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Influence of New Trends in Wine Technology on the Chemical and Sensory Profiles

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Abstract

The search for nutritional and nutraceutical foods and their health benefits beyond basic nutrition is constantly increasing. Among beverages, many of these beneficial characteristics are present in wine. Wines contain phenolic compounds in their composition, which are responsible for several health benefits due to their antioxidant capacity. However, as wine technology is complex and involves several steps, wineries encounter problems with the degradation of these compounds, which adversely affect wine quality. Basically, wine is composed of water and ethanol, but it is nevertheless considered to be one of the most complex beverages due to the presence of minor compounds such as phenolic and volatile substances, which interfere in both the chemical and sensory profiles. Wine technology demands intensive care since it involves great numbers of chemical reactions during the two fermentative steps: alcoholic fermentation which involves the yeast metabolism of sugars, and malolactic fermentation which involves malic acid decarboxylation into lactic acid by lactic acid bacteria. In this context, new trends in wine making are the object of several scientific studies. Among them, thermovinification, grape pre-dehydration, cold soaking, carbonic maceration, submerged cap and the application of selected yeasts and pectinases are examples of techniques applied by Latin American wineries in order to study the reactions involving changes in these minor compounds, assessing the antioxidant capacity as the main nutritional factor and providing results about the improvement of wine sensory quality. The wine making techniques reported in this chapter bring relevant results about the chemical behavior of all wine composition, mainly regarding phenolic compounds, such as anthocyanins and tannins. In

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addition, all the chemical changes described were correlated with the wine sensory features, which allow for the assessment of sensory quality. The present chapter aims to present a discussion of the state of the art of alternative wine making technologies and their effects on the chemical, sensory and nutritional profiles of the wines, the latter through the analysis of the antioxidant capacity.

Introduction

Wine making is a complex process involving several steps which frequently overlap. The transformation of the grapes into wine presents variations according to the region, due to the climate, soil type, grape cultivar and vine management (Jackson 2008). The traditional wine making process (Fig. 8.1), basically, followed the steps of destemming and crushing the grapes, which allow the release of the juice (must). The must and pomace (solid part) are fermented together or not and the production of red or white wines depends on the choice of the wine maker. The mixture is placed in fermentative vessels and treated with sulfur dioxide in order to avoid opportunist contamination. The alcoholic fermentation is performed by yeasts inoculation (*Saccharomyces cerevisiae*) or takes place spontaneously.

At the time of the alcoholic fermentation, the must is separated from the pomace by the dejuicing step thus allowing the chaptalization, if necessary. The solid part can be pressed, which allows the release of 10 to 15% of the juice, which remain adhered in pomace. Usually, three racking are done during the wine making process aiming to separate all the compounds that can haze the wine. The first one is performed after the protein and the phenolic stabilization which is followed by filtration or centrifugation. After the first racking, the wine is submitted to the malolactic fermentation, spontaneously or induced by the inoculation of *Oenococcus oeni*, aiming to provide low acidity to the wine. After the second racking the wines are sulphitated and the blending step can be done in order to improve wine features, followed by the cold stabilization or tartrate crystallization. In order to separate the tartrate crystals from the wine, the third racking is carry out and the wines are submitted to the oak maturation followed by bottling.

The process described above was intensely mapped in order to obtain information about the changes in the chemical profile, which result in sensory changes, altering the final quality of the wines. The wines were generally composed of water and ethanol and other minor compounds such as acids, pectins, carbohydrates, minerals and phenolic compounds. The latter is responsible for the main changes in wine sensory features during the wine making process and wine aging (Jackson 2008; Coombe and Dry 2006). Table 8.1 summarizes the composition of wine and juice.

Amongst these chemical substances present in grapes, one can highlight the phenolic compounds, which comprise anthocyanins, flavonols and flavan-3-ols as well as other compounds such as hydroxycinammic acid derivatives and stilbenes. Other phenolic compounds can be incorporated into the wine from additional sources, such as the ellagitannins and volatile phenolic compounds released from the cooperage wood used for the aging process of several wines. Moreover, all the aforementioned phenolic compounds are subject to change, which can be intensified as a result of reactions occurred throughout the wine making process, and the main goal of current studies is to control them in order to obtain a wine with singular sensory features and high antioxidant capacity.

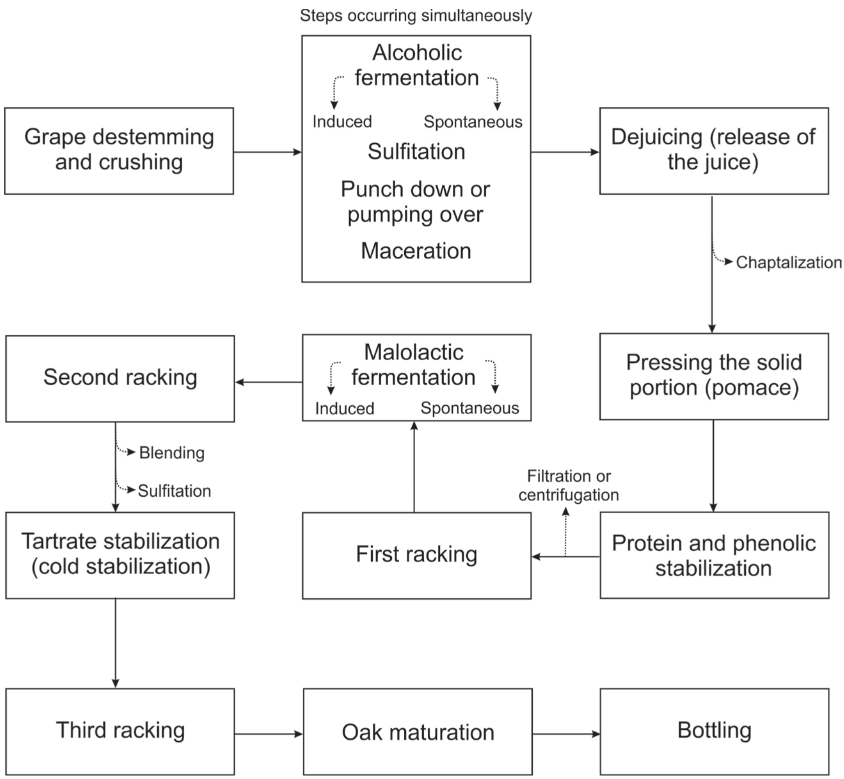

FIGURE 8.1 Traditional wine making steps. Source: Adapted from Jackson (2008) and De Castilhos et al. (2012).

The anthocyanins and other phenolic compounds present antioxidant capacity, and played an important role in the prevention of cardiovascular diseases, arteriosclerosis and thrombosis, controlling diabetes and reducing the risk of some types of cancer (Wang et al. 2006). In addition to the aforementioned antioxidant property, the phenolic compounds have been a focus of several other studies since they present intense correlation with astringency, mouth feel enhancement, color impact and also with the aging potential (Sacchi et al 2005; Gonzalo-Diago et al. 2014; Ma et al. 2014; Chira et al. 2011; Lago-Vanzela et al. 2014a). However, the wine antioxidant capacity, sensory attributes and aging potential of these compounds depend on the wine making procedure applied by the winery. In this context, several wine making techniques have been the focus of studies in order to enhance the concentration of these compounds and the sensory quality of the wines.

The largest wine producers in the world are located in Europe: France with 46.2 million hectoliters, Italy with 44.4 million hectoliters and Spain with 37.0 million hectoliters (OIV 2014). In these countries, the study of the various wine making procedures is carried out in a more intensive way, since they present a huge grape production and are known as the countries that produce the best quality wines in the world. However, Latin America has been associated with viticulture and wine making almost since its discovery and colonization by the Spanish and Portuguese.

Nevertheless, the emergence of Latin America as an important wine producer is recent when compared to the traditional aforesaid countries. While most Latin American countries present insignificant wine production, four South American countries stand out as great wine producers: Chile, Argentina, Uruguay and Brazil. In these countries, the climate and soil promote the production of *Vitis vinifera* cultivars, although, in Brazil, the cultivation of these species is restricted to the south of the country. As the greater part of Brazil has a tropical climate, the American grapes known as *Vitis labrusca* and their hybrids are gaining relevance on the wine panorama, because their wines present features truly appreciated by Brazilian consumers (De Castilhos et al. 2013; Jackson 2008).

TABLE 8.1 Typical concentration ranges of the major and minor chemical components of grape juice and dry wine. Source: Adapted from Coombe and Dry 2006.

Chemical component	Juice (g.L^{-1})	Wine (g.L^{-1})
Water	700–850	800–900
Carbohydrates	150–270	1–10
Pectins	0.1–0.8	Trace
Acids	3–15	4.5–11
Tartaric acid	3–12	1–6
Malic acid	1–8	0–8
Lactic acid	Trace	1–5
Acetic acid	Trace	0.2–1.5
Ethanol	Trace	80–150
Glycerol	Trace	3–14
Anthocyanins	0–0.5	0–0.5
Tannins	Trace–5	Trace–5
Nitrogen compounds	0.2–2	0.1–1
Inorganic constituents	3–5	1.5–4

Chile has a unique and extensive coastline that provides favorable conditions for the production of premium quality wines. Among the several wine regions in Chile, the most highly regarded section is called Regadio, which comprises the central region of Chile from the north of the Aconcagua River to the south of the Maule River. Most Chilean vineyards are associated with river valleys including the Maipo, Cachopoal, Tinguiririca, Lontué and Maule rivers. Most of the grape varieties cultivated in Chile is red type (75%), Cabernet Sauvignon being the most cultivated grape followed by Merlot and Carménère cultivars. Among the white varieties, Chardonnay and Sauvignon Blanc are of the most appreciated quality (Jackson 2008).

Viticulture and wine production are the third largest industry in Argentina. The vineyards are located mainly in the rain shadow of the Andes and the major wine producer region is the province of Mendoza. Vine management and wine making procedures follow the Spanish style and the grapes are harvested early in the Mendoza region to prevent malolactic fermentation. The grape varieties commonly cultivated in the region of Mendoza are Cabernet Sauvignon and Malbec, and in the central regions, Tempranillo and Semillon are the most representative cultivars. Nearly 50% of the grape varieties cultivated in Argentina produce red wines, 20% are used to produce white wine, and the remaining grape cultivars, such as Criolla and Cereza, are mainly cultivated in regions of the country. The slight oxidized character is appreciated by the Argentinean consumer. This feature can be mainly due to the prolonged aging of the white wines in oak cooperage (Jackson 2008).

Among the countries which produce grapes and wine in Latin America, Uruguay has the longest history, since it began its wine making history with the Spanish colonizers. Most of the vineyards are located near Montevideo in the southern part of the country. The most important cultivar is Tannat, accounting for approximately 32% of the total production (Jackson 2008).

In addition to these three Latin American countries, Brazil has been emerging as a wine producer due to its classic and emerging viticulture regions. Brazil is characterized by a considerable production of *Vitis labrusca* grapes and their hybrids, and has focused on the production of juices and wines. Among them, the Bordô, Isabel and Niágara grapes are the most important *Vitis labrusca* representative cultivars. However, these grapes present some disadvantages when compared with *Vitis vinifera* grapes, since they present weak color features and low soluble solids content in their optimal stage of ripening, and consequently, chaptalization is a necessary practice in Brazilian wines produced from these grape cultivars. In this context, the Brazilian Agro-farming Research Agency (Embrapa) has been developing new grape cultivars, known as BRS type grapes, produced by genetic improvement, in order to enhance the grape color features and the soluble solids content for avoiding the practice of chaptalization (De Castilhos et al. 2012, 2013; Lago-Vanzela et al. 2014a).

Among the Brazilian wine regions, the state of Rio Grande do Sul, located in the south of Brazil, is the most important and classical wine producer region. It is characterized by the production of wines from *Vitis vinifera* grape cultivars, since the climate allows for their cultivation. Many grape cultivars are harvested in this region, with a highlight on Cabernet Sauvignon and Merlot. Chardonnay and Sauvignon Blanc are the main white grape representative cultivars. However, other tropical regions of brazil are emerging as great wine producers, mainly for the vinification of *Vitis labrusca* wines, such as the Northwest of São Paulo state and the São Francisco river valley located in the Northeast of the country. In these regions, the BRS type cultivars have gained importance due to their intense color features and typical aromas related to red fruits such as strawberry and raspberry. Among them, BRS Cora (Camargo and Maia 2004), BRS Rúbea (Camargo and Dias 1999), BRS Violeta (Camargo et al. 2005) and BRS Carmem (Camargo et al. 2008) are the most important representative cultivars.

In this context, the present chapter summarizes and critically reviews the literature on the impact of wine making procedures on the chemical, mainly phenolic, and sensory profiles. Different procedures were considered in this chapter, describing their application at different moments in the wine making process. Many studies focused on the more important wine countries in the New World: Chile, Argentina, Brazil and Uruguay, but in addition, other studies from North America and Europe were included, in order to compare the results found in the different regions.

Wine making Procedures

Thermovinification and grape pre-dehydration

Two wine making procedures comprised the use of high temperatures: thermovinification and grape pre-dehydration. These thermal processes have the advantage of reducing the fruit microbial population, inhibiting undesirable enzymes, such as polyphenol oxidase (PPO) and laccase, and also reducing the wine making time (Andrade Neves et al. 2014).

Thermovinification is described as an alternative to the traditional maceration process for the extraction of phenolic compounds from the grape skins. Basically, it consists of submitting the grapes, after crushing, to high temperatures of around 60 to 70 ºC for a short time, extracting the compounds from the grape skins with the juice, and cooling them before fermentation. The heat damages the cell wall membranes of the grape skins, releasing the phenolic compounds (mainly anthocyanins, but also flavonols, flavan-3-ols and hydroxycinnamic acid derivatives), and also avoiding the effect of enzimatic oxidation due to their thermal inactivation, as mentioned previously. Since this process is only applied for a short time, and there the absence of alcohol during heating, tannin extraction from the seeds is avoided and the extraction of the anthocyanins is improved, although the extraction of other phenolic compounds is reduced (Sacchi et al. 2005).

Thermovinification is also a wine making procedure indicated for red grapes that present a low color potential and bad sanitary conditions. The application of the usual range of temperatures allows for denaturation of the oxidative enzymes, but it preserves the proteolytic and pectinolytic enzymes, which present great technological importance in wine making and facilitates the fining process (Rizzon et al. 1999).

Grape dehydration before fermentation uses higher temperatures in order to enhance the soluble solids content of the grapes avoiding the step of chaptalization in wine produced from *Vitis labrusca* and its hybrids (De Castilhos 2012, 2013). This procedure is the focus of some studies which evaluated its influence on the extraction of phenolic compounds (De Castilhos et al. 2015a, b).

In addition, drying cause irreversible damage to the grape skins due to water evaporation from the grapes, and these changes decrease their resilience and allow for cell rupture, which in turn allow contacts between PPO and its substrates. Moreover, drying causes structural changes to the grape skins and allows for diffusion of anthocyanins and other phenolic compounds present in the skin to the pulp, thus transferring them during alcoholic fermentation. The temperature used in grape pre-dehydration leads to the formation of oxidative compounds due to the activity of PPO, and to other products resulting from the non-enzymatic Maillard reaction. Low temperatures ranging from 30 to 40 ºC allow for PPO activity, causing the formation of oxidative compounds that lead to wine browning (Marquez et al. 2012, 2013); temperatures above 40 ºC cause PPO denaturation, but the Maillard reaction occurs in an extensive way, allowing for the formation of melanoidins, which have antioxidant capacity (De Castilhos et al. 2015a).

Many studies have focused on these thermal processes. Rizzon et al. (1999) studied the effect of thermovinification on the chemical and sensory profiles of Cabernet Franc red wines. The grapes were harvested in their optimal ripening stage and under optimal sanitary conditions, de-stemmed, crushed and heated to 65 ºC for

2 hours in stainless steel fermentation vessels, which contained a pumping device. The juice was then drained off and the pomace pressed. Alcoholic fermentation was carried out in the presence of the solid parts (pomace), which is necessary in red wine making processes. Fermentation was induced by the inoculation of 200 ppm of dry active yeast and the temperature controlled at 20 ºC. Malolactic fermentation occurred spontaneously and was monitored by thin layer chromatography. At the end of the alcoholic fermentation the wines were treated with 50 $mg.L^{-1}$ of sulfur dioxide and refrigerated at –4 ºC for 10 days to allow for tartrate stabilization. They were then bottled.

In the aforementioned study, the thermovinification procedure provided wines with higher phenolic and anthocyanin contents, as well as greater color intensity in compared to the traditional treatment of wine. The sensory attributes were also assessed and the color intensity, aroma quality, body, acidity and flavor attributes obtained higher scores when compared to the other wine making procedures. The wines produced from thermovinification presented an intense color feature and high amount of dry extract, which was responsible for enhancement of the mouthfeel and body. As a result the thermovinification wines presented higher global acceptance.

Another study showed relevant results for the application of the thermovinification procedure as an alternative wine making for Cabernet Sauvignon and Pinot Noir grapes (Andrade Neves et al. 2014). The grapes were harvested in their optimal maturity stage and good sanitary conditions, and after crushing, the juice and pomace (skins and seeds) were separated into two different fermentation vessels. The solid part was then immersed in 10% juice and submitted to a heating process at 95 ºC for 10 minutes. After cooling, the extract was added to the juice and completed the volume of the must. Alcoholic fermentation was induced by inoculating with dry active *Saccharomyces cerevisiae* yeasts at 25 ± 5 ºC and monitoring at 12-hour intervals. After complete alcoholic fermentation, the must was transferred to PET bottles, 50 $mg.L^{-1}$ of potassium metabisulfite added, and stored under refrigeration at 4 ºC for 30 days for tartrate stabilization. The wines were then bottled and stabilized for 6 months at 22 ºC.

In the aforementioned study the authors stated that the thermovinification process enhanced the extraction of the anthocyanins from the skins, which was responsible for the wine color. However, it was not effective in extracting the tannins from the seeds, and this low concentration of tannins negatively influenced wine aging, since there was a significant decrease in the anthocyanin and flavonol contents after six months of stabilizing. It has been also noted that the thermovinification significantly reduced the wine production time and the use of 95 ºC for a short time provided good acceptability for the wines, and was considered an alternative for young wines with different features (Andrade Neves et al. 2014).

In addition to thermovinification, grape pre-dehydration is another alternative step in the wine making process that has been a focus of studies in order to avoid the chaptalization process and to enhance the extraction of the phenolic compounds responsible for color and mouthfeel. De Castilhos et al. (2013) reported that pre-drying significantly influenced the chemical profile and sensory acceptance attributes of Bordô (*Vitis labrusca*) and Isabel (hybrid *Vitis labrusca*) grapes. In the study, the grapes were dried to 22 ºBrix of soluble solids content using a tray dryer at 60 ºC and of air flow 1.1 $m.s^{-1}$ in order to avoid chaptalization of the grape musts. As soon as the

22 °Brix was reached, the grapes were crushed and placed in fermentation vessels in order to begin the alcoholic fermentation. This process was induced by the inoculation of 200 ppm of dry active yeasts and the wines treated with 150 ppm of potassium metabisulfite. After 7 days of alcoholic fermentation they were racked and malolactic fermentation was induced by the inoculation of *Oenococcus oeni*. The final stage of the second fermentation process was monitored by thin layer chromatography. After this, the wines were stored in a refrigerator (3 °C) for 10 days to allow for tartrate stabilization, and then bottled in 750 mL glass bottles and stored at 18 °C until the chemical and sensory analysis. The grape pre-dehydration significantly increased almost all the chemical properties of the wines, highlighting the total acidity and the dry extract due to the evaporation of water during the drying process. In addition, the authors found that the drying process enhanced the total phenolic content of both Bordô and Isabel red wines. The dried grape wines also presented higher values for color intensity. The results showed that drying could improve the body of the wines, since the dry extract and acidity content were correlated with this sensory attribute. In addition, dried grape wines, regardless of the grape cultivar, presented higher sensory acceptance scores when compared with commercial wines. This result showed the great potential of the drying process as an alternative to produce red wines with good body and structure, despite their lower yields (De Castilhos et al. 2012, 2013, 2015a, 2015b).

A recent study presented data about the phenolic composition of red wines produced from the hybrid BRS Rúbea and BRS Cora grape cultivars submitted to pre-dehydration. The pre-dehydration process significantly degraded the anthocyanins, resulting in a decrease of their concentration; however, the grape pre-dehydration did not significantly affect the flavan-3-ols content (De Castilhos et al. 2015). The anthocyanin degradation was related to thermal degradation and the heat also affected the color of the red wines, changing the characteristic red-purplish color to a brown hue. The browning was produced by the Maillard reaction products, which took place in a more extensive way than the oxidative reactions caused by PPO, since at 60 °C the PPO loses its oxidative action (Patras et al. 2010).

According to Figueiredo-González et al. (2013), heating the grapes causes a loss of their physiological integrity, thus favoring the diffusion of anthocyanins and flavan-3-ols from the grape skin into the pulp. The same authors suggested that a prior contact between these compounds could cause well-known reactions between the anthocyanidin 3-glucosides and flavan-3-ols producing polymeric pigments, with a decrease in the flavan-3-ol contents. However, De Castilhos et al. (2015a) reported that the flavan-3-ols content was not significantly decreased by the grape pre-dehydration. The authors suggested that the copigmentation reaction between monoglucoside anthocyanins and flavan-3-ols was probably stopped because the wines produced using hybrid grape cultivars were composed mainly of 3,5-diglucoside anthocyanins.

De Castilhos et al. (2015) also reported that the antioxidant activity of dried wines was not significantly affected by the heat, suggesting that their antioxidant capacity could be explained by the balance between the loss of antioxidant phenolic compounds and reactions that produced compounds with antioxidant properties such as melanoidins, a Maillard reaction product (Delgado-Andrade and Morales 2005). In the same study, the authors reported on the potential of grape pre-dehydration as an alternative wine making procedure before alcoholic fermentation, in order to produce more structured wines with good body and intense astringency and bitterness, due to their high flavan-3-ol contents.

The results obtained in the above mentioned studies showed the potentials of grape pre-dehydration and thermovinification as alternative wine making procedures aiming at enhancing the phenolic compounds. In the latter case, the increase in the anthocyanin concentration resulted in the intense color of the red wines. Grape dehydration caused anthocyanin degradation and the flavan-3-ols content was apparently not affected by the heat. Sensory approaches showed that thermovinification could enhance the color features of the red wines and grape pre-dehydration could improve the body and structure of the wines, i.e., improving the mouthfeel sensations.

Submerged cap technique

The submerged cap technique aims to provide a constant contact between the pomace and must, avoiding the rise of solids due to the carbon dioxide produced by alcoholic fermentation. This procedure has been a focus of some studies aimed at assessing the enhancement of the extraction of the phenolic compounds as a result of the constant contact of the juice with the grape skins and seeds. Submerged cap technique limits contact with the existing oxygen inside the fermentation vessel and reduces the mechanical operations such as pumping down and pumping over (De Castilhos et al. 2012, 2013; Bosso et al. 2011; Suriano et al. 2012).

De Castilhos et al. (2013) described the influence of submerged cap on red wines produced from Bordô (*Vitis labrusca*) and Isabel (hybrid grape *Vitis labrusca* x *Vitis vinifera*) grapes. This procedure was carried out using stainless steel screens inside the fermentation vessels, aiming to hinder displacement of solids to the upper part of the vessel due to the carbon dioxide produced during alcoholic fermentation. The procedure was carried out on a microvinification scale and information about the enological parameters and sensory acceptance attributes was collected. The submerged cap wine making did not significantly change the results of the chemical analyses, since the acidity, dry extract and alcohol contents of the wines were lower than those of wines produced using the traditional treatment. In addition, the expected effect of enhanced extraction of the phenolic compounds due to the constant contact between the pomace and the must during alcoholic fermentation was not observed, since the total phenolic content and all the color indexes (absorbance at 420, 520 and 620 nm) presented no significant differences when compared with those of traditional wine making. The sensory acceptance of the submerged cap wines also presented no significant differences in comparison with those of the traditional treatment, although these wines were significantly better accepted when compared to the commercial wines. This result explained the modest potential of the submerged cap wine making when applied to Bordô and Isabel red wines, since there were no significant differences between them and the traditional treatment. A positive outcome of the study was the higher yield of the wine produced in relation to the initial amount of grapes when the submerged cap wine making procedure was used, i.e., about 1.5-fold that observed for the traditional treatment.

In another study, Bosso et al. (2011) applied the submerged cap technique in the production of wines from Barbera grapes, and compared it with the floating-cap wine making procedure. The method used to apply the submerged cap wine making technique was the use of a grate fixed at the top of the fermentation vessel to keep the cap submerged at approximately 10 cm depth in the must during alcoholic fermentation. They applied a short pump-over with 25 to 30% of the total volume of the must twice a day with no air, up to the end of maceration. The wine composition, and the polyphenolic and free volatile compounds were analyzed. The submerged cap technique increased the total extract and total acidity, and presented higher total anthocyanin and total flavonoid contents. All the color parameters were higher than those of the floating cap wines. The pressed wines and the pomace resulting from these two wine making procedures were also evaluated. The pressed wines and pomace from submerged cap presented significantly higher anthocyanin, total flavonoid and proanthocyanidin contents in comparison with the floating cap treatment.

Considering the obtained free volatile compounds, Bosso et al. (2011) noted lower C6 alcohol, acetate and higher alcohol concentrations in the submerged cap Barbera wines. The C6 alcohols are produced during the prefermentative phase by enzymatic lipoxidation of the unsaturated fatty acids, and the concentration of these compounds is closely related to the grape variety, mechanical operations and oxygen intake during the fermentative process. The lower concentrations of these compounds were due to the different oxygen intakes during alcoholic fermentation. In addition, the authors stated that the Barbera grape cultivar had poor amounts of terpenic and norisoprenoidic compounds such as linalool, α-terpineol, citronellol and β-damascenone; however, the submerged cap treatment increased the concentration of these compounds, and is considered an alternative wine making procedure to obtain wines with intense varietal features.

As another example, Suriano et al. (2012) provided relevant information about the chemical profile and sensory assessment of Nero di Troia wine produced using two alternative wine making procedures: submerged cap (horizontal winemaker-tank) and vertical winemaker-tank, and both procedures were compared to a traditional wine making technique. In their study, the submerged cap treatment was obtained using a horizontal winemaker-tank with a rotary steel vat composed of two horizontal and concentric cylindrical tanks. The tank worked with a 1 minute rotation every 3 hours at minimum speed, and after two turns, the direction of the rotation changed. The submerged cap treatment differed from those two aforementioned studies in which the behavior of the wine making procedure was assessed on an industrial scale using a rotary tank. The submerged cap technique significantly enhanced the dry extract of the wines as well as presented higher concentrations of total polyphenols, total flavonoids, flavans and proanthocyanidins. In addition, the wines presented higher concentrations of total anthocyanins and monomeric anthocyanins. The authors noted the potential of submerged cap wine making to produce high color wines, and to allow for a better dissolution of tannins and other substances responsible for the wine color intensity. The chemical results were confirmed by the sensory results, since the submerged cap wines presented relevant color intensity and more marked purple hues. In addition, they also presented intense cherry and dried prune olfactory features. With regard to the flavor descriptors, the wines were astringent, bitter and structured, due to the high extraction of catechins and tannins.

De Castilhos et al. (2015a) also evaluated the polyphenolic composition and sensory descriptors of wines produced from BRS Rúbea and BRS Cora hybrid grape cultivars, cultivated in Brazil using the submerged cap technique. They reported the potential of submerged cap as an alternative wine making procedure aiming at producing wines with intense color features, since they present higher anthocyanin concentrations. The findings showed the importance of the submerged cap to obtain highly colored wines, and this wine making procedure could be an alternative for grapes that present weak color features and varietal characteristics, since it could also enhance compounds that are intrinsic to the grape cultivar.

Cold soaking technique

The cold soaking procedure has received much attention worldwide and has been applied in most wine-growing regions, which are typically great producers of different grape cultivars. Prefermentative cold soaking consists of the contact of the fermentation solids (skins and seeds) with the must in a non-alcoholic and low-temperature environment (Casassa and Sari 2015). The absence of ethanol was assured by keeping the must at low temperatures, ranging from 5 to 10 ºC (Casassa et al. 2015) or from 10 to 15 ºC (Sacchi et al. 2005), for a period of time ranging from 3 to 5 hours per day for up to 10 days (Gil-Muñoz et al. 2009; Gordillo et al. 2010; Ortega-Heras et al. 2012). The chilling to which the pomace and the must were submitted ensured the lack of fermentative activity, avoiding the development of *Saccharomyces cerevisiae* (Zott et al. 2008) and, in contrast, favors the development of non-*Saccharomyces* yeasts, which would probably modify the volatile profile of the wine (Charpentier and Feuillat 1998). In addition, the absence of ethanol allows for a selective extraction of high water solubility compounds, including anthocyanins, free and glycosylated-bound aroma compounds and low molecular weight tannins (Apolinar-Valiente et al. 2013).

Some studies have reported that the extraction of anthocyanin and tannin due to the application of cold soak wine making was related to an intrinsic feature of the grape cultivar; however, several reports in the literature have presented controversial results. Some studies reported an increase in the extraction of phenolic compounds (Busse-Valverde et al. 2010; González-Neves et al. 2013; Favre et al. 2014), others reported a decrease (Budic-Leto et al. 2003; González-Neves et al. 2012) and no effect of cold soaking on the extraction of phenolic compounds (Ortega-Heras et al. 2012; Pérez-Lamela et al. 2007). The contradictory results found in the above mentioned studies led the researchers to search for an expansion of their knowledge in order to evaluate the real effects of cold soaking on the extraction of the phenolic compounds. Despite the results of the chemical assessment of the products from the cold soaking procedure, some authors were unable to agree about its effects on the sensory attributes. The authors reported that the negative impact of the non-*Saccharomyces* yeasts on wine flavor was due to the formation of ethyl acetate and acetaldehyde (González-Neves et al. 2013; Casassa and Sari 2015).

Casassa et al. (2015) provided information about the phenolic compound and chromatic compositions, Using the CIELab parameters, of six wines produced from Barbera, Cabernet Sauvignon, Malbec, Merlot, Pinot Noir and Syrah grape cultivars submitted to the cold soaking prefermentative treatment. The wine making procedure consisted of de-stemming the grapes and crushing them, allowing for the release of the must, which was treated with 80 $mg.L^{-1}$ of sulfur dioxide. The cold soaking treatments consisted of 4 days at approximately 9 ºC, achieved by the insertion of CO_2 pellets. The 4-day cold soaking period was followed by a 10-day maceration process carried out by two pumping-overs followed by two pumping downs. The alcoholic fermentation was initiated by the inoculation of active dry *Saccharomyces cerevisiae* yeasts, and after the maceration process, malolactic fermentation was also induced by inoculation with *Oenococcus oeni*. At the end of the malolactic fermentation, the wines were racked and stored at 1 ºC for 45 days to allow for the tartrate stabilization. The free SO_2 content was then adjusted and the wines bottled and stored at 12 ºC until analyzed. The cold soaking maceration technique significantly increased the anthocyanin concentration as compared to traditional wine making, as well as increasing the Chroma and redness of the wines. The total phenolic compounds, tannin concentration and the other CIELab parameters (luminosity, hue angle and yellowness) were not affected by the cold soaking procedure. The sensory approach only showed the influence of cold soaking on two attributes: color intensity and violet hue. Positive effects of the cold soaking treatment were observed for the Barbera and Cabernet Sauvignon wines; the Barbera wines obtained higher scores for color intensity and violet hue, and the Cabernet Sauvignon wines for color intensity. In contrast, cold soaking produced negative effect for the Pinot Noir wines, which showed lower scores for color intensity when compared with the traditional treatment. The cold soaking technique had also no effect on the aroma attributes, astringency, bitterness and body; these sensory attributes presented no significant differences when comparing the traditional and cold soaking treatments.

Casassa et al. (2015) have also applied the Principal Component Analysis to obtain relevant information about the relationship between the treatments and the wines produced from the different grape cultivars. The Principal Component Analysis explained the major data variance and allowed for differentiation of the grape cultivars, allocating them in two different clusters. The cold soaking was greatly influenced by the intrinsic features of the grape cultivars.

Other pertinent studies showed a lack of differences between the traditional and cold soaking wine making procedures, mainly concerning the sensory assessment. Using a triangle test, Gardner et al. (2011) described the failure of consumers to detect differences between Cabernet Sauvignon wines produced using the traditional and cold soaking treatments. In another study, Casassa and Sari (2015) reported lower color intensity and reduced fruity flavor in Malbec red wines submitted to cold soaking, as well as a noticeable acetaldehyde character.

Favre et al. (2014) described the application of a cold prefermentative procedure to Tannat wines and compared it to three other wine making procedures: traditional maceration, maceration with the addition of enzymes and maceration with the addition of seed tannins. The wine making procedure consisted of de-stemming the grapes followed by crushing, allowing for the release of the must. Alcoholic fermentation was induced by inoculation of yeasts, and the must was then drained and the pomace pressed. The free-run must was mixed with the pressed must and they were stored in stainless steel fermentation vessels until the end of the alcoholic fermentation. After this, sulfur dioxide was added to inhibit malolactic fermentation and the wine was bottled. The cold soaking procedure consisted of promoting the contact of the skins with the must at 10–15 ºC for 5 days prior to alcoholic fermentation, using frozen water as the cooling agent. The total phenolic compound content, anthocyanidins, flavan-3-ols and proanthocyanidins were assessed, as well as the low molecular weight non-anthocyanin phenols. The color features were also evaluated according to the CIELab parameters. The authors demonstrated that cold soaking increased the total phenolic compound and anthocyanin contents of Tannat red wines. The five anthocyanidins (cyanidin, delphinidin, petunidin, peonidin and malvidin), detected and quantitated by HPLC in cold soaked Tannat wines, presented higher concentrations when compared with the traditional maceration process, with the exception of delphinidin. In addition, among the low molecular weight non-anthocyanin phenols, the cold soaked wines showed higher concentrations of stilbenes, flavan-3-ols and flavonols when compared with the traditional procedure. The color features of the cold soaked wines seemed to be less influenced by the treatment, since the CIELab parameters presented no significant differences when compared to the traditional wines. Another important result was the high concentrations of tyrosol and triptophol in the cold soaked wines, which are compounds derived from yeast metabolism, probably due to the activity of the native yeast strains in the prefermentative phase.

Based on the above mentioned studies, the application of cold soaking as a potential alternative wine making procedure needs to be further discussed and expanded by other studies. The grape cultivar used in cold soaking wine making technique seems to enhance the phenolic compounds extraction. In addition, important factors such as the application of low temperatures by the addition of CO_2 pellets or external refrigeration need to be intensely discussed (Casassa et al. 2015).

Carbonic maceration

Carbonic maceration aimed to produce lighter and fruitier wines that are indicated for young consumption. In this procedure, whole berries or grape clusters were submitted to a carbon dioxide saturated (CO_2) atmosphere, which allowed for the activity of the glycolytic enzymes proceed from the grapes (Sacchi et al. 2005). After one or two weeks, the grapes were then pressed and the must inoculated to complete the alcoholic fermentation. In fact, a natural enzymatic transformation occurs in the grape skins due to the anaerobic conditions promoted by the saturated CO_2 environment. The berries suffered an intracellular fermentation in which the malic acid is metabolized to ethanol and other substances (Rizzon et al. 1999).

According to several studies, it is difficult to draw secure conclusions about the effect of carbonic maceration procedure on the phenolic compounds of wines. Very different results have been found with different grape cultivars. Some studies showed that carbonic maceration caused a decrease in the phenolic compound concentrations (Sun et al. 2001; Timber lake and Bridle 1976), while other studies showed an increase in the phenolic composition (Lorincz et al. 1998). In addition, the carbonic maceration process could lead to the production of lighter wines with reduced body and structure and an intense fruity aroma, especially for strawberry and raspberry aromas (Etaio et al. 2008).

Rizzon et al. (1999) applied carbonic maceration to the production of Cabernet Franc wines, placing the grapes in a 4,000 liter fermentation vessel and injecting carbon dioxide to an internal pressure of 0.5 atm. This pressure was maintained for 10 days, and the temperature varied from 25 to 30 ºC. The whole grapes were then pressed to obtain the fermentative must and alcoholic fermentation was induced by the inoculation of dry active *Saccharomyces cerevisiae* yeasts. After the final stage of alcoholic fermentation, the wines were refrigerated at –4 ºC to allow for tartrate stabilization, and then bottled.

According to the authors, the carbonic maceration wines presented lower anthocyanin concentrations and reduced color features. These wines showed an intense oxidized tonality when compared with the traditional and thermovinification procedures. In addition, they presented a higher pH and lower total acidity, since the malic acid was enzymatically transformed into ethanol inside the grape berries, and caused a decrease in the total acidity of the wines (Rizzon et al. 1999). The wines submitted to the carbonic maceration procedure presented weak aroma quality and a lower score for the flavor and olfactory balance. They were also lighter, less structured and with reduced body, due to the lower acidity and phenolic compound concentrations.

Bertagnolli et al. (2007) presented results concerning the influence of carbonic maceration and ultraviolet irradiation on the *trans*-resveratrol levels in Cabernet Sauvignon red wines. The grapes were submitted to different treatments and carbonic maceration was carried out at a temperature of 0.5 ºC in an environment of 10% CO_2. After the treatment, the grapes were de-stemmed and crushed, allowing for the release of the must. The must was treated with SO_2, chaptalized, and alcoholic fermentation induced by inoculation with *Saccharomyces cerevisiae* yeasts. At the end of the alcoholic fermentation, the wines were racked, submitted to malolactic fermentation, racked again and bottled for 2 months. The carbonic maceration wines presented an increase in the *trans*-resveratrol concentration in the first days of alcoholic fermentation, but afterward it decreased. This behavior was probably due to the saturated CO_2 environment that changed the reaction for the synthesis of *trans*-resveratrol, since its synthesis needs the elimination of four molecules of CO_2. One positive outcome of the use of carbonic maceration was related to the reduction in processing time, i.e. the pre-fermentation occurred inside the grape berries due to the influence of the carbon dioxide, allowing for a decrease in the wine making time. In addition, Bertagnolli et al. (2007) also analyzed the *trans*-resveratrol concentration in the grapes right after the treatments, and reported that grapes submitted to carbonic maceration showed the lowest *trans*-resveratrol concentrations in comparison to the other treatments assessed.

Fuleki (1974) analyzed the effect of carbonic maceration on wines produced from the Concord grape cultivar (*Vitis labrusca*). The grapes were submitted to a saturated CO_2 environment at 15 ºC for one or two weeks. After the treatment, the grapes were pressed and the must treated with sulfur dioxide. Alcoholic fermentation was then induced, the pomace was pressed and drained, the must was chaptalized and the alcohol content adjusted to 13% v/v. The wines were then maintained at 2 ºC for two weeks to allow for tartrate stabilization, and then racked, bottled and stored at 15 ºC. A chemical and sensory evaluation was carried out to evaluate the effect of carbonic maceration on the wines produced from the Concord grape cultivar.

The carbonic maceration has appeared as a suitable technique to produce Concord wines without the Concord character, i.e., the intrinsic aroma compounds of the Concord grape were degraded by carbonic maceration and a new flavor was developed. The taste of the wines was described as softer as a result of the decrease in acidity and lower tannin content. In addition, carbonic maceration probably degraded the methyl anthranilate, which is a volatile compound typical from *Vitis labrusca* grapes, including Concord, since the produced wine showed low concentrations of this compound when compared to the control treatment.

In summary, the studies concerning the application of carbonic maceration presented a slightly positive outcome, i.e. this technique reduced the production time because the pre-fermentation occurred in the grape berries before crushing and alcoholic fermentation. The wines presented low tannin concentrations, were light-structured and light-bodied, and the aroma profile was different from the one expected due to compounds which are naturally present in the grape cultivars.

Application of Different Yeast Strains for Alcoholic Fermentation

Important studies have provided information about the effect of the selected yeast on the chemical and sensory profiles of wines, mainly regarding the effect on the phenolic compounds and color features. It has been suggested that yeast lees absorb anthocyanins (Morata et al. 2003) and that macromolecules released on yeast autolysis can bind polymeric phenols, resulting in an effect similar to that provided by protein-fining agents (Sacchi et al. 2005). The mannoproteins released from different yeast species have been examined and there are hypotheses of the enhancement of the anthocyanin-tannin condensation reaction, resulting in a decrease in wine astringency (Escot et al. 2001). Mannoproteins are glycoproteins with a high glycosylation level, mainly composed of mannose (approximately 90%), glucose and proteins (approximately 10%) (Guadalupe et al. 2010; Vidal et al. 2003). The amount of mannoproteins released depends on the yeast strains and the wine making conditions (Giovani et al. 2010).

Studies have shown that the mannoproteins might improve certain negative sensory attributes such as bitterness and astringency, enhancing the persistence and mouthfeel of wines. In addition, they appear to improve color stabilization due to the colloid protector activity provided by these yeast metabolites (Del Barrio-Galán et al. 2012). Other studies have shown that these complex polysaccharides could avoid the interaction between the flavan-3-ols and the salivary proteins via different mechanisms, decreasing the astringency sensation (Carvalho et al. 2006).

Del Barrio-Galán et al. (2015) analyzed the chemical and sensory profiles of Syrah red wines fermented by different strains of *Saccharomyces cerevisiae*, which presented different capabilities for polysaccharide liberation. The wines were produced following a standard wine making procedure applied in the Caliterra winery, Chile. Briefly, the grapes were harvested at their optimal ripening stage and crushed allowing for liberation of the must, which was treated with sulfur dioxide. Alcoholic fermentation was induced by the inoculation of two *Saccharomyces cerevisiae* strains: a conventional one used in the Caliterra winery, and another one which produces high levels of complex polysaccharides during alcoholic fermentation. Fermentation was carried out at a controlled temperature ranging from 21 to 25 ºC, and once fermentation was completed, the wines were racked, allowing for malolactic fermentation which took place spontaneously. After the malolactic fermentation, the sulfur dioxide concentration was corrected and the wines bottled. The different yeast strains resulted in no significant differences in the oenological parameters. The wine fermented by the yeast that produces high levels of complex polysaccharides presented lower contents of all phenolic compounds, except for the hydroxycinnamic acids, which presented similar amounts in both treatments. These results could be due to the interaction or adsorption phenomena that occurred between the mannoproteins released by the yeasts and the phenolic compounds, mainly anthocyanins, during alcoholic fermentation. In addition, the sensory results indicated lower values for alcohol, bitterness and astringency, and higher values for red fruit flavor, persistence and mouthfeel for the wine which was fermented with complex polysaccharides producing strains. These results were in agreement with other studies which reported the effect of mannoproteins in minimizing astringency by complexation with the tannins (Del Barrio-Galán et al. 2011; Del Barrio-Galán et al. 2012). In summary, the application of a large amount of mannoproteins producing yeast strain could improve the wine palate by decreasing astringency and bitterness. However, this alternative wine making procedure could present undesirable effects on the color intensity of red wines, since the phenolic compounds, mainly anthocyanins, were also complexed by the polysaccharides produced by these *Saccharomyces* yeast strains.

Saberi et al. (2012) reported on the volatile and sensory profiles of Chardonnay white wines fermented with individual commercial, individual Burgundian and mixed Burgundian yeast strains. The novel *Saccharomyces cerevisiae* strain was isolated from a vineyard in the Burgundy region, France, and the strains were mixed in different proportions–1:1, 1:2, 1:3 and 2:3. These strains were recommended for white wines, especially Chardonnay, aiming to increase the fruity aroma and complexity. The Chardonnay fermentation was carried out at 16 and 20 ºC aiming to optimize the retention of volatile compounds. The musts were inoculated using the ratios described above, but the yeast strains were not mixed before inoculation. The fermentation bottles were capped to provide an anaerobic environment and when fermentation was completed, the wines were treated with 100 $mg.L^{-1}$ of potassium metabisulfite to avoid oxidation. The volatile compounds were analyzed via the headspace and the compounds identified and quantitated by gas chromatography-mass spectrometry. No significant differences were observed in volatile composition when the fermentation temperatures were compared. Eighteen compounds were identified and quantitated, including eight higher alcohols, five ethyl esters, three acetate esters, one aldehyde and one organic acid. The concentration of the volatile compounds for the individual and mixed Burgundian yeast strains was compared to industrial ones. The Burgundian strains produced more berry, fusel oil, candy and balsamic aromas, but the levels were below the human perception threshold. These strains also produced lower levels of nail polish and vinegar aromas. The sensory profile revealed that the individual and mixed Burgundian yeast strains produced wines with fruity aromas such as sweet fruit, strawberry, green apple, pear and banana. The Principal Component Analysis was successfully applied and it differented the Burgundian and commercial yeasts. The positive outcome of the mixed Burgundian strains may have been due to metabolic interactions between strains, allowing them to respond in a different form according to the different substrates. This study showed the high potential of applying an isolated yeast strain to obtain fruity, more balanced and complex wines with unique features (Saberi et al. 2012).

Pectinolytic Enzymes

The use of pectinolytic enzymes in the wine industry has been the focus of many studies since it maximizes the extraction of free-run juice during maceration and helps in the wine clarification and filtration (Ducasse et al. 2010). In addition, the pectinolytic enzymes, also known as pectinases, have been used in wine making trials to obtain an increase in wine color due to rupture of the skin cell walls, releasing the pigments. These enzymes are carbohydrases that catalyze the breakdown of pectic substances. Both the grape and the yeast showed pectinase activity, although their effect can be limited by the sugar and alcohol contents. The use of pectinases in wine making brings several benefits including the faster start of fermentation, higher yield of must flow from the dejuicing step, easier pressing of the pomace, enhancement of the clarification activity and an important extraction of phenolic and aroma compounds from the grape skin (Pardo et al. 1999).

Studies on the effect of enzymes on wine color and phenolic compounds have led to controversial results: some have indicated an increase in wine anthocyanin content and an improvement in wine color due to the application of pectinolytic enzymes (Kelebek et al. 2007; Bakker et al. 1999), some studies have shown a decrease in these compounds (Bautista-Ortin et al. 2005; Wightman et al. 1997), and others showed no significant effect (Ducasse et al. 2010). These discrepancies could be explained by several factors, such as different compositions of grape polyphenolics, the extraction rate in the maceration step and reactions between them during the wine making process and aging (Ducasse et al. 2010).

Many of the enzymatic preparations are enzyme complexes, which can promote some collateral effects in musts and wines, such as loss in the anthocyanins stability promoted by cinnamate-esterase activity (Günata et al. 1986), intense turbidity and loss of color promoted by β-glucosidase activity (Somers and Ziemelis 1985) and pectin hydrolysis in the grape skin by pectin methylesterase, promoting an increase in methanol in the juices and wines (Lago-Vanzela et al. 2014b). According to the latter authors, the toxicity of methanol is low; however, other compounds are produced in the metabolic process, such as formic aldehyde and formic acid. The methanol content and phenolic extraction depended on the type and amount of added enzymes, the grape cultivars and the time of contact between the grapes and the enzymes. Enzymatic preparations with low pectin methylesterase activity and high pectinlyase activity could minimize the formation of methanol in wines submitted to this treatment (Gómez-Plaza et al. 2001).

Echeverry et al. (2005) reported changes in the antioxidant activity of Tannat red wines during early maturation. The wines were submitted to different maceration times and treated with pectinolytic enzymes. The musts were treated with SO_2 and alcoholic fermentation was induced by the inoculation of *Saccharomyces cerevisiae* (15 $g.hL^{-1}$). The use of a pectinolytic enzyme with color extractor features enhanced the anthocyanin, flavonol, flavan-3-ol and proanthocyanidin contents, but the results provided no information about the presence of significant differences between the treatments. The authors also reported the higher antioxidant capacities of wines with higher polyphenol content, but stated that the wine antioxidant capacity was determined not only by the grape variety, vineyard methods and wine making techniques, but also by the reactions occurring during the wine making process.

Conclusions

Considering the alternative wine making techniques reviewed in this manuscript, thermovinification and submerged cap allowed for enhancement of the color features by increasing the anthocyanin contents. Cold soaking and carbonic maceration presented distinct results and seemed to be influenced by the intrinsic factors of the grape cultivars. Carbonic maceration could be considered as an alternative wine making procedure that degrades the varietal features of the grape, since these characteristics were not transferred to the wine. Further studies need to be developed aiming at evaluating the real effect of cold soaking and carbonic maceration on the chemical and sensory profiles as well as on the wine polyphenolic compound concentrations, since there seemed to be a strong effect of the grape on these two wine making procedures. The yeast selection procedure and the use of mixed yeast strains resulted in a decrease in the color features due to the prior stabilization of the phenolic compounds by the mannoproteins. In addition, the wines produced from isolated yeasts showed good structure and mouthfeel and enhancement of the fruity aromas. The application of pectinolytic enzymes could improve the color features of red wines, since they cause rupture of the skin cell walls releasing the anthocyanins, which are responsible for the attractive color of red wines. In addition, other phenolic compounds could be released by the use of pectinolytic enzymes, enhancing the antioxidant capacity of the wines. A negative outcome is related to the formation of considerable methanol by these enzymes, since methanol is highly toxic.

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