine survey; nutritional composition of wine made from common local grape varieties produced in organic and conventional vineyards in Crete

6.1 Results

Wines were collected from wineries in the same region of Crete where the farm survey of grapes was carried out (see Chapter 5 above). Wines were made from two of the three grape varieties, Kotsifali (red) and Vidiano (white), that were also used in the farm survey, or mixtures of grapes in which these two varieties accounted for 70% of the grapes used (see below).

Three separate 2-way ANOVA with management systems and grape variety as factors were carried out comparing data from:

(a) Wines made from 100% Kotsifali (red) or 100% Vidiano (white) grapes;

(b) Wines made from mixtures of grapes where Kotsifali or Vidiano accounted for 70% of the grapes used; the following Kotsifali and Vidiano mixtures were included:

- Kotsifali (70%):
  Kotsifali (70%)/Cabarnet (30%); Kotsifali (70%)/Syrah (30%); Kotsifali (70%)/Mandilari (30%)
- Vidiano (70%):
  Vidiano (70%)/Savignon (30%); Vidiano (70%)/Plyto (30%)

(c) Wines made from 100% Kotsifali grapes and wines made from a 70% Kotsifali and 30% Syrah grapes; Only the Kosifali/Syrah mixture was used in this analysis, because for all other grape mixtures, less than 3 matching vintage and management pairs were available.

Since wine quality is known to vary substantially between years/vintages, only wines for which matching organic – conventional vintages were available were included in the analyses. Total anthocyanin concentrations were only assessed in the red variety (Kotsifali), since anthocyanin concentrations are very low in white wines.

For antioxidant activity and concentrations of all composition parameters assessed activity/concentrations in white wine samples (see results below) were substantially lower than those measured in white grape samples (see chapter 5).

Table 6.1 shows the results for wines made from only Kotsifali (red) and only Vidiano (white) grapes. No significant main effect of management systems was detected for the composition parameters assessed. However, significant main effects of variety were detected for total phenolic content and total antioxidant activity (by DPPH/ABTS assays). Kotsifali, the red variety, had higher total phenolic concentrations and higher antioxidant activity (DPPH and TEAC) than Vidiano, the white variety.

Interaction between factors

A 2-way interaction was detected only for total phenolic concentrations (Table 6.1). Concentrations were significantly higher in wines made from conventional than organic Kotsifali grapes, while there was not significant differences in total phenolic content in wines made from organic and conventional Vidiano grapes (Figure 6.1).

Table 6.1 Effect of, and interaction between, management system and variety for the total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /ABTS assays and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) of only Kotsifali (red) and only Vidiano (white) made local wine samples

	TPC mg GAE/ L	**TAA (DPPH) mM TE/l**	**TAA (TEAC) mM TE/l**	**TAC (red) mg cyan/ L**	**TAC (red) mg mal/ L**
Management (man)					
ORG **(n=9)**	1108.1 ± 305.4	3.6 ± 1	3.9 ± 0.4	108.9 ± 51.9	114.9 ± 54.7
CONV **(n=9)**	1119.9 ± 339.2	3.9 ± 1.2	3.7 ± 0.4	110.8 ± 15.7	117 ± 16.5
Wine					
Kotsifali **(n=6)**	2387.1 ± 98.4 **a**	7.9 ± 0.4 **a**	5.2 ± 0.002 **a**	109.8 ± 24.2	115.9 ± 25.6
Vidiano **(n=12)**	477.4 ± 30.72 **b**	1.7 ± 0.3 **b**	3.2 ± 0.2 **b**		
ANOVA (P values)					
Man	NS	NS	NS	NS	NS
Var	**<.0001**	**<.0001**	**<0.0001**		
Interaction					
man x var	**0.0468**	NS	NS		

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

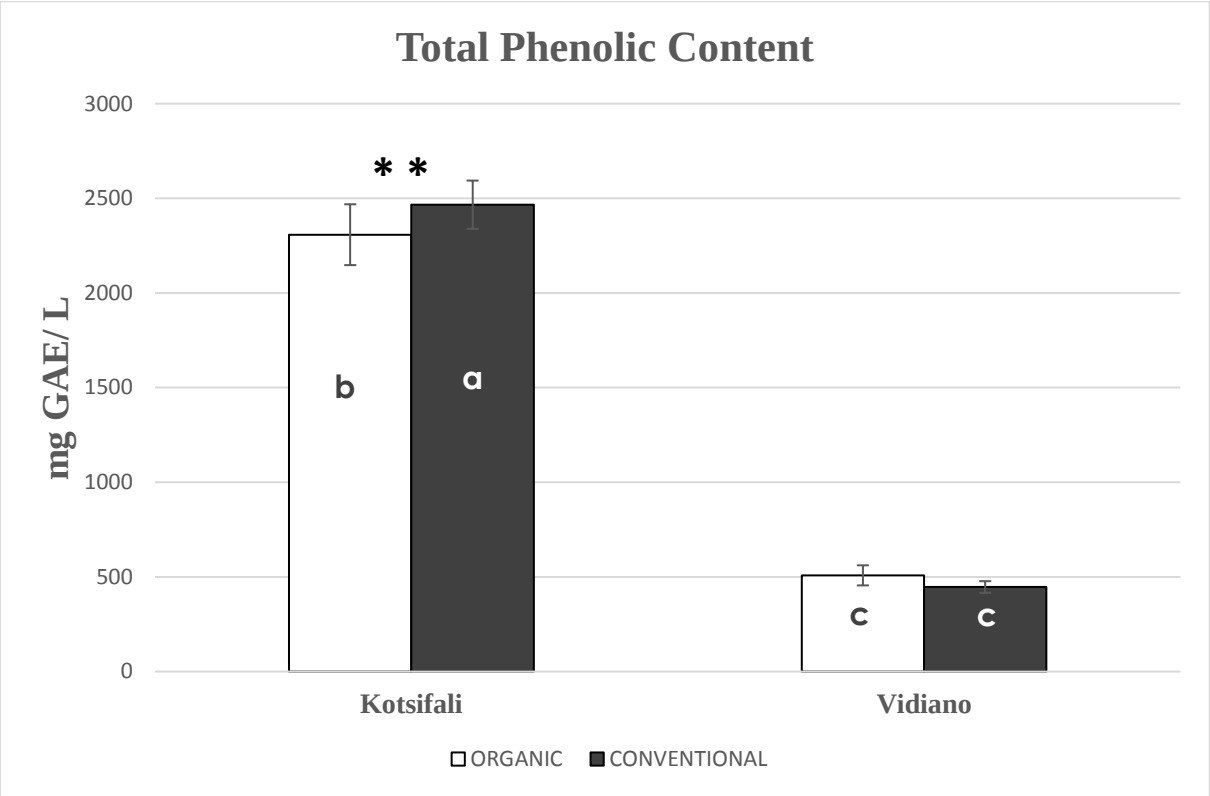


Figure 6.1 Level of total phenolic content; a, b, c correspond to significantly different mean values in different varieties and **-indicates significant interaction in one variety by 2-way ANOVA and Tukey post hoc test for $p<0.05$;

When wines made from Kotsifali (70%) or Vidiano (70%) based grape mixtures were compared, the same results as for wines made from Kotsifali or Vidiano grapes only, were obtained, except for the interaction between management and variety for total phenolic content not being significant (Table 6.2).

Table 6.2 Effect of, and interaction between, management system and variety on the total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /TEAC and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) in red and white wines made from mixtures of red or white grapes; Mixed red wines were made from 70% Kotsifali plus 30% Cabarnet, 30% Syrah or 30% Mandilari grapes and mixed white wines were made from 70% Vidiano plus 30%Savignon or 30% Plyto grapes

	TPC mg GAE/ L	**TAA (DPPH) mM TE/l**	**TAA (TEAC) mM TE/l**	**TAC (red) mg cyan/ L**	**TAC (red) mg mal/ L**
Management (man)					
ORG **(n=19)**	1508.3 ± 216.2	5.2 ± 0.7	4.4 ± 0.2	65.7 ± 17.1	69.4 ± 27.4
CONV **(n=19)**	1540.5 ± 227.7	5.4 ± 0.7	4.2 ± 0.3	105.7 ± 13.9	111.6 ± 8.3
Wine					
Kotsifali (70%) **(n=22)**	2289.4 ± 80.9 **a**	7.8 ± 0.3 **a**	5.2 ± 0.001 **a**	85.7 ± 11.7	90.5 ± 12.8
Vidiano (70%) **(n=16)**	472.5 ± 23.5 **b**	1.8 ± 0.2 **b**	3.1 ± 0.2 **b**		
ANOVA (P values)					
Man	NS	NS	NS	*T*	*T*
Var	**<.0001**	**<.0001**	**<.0001**		
Interaction					
man x var	NS	NS	NS		

The values presented as means±SE; Different letter values are significantly different according to THSD test *(p<0.05)*

When wines made only from Kotsifali variety where compared with wines made from a mixture of red varieties (70% Kotsifali plus 30% Syrah), no significant main effect of factors and no significant 2-way interaction was detected. However, there were trends towards a significant differences for total antioxidant activity (DPPH) and total anthocyanin content (Table 6.3).

Table 6.3 Effect of, and interaction between, management system and variety on the total phenolic content (TPC), total antioxidant activity (TAA) by DPPH /TEAC and total anthocyanin content (TAC) (cyanidin 3-glucoside/malvidin 3-glucoside equivalent) in red wine made from 100% Kotsifali grapes and red wine made from a mixtures of 70% Kotsifali plus 30% Syrah(30%) grapes

	TPC mg GAE/ L	**TAA (DPPH) mM TE/l**	**TAA (TEAC) mM TE/l**	**TAC (red) mg cyan/ L**	**TAC (red) mg mal/ L**
Management (man)					
ORG **(n=8)**	2328 ± 72.4	8.09 ± 0.21	5.16 ± 0.002	76.7 ± 20.9	80.9 ± 22
CONV **(n=8)**	2359 ± 113.9	7.97 ± 0.33	5.16 ± 0.001	111 ± 11.9	117 ± 12.5
Wine					
Kotsifali **(n=12)**	2279 ± 70.2	7.79 ± 0.21	5.15 ± 0.001	91.5 ± 16.4	96.6 ± 17.3
Kotsifali (70%) **(n=4)**	2537 ± 120.4	8.75 ± 0.06	5.16 ± 0.002	102 ± 10.9	107 ± 11.5
ANOVA (P values)					
man	NS	NS	NS	*T*	*T*
var	NS	*T*	NS	NS	NS
Interaction					
man x var	NS	NS	*T*	NS	NS

The values presented as means±SE; Different letter values are significantly different according to THSD test *($p<0.05$)*

HPLC results for individual anthocyanins of red wine samples in different vintage years

No significant main effects and interactions between management system and variety (Kotsifali vs Kotsifali (70%)/ Syrah (30%)) were detected for individual anthocyanins (Table 6.4).

Moreover, in two additional 2-way ANOVAs (in which wines made only from Kotsifali grapes; and wines made from mixture of Kotsifali and other red grapes compared), no significant main effect of variety was detected, which can be found in APPENDIX B (Table B2 and B3).

Table 6.4 Effect of, and interaction between, management system and variety for the individual anthocyanin compounds (*Delphinidin 3-O-glucoside, Cyanidin 3-O-glucoside, Petunidin 3-O-glucoside, Peonidin 3-O-glucoside, Malvidin 3-O-glucoside, Peonidin 3-O-p-coumaroylglucoside, Malvidin 3-O-p-coumaroylglucoside)* of red variety Kotsifali and Kotsifali(70%)/Syrah(30%) (mix) made wine samples (2-way ANOVA)

	Delphinidin 3-O-glucoside	**Cyanidin 3-O-glucoside**	**Petunidin 3-O-glucoside**	**Peonidin 3-O-glucoside**	**Malvidin 3-O-glucoside**	**Malvidin 3-O-p-coumaroylglucoside**
Management (man)						
ORG **(n=8)**	1.04 ± 0.47	0.21 ± 0.1	1.19 ± 0.57	1.45 ± 0.62	7.64 ± 3.48	0.62 ± 0.3
CONV **(n=8)**	0.9 ± 0.18	0.24 ± 0.03	1.2 ± 0.25	1.81 ± 0.36	9.87 ± 2.41	0.77 ± 0.19
Wine						
Kotsifali **(n=12)**	1.09 ± 0.32	0.25 ± 0.07	1.31 ± 0.39	1.75 ± 0.46	9.52 ± 2.74	0.74 ± 0.23
mix **(n=4)**	0.61 ± 0.07	0.16 ± 0.03	0.85 ± 0.14	1.26 ± 0.24	6.46 ± 0.77	0.57 ± 0.09
ANOVA (P values)						
man	NS	NS	NS	NS	NS	NS
var	NS	NS	NS	NS	NS	NS
Interaction						
man x var	NS	NS	NS	NS	NS	NS

The values presented as means±SE; Mean values are expressed as mg/L

6.2 Discussion

Concentration of **total phenolics** were significantly higher in red wines made from Kotsifali, than in white wines made from Vidiano grapes. These results are consistent with the findings in several previous studies (Tinttunen and Lehtonen, 2001; Minussi *et al.*, 2003; Zafrilla *et al.*, 2003b; Lante *et al.*, 2004; Yıldırım *et al.*, 2005; Vrček *et al.*, 2011; Tassoni *et al.*, 2013). For instance, it was reported by Minussi *et al.* (2003) that the concentration of total polyphenols ranged between 1645-3791 mg/L for red wines and 216-854 mg/L for white varieties.

Previous studies about the effect of conventional vs. organic management systems reported contrasting results for phenolic content. A significant interaction between management system and variety choice was observed only for a data comparing 100% Kotsifali made red with 100% Vidiano made white wine samples. Based on this interaction, a significant difference was detected only for red wine samples (Kotsifali), where conventional wines had higher concentrations of total phenolic content compared to organic wines. Akçay *et al.* (2004), also reported higher total phenolic concentrations in conventionally compared to organically produced red (Cabernet Sauvignon) wines (40.2 mg/ml vs 18.3 mg/ml respectively). Similarly, Miceli *et al.* (2003) reported a 7% higher concentration total phenolic content in Controlled Denomination of Origin (DOC) wines, compare to organic wines. In contrast, studies by Laureati *et al.* (2014), Vrček *et al.* (2011) and Tinttunen and Lehtonen (2001), reported significantly higher concentrations of total polyphenols in organic red (Sangiovese (Laureati *et al.*, 2014); Zweigelt and Plavac mali (Vrček *et al.*, 2011); Burgundy (Tinttunen and Lehtonen, 2001) wines compared to conventional, and a range of other studies (Zafrilla *et al.*, 2003b; Mulero *et al.*, 2010; Martin and Rasmussen, 2011; Tassoni *et al.*, 2013; Tassoni *et al.*, 2014; Garaguso and Nardini, 2015) did not detect significant differences between organic and conventional wines.

Total antioxidant activity (DPPH and TEAC) was higher in red wine made from 100% Kotsifali than white wine made from 100% Vidiano grapes. This trend was also true for the wines made by mixing the main local red grape variety with up to 30% of other red or mixing the main local white grape variety with up to 30% of other white grape varieties. Although these results are consistent with most previous studies, including Tassoni *et al.* (2013), Tassoni *et al.* (2014), Minussi *et al.* (2003) and Yıldırım *et al.* (2005), it is not quite similar to the results obtained in the grape survey (see Chapter 5), where the white grape variety (Vidiano) had a slightly higher concentrations of total polyphenols compared to red grape variety (Kotsifali).

The levels of antioxidant activity in red wine samples, detected with the DPPH assay (7.8-8.7 mM TE/l) were within the range reported in other related studies (Mulero *et al.*, 2010; Tassoni *et al.*, 2013; Büyüktuncel *et al.*, 2014). However, the levels of antioxidant activity measured using the ABTS assay were lower (around 5.2 mM TE/l) than those found in previous studies of red wine (12-32.2 mM TE/l) (Minussi *et al.*, 2003; Büyüktuncel *et al.*, 2014; Garaguso and Nardini, 2015), except for the study by Vrček *et al.* (2011), which reported activity levels between 5.2-11.7 mM TE/l.

For the white wines activity levels detected by both assays (DPPH and TEAC) were within the range reported in previous studies (Simonetti *et al.*, 1997; Alonso *et al.*, 2002; Tassoni *et al.*, 2013; Tassoni *et al.*, 2014).

Anthocyanin concentrations and anthocyanin profiles in red wines were not affected by the production system overall, although concentrations of total anthocyanins were lower (85.7-115.9 mg/L) than those reported in several other studies (172-741 mg/L) (Zafrilla *et al.*, 2003b; Mulero *et al.*, 2009; Mulero *et al.*, 2010; Tassoni *et al.*, 2013; Laureati *et al.*, 2014). However, according to Arnous *et al.* (2002) and Kallithraka *et al.* (2006), which investigated Greek wines made from local wine varieties, the content of total anthocyanins may be as low as 53.6 mg/l and 18 mg/l. Previous studies which investigated anthocyanin concentrations during storage, showed that anthocyanin concentrations in wine decrease over time and concluded that there can be many reasons for different concentrations of total anthocyanin, especially variety choice (Zafrilla *et al.*, 2003b; Monagas *et al.*, 2006; Mulero *et al.*, 2009). For example, Zafrilla *et al.* (2003b) reported a 88-95% decrease in anthocyanin content during a seven month storage period of wine in the dark.

As mentioned above, nonsignificant results were also detected for individual anthocyanin compounds, with the highest concentration of malvidin 3-glucoside and the lowest of cyaniding 3-glucoside among the 6 compounds.

Similar to the farm survey (see Chapter 5), there is a positive correlation between total phenolic content and antioxidant activity of wine samples, which is consistent with the results of several other studies (Yıldırım *et al.*, 2005; Orak, 2007; Vrček *et al.*, 2011; Büyüktuncel *et al.*, 2014). For future concern, storage conditions, grape variety and its growing conditions should be considered while comparing polyphenol composition of wine, as it was limitations for this experiment.

GENERAL DISCUSSION AND CONCLUSION

Uncertainty about meta-analyses results

A range of epidemiological/cohort and dietary intervention studies carried out over the last two decades have reported positive associations between high dietary intakes of antioxidant/(poly)phenol-rich foods (whole grain cereals, fruit and vegetables) and lower risk of cardiovascular disease, other oxidative stress related chronic/degenerative diseases and overall mortality (Muntwyler *et al.*, 1998; Beckman, 2000; Scalbert *et al.*, 2005; Katiyar, 2008; Lindberg and Amsterdam, 2008; Vislocky and Fernandez, 2010; Nassiri-Asl and Hosseinzadeh, 2016).

Grapes and grape products, such as grape juice and wine are considered antioxidant -rich foods containing high levels of a range of different (poly)phenolic compounds (e.g. anthocyanins, resveratrol, stilbenes, flavonoids) (Wang *et al.*, 2002; Fuleki and Ricardo-Da-Silva, 2003). The antioxidant properties of (poly) phenolic compounds in grapes and grape based foods were investigated in a wide range of previous studies (German and Walzem, 2000; Yang and Xiao, 2013; Toaldo *et al.*, 2015; Rasines-Perea and Teissedre, 2017).

More recently, there has been an increase in consumer demand for organic foods, which are produced without the use of synthetic chemical mineral N and P fertilisers, pesticides, and plant growth regulators. This increase in demand for organic foods was due, at least partially, to consumer perception that organic farming practices **(a)** result in "*a nutritionally more desirable food composition*" (e.g. higher levels of desirable compounds such as antioxidants and lower levels of undesirable potentially toxic agrochemical residues) and **(b)** improve farm animal and human health (Forman and Silverstein, 2012; Oates *et al.*, 2012; Baudry *et al.*, 2015). This triggered a wide range of studies to investigate the effects of agronomic management practices (organic vs conventional) on **(a)** nutritional quality of crop plants from the mid-1990's onwards (Dani *et al.*, 2007b; Corrales *et al.*, 2010; Brandt *et al.*, 2011; Barański *et al.*, 2014) and more recently (from 2012 onwards), and **(b)** cohort/epidemiological studies aimed at identifying associations between organic food consumption and health and **(c)** dietary intervention studies aimed at identifying the impact of organic feed consumption on physiological health related parameters/markers in animal models (Barański *et al.*, 2017; Mie *et al.*, 2017).

However, many uncertainties remain related to both **(a)** the impact of organic production methods on food composition and **(b)** potential impacts of organic food consumption on human health.

With respect to previous meta-analyses of comparative food composition data important cofounding effects and uncertainties were found to be associated (Baranski *et al.*, 2014) with:

- food composition data from different crops, geographic regions and pedo-climatic background conditions having to be combined in meta-analyses, because there is an insufficient number of studies for individual crops; this approach prevents (a) estimates of composition differences for specific crops and (b) confounding effects of contrasting pedo-climatic conditions and/or geographic regions to be identified
- insufficient information in most (including peer-reviewed) papers on the specific agronomic management practices (soil management and tillage, crop rotation, fertilisation and crop protection regimes and variety choice) used to produce both the organic and conventional crops; this prevents composition differences to be linked with specific management practices and confounding effects of variety choice to be identified

In this thesis carried out meta-analysis of published comparative (organic vs conventional) datasets for a specific crop (grapevine) and its product (wine), and carried out supplementary supermarket and farm surveys was meant to identify/quantify the importance of potential confounding effects of variety choice and agronomic background conditions in studies comparing the composition of organic and conventional crops.

The meta-analysis of published data on composition differences between organic and conventional fresh grapes and grape products is, to our knowledge the first meta-analyses focused on just one crop and processed foods produced from it, since previous reviews covered different crops not a single crop (Basker, 1992; Di Renzo *et al.*, 2007; Dangour *et al.*, 2009; Brandt *et al.*, 2011; Smith-Spangler *et al.*, 2012; Barański *et al.*, 2014).

The findings of the standard weighted meta-analyses produced different results from the UK supermarket and the farm survey in Greece. The meta-analysis identified significantly higher concentrations of several individual polyphenol compounds (e.g. anthocyanins, total flavonoids) in organic grapes/grape products, but no significant difference in total antioxidant activity between organic and conventional grape products. Inconsistent effects of the production systems on phytochemical concentrations (e.g. total phenolic and anthocyanin concentrations) and their total antioxidant activity could be detected in supermarket and farm

surveys. Both the supermarket and farm surveys suggest that differences in climatic conditions of production seasons and variety choice can be major confounding factors when assessing the effect of production systems (in this case organic vs conventional) on nutritionally relevant composition parameters of grapes and grape products. Most importantly, results from this study determined whether or not there was a significant effect of production system and/or whether concentrations of specific compounds were higher in organically or conventionally grown grapes in many cases depending on grape type (white, red or black) and/or variety. In addition, the results from the farm survey suggest that soil type and non-production specific agronomic parameters (e.g. the spacing between rows; whether or not irrigation is used in vineyards) may also be confounding factors.

Based on these results the following conclusions can be drawn from this study:

- results from meta-analyses of composition differences between organic and conventional grapes/grape products are currently unreliable due to **(a)** the small number of data sets/studies available, **(b)** grape/grape product samples not having been matched for variety and/or **(c)** insufficient information about potential confounding factors such as pedo-climatic conditions, non-production specific agronomic parameters
- recommendation should be developed for future retail and farm surveys, but also field experimental studies which ensure that the impact of confounding factors is minimised (e.g. by only comparing conventional and organic grapes of the same variety) and/or can be assessed by redundancy analyses (e.g. by collecting detailed information on orchard characteristics and management practices, pedo-climatic conditions during the growing season, ripening stage at harvest).

Close associations between polyphenol content and total antioxidant activity

A recent meta-analysis conducted by Wang *et al.* (2014) detected strong links between flavonoids intake and a reduced risk of cardiovascular diseases. Also, one class of flavonoids, the anthocyanins (which are found in high concentrations in red and especially black grapes) have been associated with a range of biological activities, (e.g. antioxidant/free radical scavenging and anti-inflammatory, protection against oxidative stress) that are thought to be responsible for such health benefits (Sarma and Sharma, 1999; Wang *et al.*, 1999; Georgiev *et al.*, 2014).

The results from both the retail and farm survey consistently showed a positive association between total phenolic content and antioxidant activity and thereby confirmed by previous reports (Meyer *et al.*, 1997; Minussi *et al.*, 2003; Dávalos *et al.*, 2005; Orak, 2007). The antioxidant activity of polyphenol compounds is considered to be the main protective mechanism against cardiovascular diseases (Pandey and Rizvi, 2009), although the exact mechanism underlying these effects is unclear at the present time. Consequently, whether these compounds act as 'antioxidants' once metabolised in the gut and absorbed or whether they act as signalling molecules require further investigation. The "French paradox" described by Renaud and de Lorgeril (1992) could be a good example for the positive association between moderate consumption of (especially red) wine and a relatively low risk of cardiovascular disease in France, although French diets include a relatively high saturated animal fat intake. Also, consumption of grapes and grape products was linked to a reduced risk of certain cancers, obesity, microbial diseases, aging and other degenerative diseases (Zheng *et al.*, 1993; Yuan *et al.*, 2011; Scola *et al.*, 2013; Georgiev *et al.*, 2014). Anthocyanins in particular are thought to be a good indicator for potential health-promoting effects, since the anticancer, antitumor, anti-inflammatory and anti-oxidative stress effects of anthocyanins have been reported in several epidemiological studies (Meyer *et al.*, 1997; Burns *et al.*, 2000; de Pascual-Teresa *et al.*, 2010).

However, to what extent switching to organic table grape/wine consumption may provide additional benefits remains unclear from the results reported here. It should be pointed out that there was insufficient published data to compare toxic metal concentrations and the frequency of pesticide residues in grapes/wine from organic and conventional production. Also, toxic metal and pesticide residues were not assessed in the survey based studies reported here, although both cadmium concentrations and the frequency of pesticide residues was shown to be higher in conventional crops (Barański *et al.*, 2014). These nutritionally undesirable composition parameters as well as nutritionally desirable mineral concentrations (e.g. Fe, Zn, Cu) should be compared in future studies to gain a more complete understanding of the impacts of organic and conventional grape management practices on the nutritional composition of grapes/grape products. Also, limitation in climatic data, soil properties, non-production system-specific management parameters (e.g. irrigation, tillage, ripening stage, grape age), impact of foliar diseases, should be considered for future investigation.

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APPENDIX A

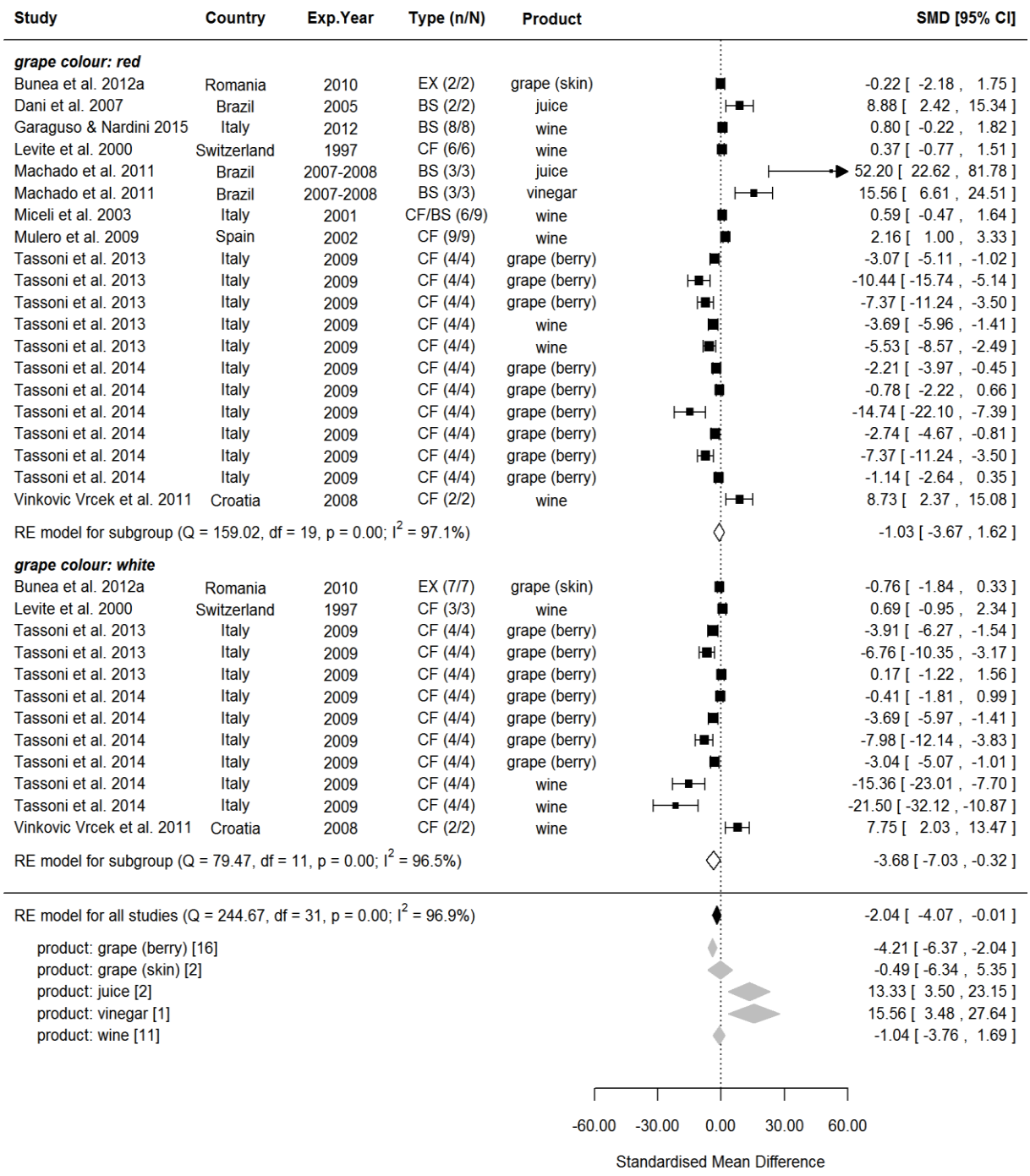

Figure A1. Forest plot showing the results of the comparison of stilbenes (group) between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods

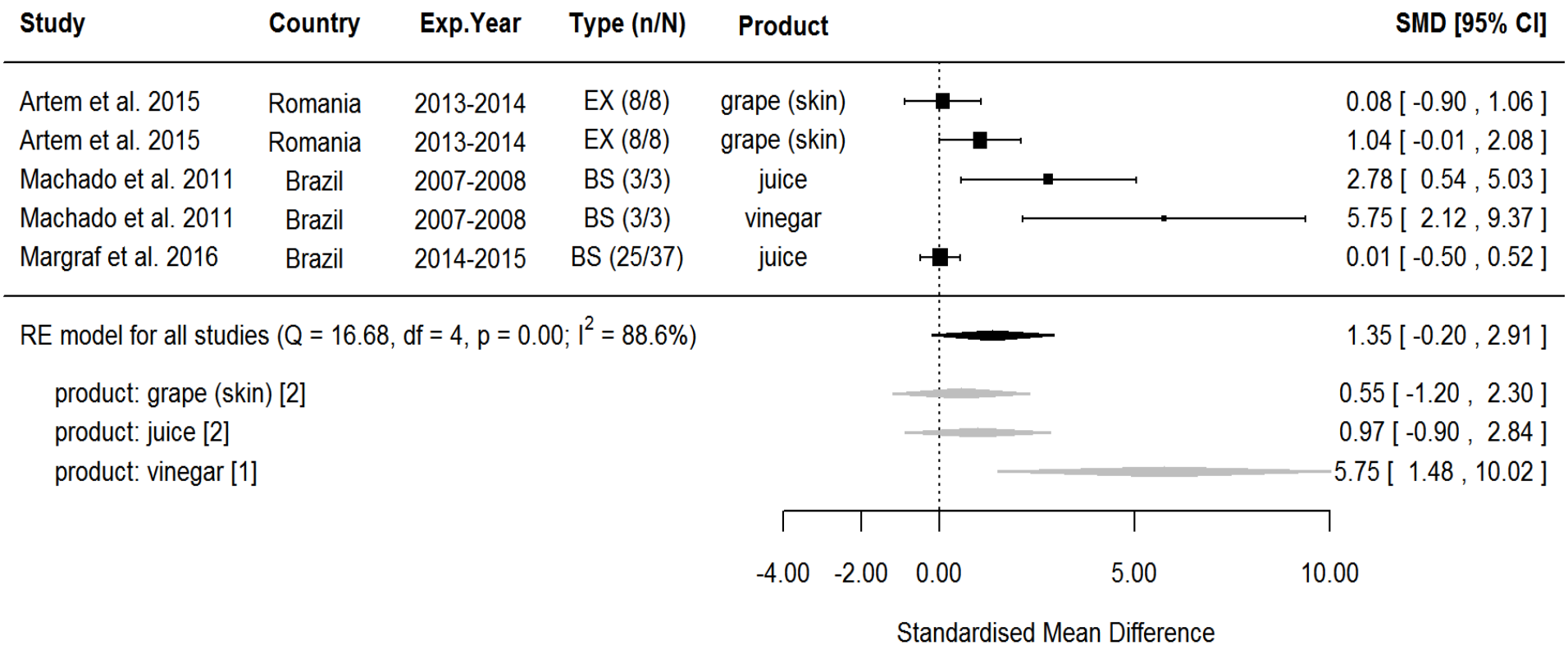

Figure A2. Forest plot showing the results of the comparison of tannins between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods.

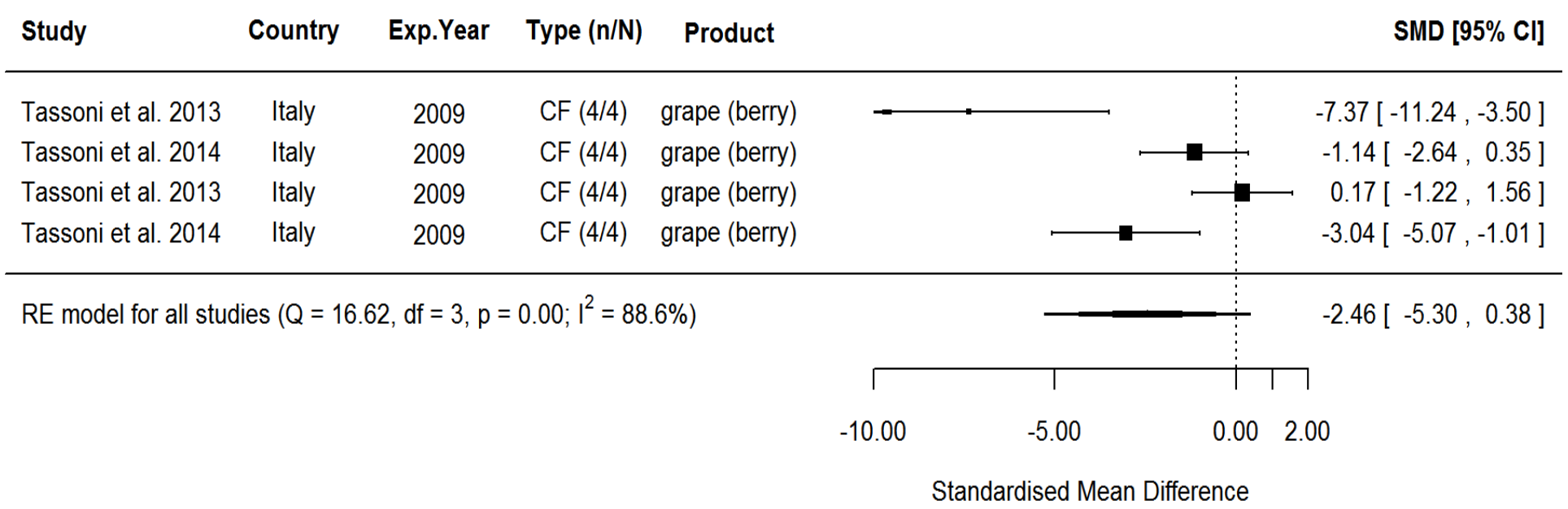

Figure A3. Forest plot showing the results of the comparison of piceatannol between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods.

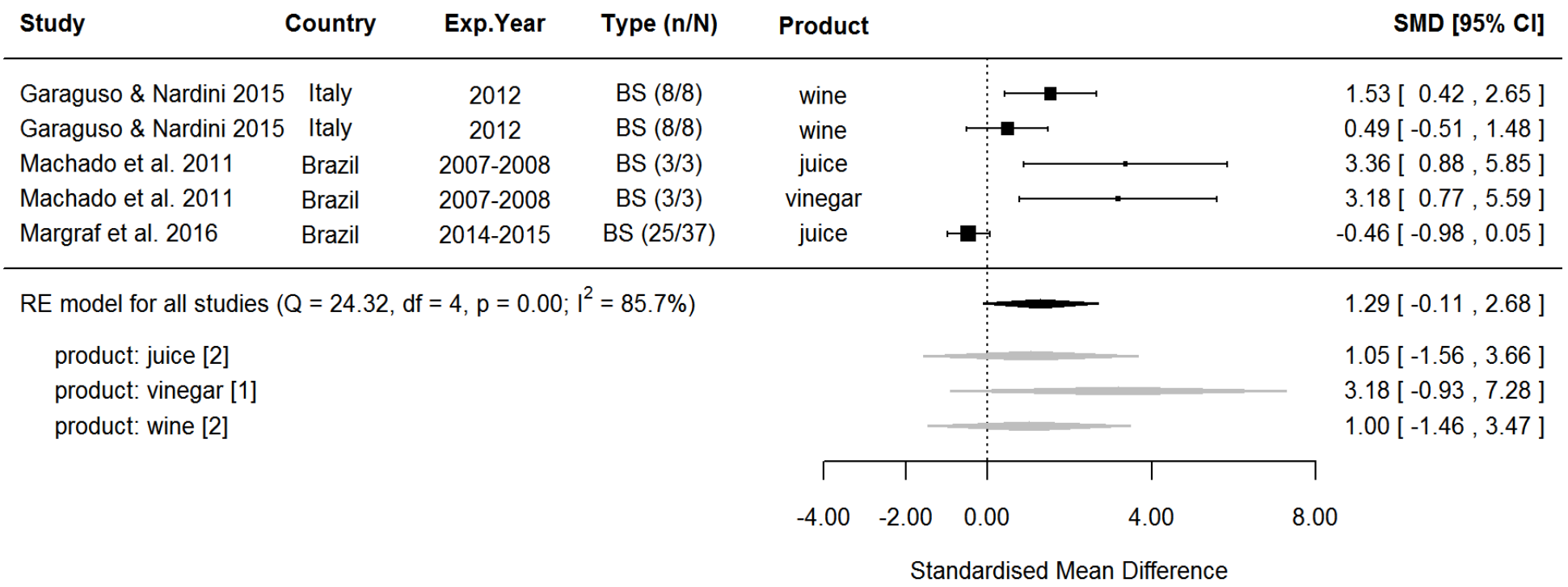

Figure A4. Forest plot showing the results of the comparison of caffeic acid between organic and conventional grape products using standardized mean differences (SMDs) with 95% confidence intervals (CI), for studies included in standard weighted meta-analysis. The estimated average SMD from Random-Effect (RE) model for all studies and SMDs for different product groups are indicated at the bottom of the figure. n-number of data point from control group (conventional); N- number of data point from intervention (organic). Sign of the SMD indicates if the analysed parameter is higher (+) or lower (-) in organic foods.

Table A1. Sensitivity analysis results of grape and grape products' composition parameters for which significant differences and trends toward significant differences were detected by weighted and unweighted meta-analysis by treating **cultivars separately and experimental years averaged**

	Unweighted meta-analysis					Weighted meta-analysis						
										Heterogeneity		
Parameter	***n***	**Ln(R)**	***P*†**	**MPD‡**	**95%CI**	***n***	**SMD**	**95%CI**	***P*†**	**§**	**MPD‡**	**95%CI**
antioxidant activity **	53	4.73	0.004	17.44	4.43, 30.44	20	-0.53	-1.95, 0.9	0.468	Yes (96%)	-3.87	-23.53, 15.79
anthocyanins *	29	4.87	0.029	42.12	-9.3, 93.54	10	4.71	2.14, 7.29	0.000	Yes (82%)	86.57	47.85, 125.28
stilbenes *	68	4.71	0.134	45.50	-4.44, 95.45	29	-3.59	-5.84, -1.34	0.002	Yes (95%)	-47.51	-85.24, -9.77
flavonoids *	55	4.63	0.077	2.49	-0.8, 5.78	25	0.42	-0.08, 0.92	0.103	Yes (69%)	4.54	0.39, 8.69
tannins	11	4.69	0.018	9.76	1.56, 17.96	10	0.18	-0.23, 0.59	0.378	No	9.72	0.66, 18.78
cis-piceid	4	4.30	0.060	-38.56	-68.08, -9.05	4	-5.87	-13.28, 1.54	0.120	Yes (97%)	-38.56	-68.08, -9.05
cis-resveratroloside	5	4.40	0.032	-22.88	-34.45, -11.31	5	-524.09	-1639.77, 591.59	0.357	Yes (100%)	-22.88	-34.45, -11.31
epicatechin	14	4.23	0.041	-69.43	-134.82, -4.03	10	-3.08	-13.25, 7.09	0.553	Yes (99%)	-90.03	-171.81, -8.25
flavonoids**	5	4.74	0.030	15.42	4.42, 26.43	4	0.74	-0.02, 1.49	0.057	Yes (51%)	11.05	2.14, 19.96
gallic acid	17	4.75	0.220	55.83	-43.99, 155.65	4	5.52	-1.89, 12.92	0.144	Yes (98%)	246.04	-147.33, 639.42
piceatannol	5	4.10	0.059	-73.49	-121.77, -25.21	4	-2.46	-5.3, 0.38	0.090	Yes (89%)	-66.87	-126.9, -6.84
resveratrol	25	4.89	0.024	60.32	-6.83, 127.48	3	7.45	-1.15, 16.06	0.089	Yes (87%)	103.98	34.97, 172.99
trans-resveratroloside	5	3.98	0.029	-101.47	-188.76, -14.17	4	-514113.42	-1599822.72, 571595.87	0.353	Yes (100%)	-117.91	-222.64, -13.17
2,3-hydroxybenzoic	6	3.25	0.018	-315.28	-453.38, -177.17							
gallic acid	5	5.50	0.033	223.26	-56.86, 503.38							
chlorogenic acid	5	5.33	0.028	111.19	58.38, 164.01							
p-coumaric acid	7	4.17	0.049	-77.31	-162.46, 7.84							
p-hydroxybenzoic	6	4.10	0.016	-73.04	-123.91, -22.18							
trans-resveratrol	4	6.55	0.062	617.12	439.08, 795.16							
syringic acid	8	4.34	0.044	-37.47	-71.58, -3.35							

n, number of data points included in the comparison; MPD, mean percentage difference; SMD, standardised mean difference of fixed-effect model. Ln(R) = Ln (ORG/CONV × 100%); †*P* value <0.05 indicates significance of the difference in composition between organic and conventional crop/crop based food; ‡Magnitude of difference between organic (ORG) and conventional (CONV) samples (value <0 indicate higher concentration in CONV, value >0 indicate higher concentration in ORG); §Heterogeneity and the I^2 Statistic; *-group of particular parameters; **-total of particular parameters.

Table A2. Sensitivity analysis results of grape and grape products' composition parameters for which significant differences and trends toward significant differences were detected by weighted and unweighted meta-analysis by treating **cultivars averaged and experimental years separately**

	Unweighted meta-analysis					Weighted meta-analysis						
										Heterogeneity		
Parameter	***n***	**Ln(R)**	***P*†**	**MPD‡**	**95%CI**	***n***	**SMD**	**95%CI**	***P*†**	**§**	**MPD‡**	**95%CI**
antioxidant activity **	35	4.68	0.098	8.32	-5.96, 22.6	30	0.52	-0.59, 1.63	0.355	Yes (95%)	7.70	-7.61, 23.01
polyphenols **	45	4.67	0.088	9.72	-4.62, 24.06	31	0.32	-1.2, 1.84	0.681	Yes (96%)	4.80	-8.24, 17.84
anthocyanins *	29	4.87	0.025	42.12	-9.3, 93.54	10	4.71	2.14, 7.29	0.000	Yes (82%)	86.57	47.85, 125.28
stilbenes*	53	4.73	0.153	63.73	-17.47, 144.94	37	-2.05	-4.08, -0.02	0.048	Yes (96%)	49.64	-61.84, 161.12
tannins	7	4.70	0.022	10.05	1.93, 18.18	7	0.86	-0.03, 1.74	0.059	Yes (64%)	10.05	1.93, 18.18
caffeic acid	9	4.58	0.462	-15.27	-89.23, 58.69	5	1.29	-0.11, 2.68	0.070	Yes (86%)	26.63	-6.93, 60.2
catechin	13	4.59	0.474	-28.45	-153.84, 96.95	8	1.42	-5.39, 8.23	0.683	Yes (99%)	-117.58	-289.34, 54.17
cis-piceid	4	4.30	0.063	-38.56	-68.08, -9.05	4	-5.87	-13.28, 1.54	0.120	Yes (97%)	-38.56	-68.08, -9.05
cis-resveratroloside	5	4.40	0.032	-22.88	-34.45, -11.31	5	-524.09	-1639.77, 591.59	0.357	Yes (100%)	-22.88	-34.45, -11.31
epicatechin	14	4.23	0.042	-69.43	-134.82, -4.03	10	-3.08	-13.25, 7.09	0.553	Yes (99%)	-90.03	-171.81, -8.25
flavonoids**	7	4.71	0.007	11.86	5.77, 17.95	7	1.04	0.33, 1.75	0.004	Yes (53%)	11.86	5.77, 17.95
p-coumaric acid	7	4.15	0.015	-76.56	-153.96, 0.84	4	-0.66	-1.08, -0.25	0.002	No	-112.70	-239.03, 13.62
piceatannol	5	4.10	0.063	-73.49	-121.77, -25.21	4	-2.46	-5.3, 0.38	0.090	Yes (89%)	-66.87	-126.9, -6.84
resveratrol	13	5.39	0.000	239.39	-24.3, 503.08	9	0.43	-0.17, 1.02	0.157	Yes (21%)	266.82	-115.32, 648.96
syringic acid	6	4.33	0.030	-37.02	-71.93, -2.1	5	-0.76	-1.16, -0.36	0.0002	No	-42.45	-83.17, -1.73
trans-resveratroloside	5	3.98	0.030	-101.47	-188.76, -14.17	4	-514113.42	-1599822.72, 571595.87	0.353	Yes (100%)	-117.91	-222.64, -13.17
ferulic acid	4	4.45	0.063	-17.01	-25.45, -8.57	4	-0.79	-1.37, -0.21	0.007	No	-17.01	-25.45, -8.57
myricetin	4	5.04	0.061	59.03	18.4, 99.66							

n, number of data points included in the comparison; MPD, mean percentage difference; SMD, standardised mean difference of fixed-effect model. Ln(R) = Ln (ORG/CONV × 100%); †*P* value <0.05 indicates significance of the difference in composition between organic and conventional crop/crop based food; ‡Magnitude of difference between organic (ORG) and conventional (CONV) samples (value <0 indicate higher concentration in CONV, value >0 indicate higher concentration in ORG); §Heterogeneity and the I^2 Statistic; *-group of particular parameters; **-total of particular parameters.

Table A3. Sensitivity analysis results of grape and grape products' composition parameters for which significant differences and trends toward significant differences were detected by weighted and unweighted meta-analysis by treating **cultivars separately and experimental years separately**

	Unweighted meta-analysis					Weighted meta-analysis						
Parameter	***n***	**Ln(R)**	***P*†**	**MPD‡**	**95%CI**	***n***	**SMD**	**95%CI**	***P*†**	**Heterogeneity§**	**MPD‡**	**95%CI**
antioxidant activity **	53	4.73	0.004	17.44	4.43, 30.44	20	-0.53	-1.95, 0.9	0.468	Yes (96%)	-3.87	-23.53, 15.79
polyphenols **	59	4.66	0.098	7.93	-4.55, 20.41	23	0.56	-1.97, 3.09	0.665	Yes (98%)	7.70	-8.72, 24.12
anthocyanins *	29	4.87	0.028	42.12	-9.3, 93.54	10	4.71	2.14, 7.29	0.000	Yes (82%)	86.57	47.85, 125.28
stilbenes *	68	4.71	0.144	45.50	-4.44, 95.45	29	-3.59	-5.84, -1.34	0.002	Yes (95%)	-47.51	-85.24, -9.77
flavonoids*	63	4.64	0.035	3.47	-0.2, 7.13	28	0.50	-0.03, 1.02	0.065	Yes (72%)	5.81	1.6, 10.03
cis-piceid	4	4.30	0.059	-38.56	-68.08, -9.05	4	-5.87	-13.28, 1.54	0.120	Yes (97%)	-38.56	-68.08, -9.05
cis-resveratroloside	5	4.40	0.032	-22.88	-34.45, -11.31	5	-524.09	-1639.77, 591.59	0.357	Yes (100%)	-22.88	-34.45, -11.31
epicatechin	14	4.23	0.041	-69.43	-134.82, -4.03	10	-3.08	-13.25, 7.09	0.553	Yes (99%)	-90.03	-171.81, -8.25
Flavonoids**	6	4.74	0.016	14.69	5.08, 24.29	5	0.82	0.04, 1.61	0.039	Yes (49%)	11.04	3.18, 18.9
piceatannol	5	4.10	0.064	-73.49	-121.77, -25.21	4	-2.46	-5.3, 0.38	0.090	Yes (89%)	-66.87	-126.9, -6.84
resveratrol	25	4.89	0.025	60.32	-6.83, 127.48	3	7.45	-1.15, 16.06	0.089	Yes (87%)	103.98	34.97, 172.99
trans-resveratroloside	5	3.98	0.028	-101.47	-188.76, -14.17	4	-514113.42	-1599822.72, 571595.87	0.353	Yes (100%)	-117.905	-222.64, -13.17
ferulic acid	11	4.44	0.010	-19.40	-32.32, -6.48	4	-2.46	-5.3, 0.38	0.090	Yes (89%)	-66.87	-126.9, -6.84
tannins	19	4.70	0.013	10.29	1.71, 18.87	9	0.43	-0.17, 1.02	0.157	Yes (21%)	266.82	-115.32, 648.96
2,3-hydroxybenzoic	6	3.25	0.015	-315.28	-453.38, -177.17	5	-0.76	-1.16, -0.36	0.0002	No	-42.45	-83.17, -1.73
gallic acid	5	5.50	0.028	223.26	-56.86, 503.38	4	-514113.42	-1599822.72, 571595.87	0.353	Yes (100%)	-117.91	-222.64, -13.17
chlorogenic acid	5	5.33	0.031	111.19	58.38, 164.01	4	-0.79	-1.37, -0.21	0.007	No	-17.01	-25.45, -8.57
p-coumaric acid	7	4.17	0.045	-77.31	-162.46, 7.84							
p-hydroxybenzoic	6	4.10	0.014	-73.04	-123.91, -22.18							
trans-resveratrol	4	6.55	0.059	617.12	439.08, 795.16							
syringic acid	8	4.34	0.049	-37.47	-71.58, -3.35							

n, number of data points included in the comparison; MPD, mean percentage difference; SMD, standardised mean difference of fixed-effect model. Ln(R) = Ln (ORG/CONV × 100%); †*P* value <0.05 indicates significance of the difference in composition between organic and conventional crop/crop based food; ‡Magnitude of difference between organic (ORG) and conventional (CONV) samples (value <0 indicate higher concentration in CONV, value >0 indicate higher concentration in ORG); §Heterogeneity and the I^2 Statistic; *-group of particular parameters; **-total of particular parameters

APPENDIX B

Table B1. Multivariate analysis of agronomic, site and soil type data of vineyards.

	Axis 1	**Axis 2**
Eigen values	17%	6%
Drivers	**f-value**	**p-value**
DISV	2.8	0.08
IRR.yes	1.5	0.196
IRR.no	1.5	unknown
ST.cl	2.1	0.132
ORI.ew	1.1	0.343
SLO	0.6	0.492
AGE	0.6	0.492
ST.acl	0.6	0.474
ELE	0.3	0.664
ST.clcc	0.4	0.56
DISR	0.8	0.412
ORI.ns	0.6	0.494

DISV-distance between vines with in rows; DISR-distance between rows; IRR-irrigation (yes/no); ST-soil type (clay loam [cl]; argil clay loam [acl], clay loam $CaCO_3$ [clcc]); ORI-orientation (east-west [ew]; north-south [ns]); SLO-slope; ELE-elevation; AGE-age

Table B2. Effect of, and interaction between, management system and variety for the individual anthocyanins (*Delphinidin 3-O-glucoside, Cyanidin 3-O-glucoside, Petunidin 3-O-glucoside, Peonidin 3-O-glucoside, Malvidin 3-O-glucoside, Peonidin 3-O-p-coumaroylglucoside, Malvidin 3-O-p-coumaroylglucoside)* of only red variety Kotsifali made wine samples (2-way ANOVA)

	Delphinidin 3-O-glucoside	**Cyanidin 3-O-glucoside**	**Petunidin 3-O-glucoside**	**Peonidin 3-O-glucoside**	**Malvidin 3-O-glucoside**	**Malvidin 3-O-p-coumaroylglucoside**
Management (man)						
ORG **(n=7)**	1.02 ± 0.55	0.21 ± 0.12	1.13 ± 0.67	1.36 ± 0.74	7.01 ± 4.15	0.58 ± 0.36
CONV **(n=6)**	1 ± 0.23	0.25 ± 0.04	1.31 ± 0.32	1.92 ± 0.47	10.88 ± 3.15	0.81 ± 0.26
Wine						
Kotsifali **(n=13)**	1.01 ± 0.3	0.23 ± 0.07	1.21 ± 0.38	1.62 ± 0.44	8.79 ± 2.62	0.69 ± 0.22
ANOVA (P values)						
man	0.8911	0.7611	0.8626	0.5376	0.4186	0.5869

The values presented as means±SE; Mean values are expressed as mg/L

Table B3. Effect of, and interaction between, management system and variety for the individual anthocyanins (*Delphinidin 3-O-glucoside, Cyanidin 3-O-glucoside, Petunidin 3-O-glucoside, Peonidin 3-O-glucoside, Malvidin 3-O-glucoside, Peonidin 3-O-p-coumaroylglucoside, Malvidin 3-O-p-coumaroylglucoside)* of all the mix red varietieties considered as a Kotsifali (70%) made wine samples (2-way ANOVA)

	Delphinidin 3-O-glucoside	**Cyanidin 3-O-glucoside**	**Petunidin 3-O-glucoside**	**Peonidin 3-O-glucoside**	**Malvidin 3-O-glucoside**	**Malvidin 3-O-p-coumaroylglucoside**
Management (man)						
ORG **(n=12)**	0.76 ± 0.33	0.15 ± 0.07	0.84 ± 0.4	1.04 ± 0.44	5.52 ± 2.45	0.43 ± 0.21
CONV **(n=12)**	0.73 ± 0.15	0.19 ± 0.03	1.02 ± 0.23	1.73 ± 0.47	9.02 ± 2.35	0.8 ± 0.26
Wine						
Kotsifali (70%) **(n=24)**	0.74 ± 0.18	0.17 ± 0.04	0.93 ± 0.23	1.39 ± 0.32	7.27 ± 1.7	0.62 ± 0.17
ANOVA (P values)						
man	NS	NS	NS	NS	NS	NS

The values presented as means±SE; Mean values are expressed as mg/L