Agricultural University of Athens

Department of Food Science and Technology & Human Nutrition

Laboratory of Food Engineering

MASTER THESIS

The contribution of volatile oxidation indicators to the description and prediction of the quality changes in preserved in bottles white wine.

Συμβολή πτητικών οξειδωμετρικών δεικτών στην περιγραφή και πρόβλεψη της εξέλιξης των ποιοτικών μεταβολών του συντηρημένου λευκού εμφιαλωμένου κρασιού.

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Food Processing and preservation

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Abstract

The flavor profile evolution and it's rate may indicate the cohesions existing in a packed wine-storage-environment system. The objective of this project was to identify the characteristic of flavor and off-flavor compounds that could be used as oxidation markers, the recording of their evolution and their impact on consumer perception. Quality parameters, evolved in glass-bottled wines, closed with two types of corks differing in their oxygen permeability properties, were in focus. Wines varieties and origin (winery) were: Assyrtiko, Malagouzia and Sauvignon blanc, all harvested, extracted and bottled in fall of 2014. Two bottles of each variety/winery were withdrawn from each of the storage areas (18-20 and 30^{o}C, all at dark) every 3 months. Isolation, detection and quantification of aroma compounds were performed by a SPME/GC-DBWAX-FID system. Resistance to oxidative degradation (absorbance at 420nm), acetaldehyde concentration, total and free-sulfur dioxide content (by iodine titration), and antioxidant potential (by resistance to oxidation) were also tested. A panel of 25 trained persons performed the organoleptic examination of all samples in order to provide the "quality limits", i.e. the acceptance of the samples and to recognize the detection threshold of un-favored aroma notes. Analysis of samples at the beginning of the storage period, showed that presence of specific flavor compounds in certain samples only. Evolution of new flavor compounds was not apparent during the first 3 months of storage, independent of the type of the corks and temperature of storage, very much in accordance to sensorial remarks. Physicochemical analysis indicated the absence of significant oxidative degradations, supporting the aforementioned conclusion regarding absence of off flavors. Samples stored for 7 months had a rather significant alteration in their flavor profile. Results will be reported and interpreted using the root cause analysis versus packaging and storage conditions.

KEYWORDS: packaging, aroma, wine, oxidation, closures

Περίληψη

Το αρωματικό προφίλ των οίνων και ο ρυθμός εξέλιξης του μπορεί να υποδεικνύει τις αλλαγές που συμβαίνουν στο σύστημα κρασί-αποθήκευση-περιβάλλον. Ο στόχος αυτού του έργου είναι ο προσδιορισμός των αρωματικών συστατικών και οι ενώσεις με δυσάρεστη οσμή που θα μπορούσαν να χρησιμοποιηθούν ως δείκτες οξείδωσης, η καταγραφή της εξέλιξης τους και το αντίκτυπό τους στην αντίληψη του καταναλωτή. Παραμέτροι ποιότητας, ελέγχθηκαν σε εμφιαλωμένα κρασιά σε γυάλινες φιάλες, με δύο τύπους φελλών που διαφέρουν στις ιδιότητες διαπερατότητας οξυγόνου τους. Οι ποικιλίες κρασιών ήταν: Ασύρτικο, Μαλαγουζιά και Sauvignon blanc παραγωγής του έτους 2014. Χρησιμοποίηθηκαν δύο φιάλες κάθε ποικιλίας σε θερμοκρασίες συντήρησης 18-20^{o}C και 30^{o}C, όλα στο σκοτάδι και η δειγματοληψία πραγματοποιήθηκε σε χρόνο 0, 3 και 7 μήνες. Ταυτοποιήθηκαν και ποσοτικοποιήθηκαν αρωματικές ενώσεις με ένα σύστημα GC-FID DBwax-SPME καθώς και η ένταση χρώματος (απορρόφηση στα 420 nm), η συγκέντρωση ακεταλδεΰδης (απορρόφηση στα 570 nm, συνολική περιεκτικότητα σε ελεύθερο και ολικό θειώδη ανυδρίτη (τιτλοδότηση με ιώδιο) καθώς και η αντιοξειδωτική ικανότητα (απορρόφηση στα 515 nm). Ακόμη μια ομάδα 25 εκπαιδευμένων ατόμων πραγματοποίησε την οργανοληπτική εξέταση όλων των δειγμάτων, προκειμένου να παρέχουν τις «όρια ποιότητας", δηλαδή την αποδοχή των δειγμάτων και να αναγνωρίσουν το όριο ανίχνευσης αρωματικών συστατικών που διαφέρουν σημαντικά μεταξύ διαφορετικών υλικών συσκευασίας. Η ανάλυση των δειγμάτων κατά την έναρξη της περιόδου αποθήκευσης, έδειξε ότι την παρουσία συγκεκριμένων αρωματικών ενώσεων μόνο σε ορισμένα δείγματα. Η εξέλιξη των νέων ενώσεων δεν ήταν εμφανής κατά τη διάρκεια των 3 πρώτων μηνών της αποθήκευσης, ανεξάρτητα από τον τύπο των φελλών και θερμοκρασία αποθήκευσης, σύμφωνα και με τις οργανοληπτικές παρατηρήσεις. Οι φυσικοχημικές ανάλυσεις έδειξαν την απουσία σημαντικής οξειδωτικής υποβαθμίσης, υποστηρίζοντας το συμπέρασμα σχετικά με την απουσία των off-flavor ενώσεων. Ωστόσο στο τέλος της συντήρησης στους 7 μήνες υπήρχε μια σημαντική αλλαγή στο αρωματικό τους προφίλ. Τα αποτελέσματα καταγράφονται και να ερμηνεύονται με τη χρήση της ανάλυσης σε σχέση με τις συνθήκες συσκευασίας και αποθήκευσης.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: συσκευασία, άρωμα, κρασί, οξείδωση, φελλοί.

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CHAPTER I

INTRODUCTION

1.1. Products preservation and packaging

Advances in food processing and food packaging play a primary role in keeping the food supply among the safest in the world. Simply stated, packaging maintains the benefits of food processing after the process is complete, enabling foods to travel safely for long distances from their point of origin and still be wholesome at the time of consumption. However, packaging technology must balance food protection with other issues, including energy and material costs, heightened social and environmental consciousness, and strict regulations on pollutants and disposal of municipal solid waste. (Marsh and Bugusu, 2007).

The principal roles of food packaging are to protect food products from outside influences and damage, to contain the food, and to provide consumers with ingredient and nutritional information. Traceability, convenience, and tamper indication are secondary functions of increasing importance. The goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, maintains food safety, and minimizes environmental impact (Marsh and Bugusu, 2007).

There is currently no product that sold bare, after it should be protected from the external environment to preserve it for the maximum time possible. Although some mechanisms alteration would take place even without mass transfer (or heat) between the outdoor and indoor environments can increase the shelf life of products with the selection and application of appropriate packing materials. The package related to food safety at two levels:

First, if the packaging does not provide the immediate barrier to microorganisms or indirectly through permeability to oxygen, moisture and light, the food will be exposed to the factors likely to favor the alteration of which would otherwise be much slower. Second, the migration of potentially toxic elements from some packaging materials to food is possible under conditions increase the risk and concerns about reduced consumer safety but also to alter the specific characteristics of the product (Καναβούρας, 2010).

To understand the effect of packaging on the product, we must first define the concept of quality of the food. One way is through the description of the main quality characteristics such as color, texture, flavor, structure, taste, appearance and nutritional value of the food. Some of them are immediately visible to the consumer while others do not (nutritional value). Knowledge of basic food spoilage reactions that affect the quality, is the first step in the design and development of food packaging. This package should ensure minimal change through unwanted changes of the aesthetic qualities of the product and maximize the development and maintenance of desired properties. Once you understand the nature of the reactions, knowledge of the factors that determine the rate of these reactions is essential to have complete control of the changes taking place in food during storage and maintenance, i.e while staying packed (Καναβούρας, 2010).

1.2. Principal deterioration reactions of food

Chemical changes. Many important deteriorative changes can occur arising from reactions within the food or from reactions of food components with external species, for example oxygen. Rancidity development is an important factor in fat – containing foods, oxidative reactions and flavor reversion reactions. Chemical hydrolysis can occur in products containing intense sweeteners, reducing sweetness and non – enzymic browning can occur in many foods from Maillard reactions. Changes can also occur on exposure to light, including color loss in natural food colors and rancidity and off flavor development (The stability and the shelf life of the food).

Color changes. Accepting the color of a product depends on many factors among them cultural, geographical and social. Nevertheless certain food groups is accepted only if they fall into defined color boundaries. The color of many foods depends on the presence of pigments such as:

a. Chlorophylls
b. Blood pigments
c. Anthocyanins
d. Carotenoids
e. Various natural dyes

Changes of "bouquet". By bouquet describe the overall acceptance of the senses of smell and taste when consumed in food (Καναβούρας, 2010).

Physical deteriorative reactions. Moisture migration is a major cause of deteriorative physical changes in food. Physical changes in packaging materials, sometimes coupled, with subsequent chemical reactions, can also limit sensory shelf – life. As an example, permeability changes with time can change the in – pack equilibrium atmosphere, giving rise to both microbiological and chemical effects. Such changes may also allow migration of external volatiles into the food, resulting in the development of taint. Migration of chemical components from the packaging material can also produce taints, and this can be particularly serious in products with a long shelf – life (The stability and the shelf life of the food).

1.3. Shelf life

The quality of most food decreases during time so the food is not fit for consumption after a certain time. The point to consider as the beginning of the shelf life of the product may vary depending on the product, the processes and accepts the management system and the movement. Typically, the shelf life begins from the moment the product is packed, for this reason the packaging should maintain the product quality intact for the maximum time possible, ie to extend the shelf life as possible. Of course, the benefits of this expansion is mainly economic losses as limited to spoiled food but growing and consumer satisfaction both through the maintainability of food and use products with high quality characteristics. The cost of the package is increased by using materials and methods that help maintain quality. This cost should be compared to the economic benefit from the extension of shelf life through the increase in sales (initial and recurrent).

In general, therefore, shelf life is the period during the food retains acceptably those key characteristics that determine quality but ensures that and the consumer safety is not at risk.

Indirectly it is concluded that the quality is feature dependent and largely determined by the consumer. Of course, there are measurable chemical, physical and organoleptic characteristics that quantified can determine the quality of a food and population growth of microorganisms in food is indisputable criterion of suitability for consumption. The factors that control the life of a product are:

a) **The characteristics of the product**
How perishable is the percentage of free space and 'wholesale' density, the phenomena of concentration of the components that affect the rate of deterioration reactions.
b) **The treatment of conditions** that affect and requirements in subsequent protection and thus determine the requirements on materials and packaging methods. A typical example is the aseptic packaging in which the product is processed and standardized to strict hygienic conditions and areas resulting in high microbiological purity which should be maintained thereafter. Thus, the packing materials should be selected based on their potential contribution to meeting this requirement.
c) **The environment** in which the product is exposed during storage and distribution of climatic factors such as those involved in the permeability of packaging materials (humidity, oxygen, carbon dioxide, etc.), and their absorption by the food itself (e.g., humidity, oxygen, etc.) and of course the temperature.
mass transfer phenomena
heat tranfer phenomena
both mass and heat transfer phenomena
d**) The properties of the package**
Moisture transfer
Gases and odors transfer
Interaction food / packaging material (Καναβούρας, 2010).

1.4. Permeability of gases and vapor

Dissolution and transport of low molecular weight substances through the materials is of primary importance for the maintenance of these packaged foods or other products (hydroscopic products). The protection of such products is also dependent on the integrity of the package.
In general the gases pass through the packaging in two ways:
a. Via resources, pinholes and cracks, which may be located in the membrane material, while the probability of the presence increases with decreasing film thickness, and
b. Through the phenomenon dissolution - diffusion, in which the gases dissolved in the in the mass of material, due to diffuse potential difference (pressure) and evaporate once they get on the other side, thereby transferred to the product or the surrounding interior. This process is described as active diffusion Permeability, P.

Under constant conditions, the gas phase of a component can be diffused in the mass of material at a steady rate since maintain the pressure difference between the two sides of the membrane (external = environment, internal = space within the package where the product is packed). Therefore, an A surface, passes a constant amount Q, at time t. Assuming that the pressure difference DP = $P_{external}$ - $P_{internal}$ and correspondingly the amount of the substance will be $C_{external}$ - $C_{internal}$ and material thickness as x, then the transmittance of the film is:

$P = \frac{Q*x}{A*t*\Delta P}$ (1.4.1)

There are some assumptions to apply the above equation:

a. Diffusion is a solid state

b. The presence of the gas within the mass of the material is linear.

c. The diffusion takes place only in one direction.

d. The solubility and diffusion is independent of concentration. This applies to gases such as O_2, H_2 and N_2, and for gases differ slightly from the laws of gases such as CO_2. But when there is a strong interaction between the gas phase and polymer as in the case of water vapor and nylon or regenerated cellulose, or many organic solvents (e.g., perfumes) which are dissolved in organic polymers, this assumption is not valid.

e. The amount of gas that permeates the membrane and reaches the space surrounding the product is consumed immediately by the product that the concentration difference (pressure) between the parts of the membrane remains constant.

Because of the finite gas diffusion mode into the bulk of the polymer, there is an initial period when the steady state has been reached. That is, the amount of gas that permeates the film gradually increased until it stabilized at the maximum price set by the factors described in equation 2.1. This time L, depends on the thickness of the material and the diffusion coefficient D:

$L = \frac{X^2}{6D}$ ή $X = \frac{X^2}{6L}$ (1.4.2.)

The diffusion coefficient D, is given in units of thickness^2 / year, typically in cm^2 / sec. (Καναβούρας, 2010).

1.5. Wine packaging

The history of humans and wine goes back a long way. Indeed, wine has been a part of human culture for almost 6000 years. In that time, many improvements have been made in both viticulture and winemaking techniques, from the domestication of *Vitis vinifera* through the development of systematic written studies by different monastic orders beginning in the 10th century. Biological understanding of the fermentation processes occurring during winemaking took a leap forward with the remarkable work of Pasteur in the middle of the 19th century, when the scientist became the first to consider the importance of oxygen for wine production and ageing. Since the 1960s, researchers have collaborated with winemakers to systematically identify wine compounds, especially phenolic compounds, to better understand mechanisms of oxidation occurring in wine. These include processes from harvest through wine ageing in bottles, and are often associated with wine coloration However, while detrimental effects of excessive exposure are well established, little is known about the exact impact on wine quality of low levels of oxygen exposure. The first sporadic reports of white wine oxidation as a major organoleptic fault appear in the 1990s, when the problem drew attention due to increasing economic impact. The random nature of the problem makes it difficult to analyze. Research on wine oxidation has been approached on many scales. From a macroscopic point of view, modifications of sensory perceptions are considered, while work on the microscopic scale attempts to delineate the step-by-step mechanisms involved in oxidation. Experimentally, two schemes can be considered, one

working on the real product and its global evolution, and the second on simplified systems in order to model what can occur in the much more complicated product. Sensory experiments in this field are most useful for assessing possible correlations with physicochemical parameters, as, in the guise of the wine product, the consumer is actually buying a sensory experience. While a basic understanding of key factors influencing the sensory perception of wines has been achieved, other important aspects are still not yet well understood, including the particular role of oxygen. In a more fundamental way, the molecular approach aids in clarifying the underlying mechanisms of oxidation in wine, and allows the inference of the overall impact on wine quality. Even as elucidation of basic wine oxidation mechanisms begins, the extreme complexity of this medium and the range of variables affecting its makeup suggest that much more work will be done before all aspects are fully understood (Karbowiak et al., 2010).

Wine is an alcoholic beverage composed of water (80–85%), alcohols (the major one being ethanol, 9–15%) and a variety of minor constituents (3%). Such minor constituents include organic acids, sugars, phenols, nitrogenous compounds, enzymes, vitamins, lipids, inorganic anions and cations and a large number of volatile compounds. Amongst these, organic acids and phenolics play a critical role directly affecting product quality. The major organic acids include tartaric, malic, citric acid and acetic acid. Of these, tartaric acid and its salts give rise to wine total and titratable acidity whilst acetic acid is mostly responsible for wine's volatile acidity. On the other hand phenolic compounds, besides their contribution to astringency, are responsible for the characteristic colour and antioxidant activity of wines (Revi et al., 2014).

The primary objective of packaging is to protect and retain, as much as possible, the initial quality of foods and beverages. Key physicochemical properties that enable the packaging to fulfill its mission are its barrier properties to oxygen, carbon dioxide, moisture, light and aroma compounds. Its inertness, with respect to the migration of low molecular weight compounds from the package to the product and/or flavour scalping (sorption of volatile aroma compounds of the product by the packaging material) is also of paramount importance (Revi et al., 2014).

A package can be used to display a product and encourage its purchase, it is primarily an enclosure used to protect, store and transport a product. A basic packaging material is that which is used to fabricate the walls of such an enclosure, auxiliary packaging materials are those used to combine decorate, adhere, close, cluster, or permit easy opening of the basic package structure. A label would be an auxiliary packaging material attached to a basic packaging material such as bottle (Principles of package development, second edition).

The basic packaging materials fall into four major categories: ceramics, metals, vegetable products and plastics. Ceramics include pottery, chinaware and glassware. Metals include tinplate (steel), aluminium and occasionally copper, brass, pewter and more precious alloys. Vegetable products include wood, wood fiber, other vegetable fibers, cork, rubber and the like. Plastics encompass a whole family of natural and man – made substances. In parallel with the development of basic packaging materials and forms, it was necessary to develop methods and materials that could be used to join and fasten them. The early plugs, bungs, corks, and lead seals led ultimately to the modern closure industry, which produces a wide variety of caps, plugs, seals and ties (Principles of package development, second edition).

Packaging plays a key role in food manufacturing and marketing strategy. However, the interactions of packaging/sealing materials with foods and wine in particular arises different concerns, including the environmental impact and health issues. Cylindrical cork stoppers are the

classic closure used in the wine industry. The impermeability of cork to liquids and gases and its high compressibility and flexibility, make it ideal for sealing bottles. However, it is well known that in bottled wines sealed with cork several problems may occur, including cork taint mainly due to 2,4,6-trichloroanisole (TCA), causing the rejection of wine by consumers, and the variability in transmission (i.e. diffusion and permeation) to gases that can contribute to post-bottling oxidation of wine (Giunchi et al., 2008).

1.6. The role of cork

The seal of a bottle is a vital part of the package and its integrity must be maintained throughout the distribution chain. The closure must contain the liquid within the bottle and prevent it from seeping out, particularly when the bottle is on its side or inverted. It must also provide a gas – tight seal, preventing any carbonation within the product from escaping, and any atmospheric oxygen getting into the pack. Ideally, the cap or seal must also have tamper – evident properties, so that the consumer can be sure that he is buying a full bottle, and that product within the bottle is what it claims to be on the label. It must also prevent any invasion of the package by insects or microbial agents. And, like all other packaging materials in contact with the product, the closure must be inert and not affect the flavor or aroma of the product**.** The main function of a wine bottle closure is to ensure a good seal, in order to prevent any organoleptic deterioration of the wine during storage. Unlike the glass bottle, however, the cork closure is not an inert material, and its permeability can lead to mass transfer of various small molecules, such as oxygen or water (Developments in the packaging of alcoholic drinks).

1.6.1. Physico – chemical properties of cork

Cork, commonly used for wine stoppers comes from the bark of the oak tree *Quercus suber L.* The first known use of cork as a closure dates back to the fifth century BC, when it was used with Greek amphora. Nevertheless, the rise of cork started at the fifteenth century with the beginning of glass wine bottles. For several centuries, cork was the stopper of choice for various alcoholic beverages, due to its supposedly inert nature, impermeability to liquids and gases, and flexibility. Cork harvesting only takes place every nine to twelve years, and the first harvest of useable quality generally occurs on 40- to 50-year-old trees. Once harvested cork planks are stored for six months to two years. The next step is to boil the cork in water for at least one hour in order to tighten cells and produce uniform cell structure by gas expansion, and at the same time reduce the microorganism population. After drying and several weeks of storage under controlled conditions of temperature and relative humidity, cork is then graded and cut into strips. The quality grading is based on visual analysis of transverse and tangential sections of cork planks, taking into account the three main types of defects: pores (lenticular channels), physiological anomalies (nails, clay), and pathogenic anomalies (insect galleries). The stoppers are finally punched from strips of acceptable quality, and the remaining material is commonly used for agglomerate stoppers. After cutting to proper size and cleaning, cork stoppers are visually sorted into grades of different quality, depending on the extent of holes or imperfections. Then, they may be printed and the surface treated with either silicone or paraffin, in order to improve insertion and removal from the bottleneck. In addition to the visual control, most finished cork stoppers undergo a set of standard analyses (ISO-9727) (http://www.iso.org/iso/home.html), which include dimensional measurements (diameter,

length, ovalization), mass and apparent density, moisture content (optimally between 4 to 8%), diameter recovery after compression, maximum extraction force, liquid tightness, dust content, and in some cases peroxide residue and organoleptic tests. The physical structure of cork can be considered in terms of its three axes: axial (vertical, parallel to the center of the tree), radial (horizontal), and tangential (perpendicular to the axial radial plane). Cork stoppers are punched out along the axial dimension. When viewed from a radial perspective, cork cellular structure is a homogeneous tissue of thin-walled cells orientated in an alveolar, honeycomb type pattern of hexagonal sections with no intercellular spaces. When viewed from an axial or a tangential perspective, the cells appear as rectangular prisms, stacked base to base, parallel to the radial axis. Average cork cells are 45 μm tall with a hexagonal face of 20 μm and with a thickness of 1 μm. The density of cork can vary from 120 and 240 kg·m−3, with 10 to 40 million cells per cubic centimeter. Cork always contains lenticular varying numbers of lenticular channels running radially, which are hollow and approximately cylindrical, and constitutes macroscopic porosity. The volume and number of these channels varies significantly according to different types of cork, and is directly related to its industrial quality. The composition of cork as described in literature is relatively variable, but can be summarized as follows:

- Suberin: 33–50% (w/w)
- Lignin: 13–29%
- Polysaccharides: 6–25%
- Waxes: 2–8%
- Tannins: 6–7%
- Extractables: 8–24%
- Ash: 2–3%
- Others: 6–7%

Also indicated the existence of variation in the composition within the tree and a large variability between trees. Nevertheless, the main constituents are suberin and lignin, with somewhat smaller percentages of polysaccharides and waxes. Lignin is thought to be the main constituent of the thin internal primary cork cell wall, which is surrounded by alternating suberin and wax lamella in the thick secondary wall, which is in turn contained by the thin tertiary wall composed of polysaccharides. The chemical structure of suberin and lignin in cork has not yet been fully deciphered. Suberin is thought to be a macromolecular network of aliphatic polyesters, with various long-chain fatty acids and phenolic moieties. Although covalently linked, the poly(aliphatic) and poly(phenolic) domains appear to be spatially distinct. Suberin is assumed to play an important physiological role of water retention, and also acts as an antimicrobial barrier. It is also indicated in the low permeability of cork to liquids. Cork displays a low-energy surface, with a low polarity, similar to those low density polyethylene or polypropylene packaging films. While its status as a natural product is desirable, the cork's reputation for chemical inertness has come into question, and along with it the quantity of potential extractables. Have reported more than a hundred volatile compounds identified from cork. While the interactions of these aromatic components with wine remain largely unknown, also identified, after ether extraction, various low molecular weight phenolic compounds, most of them described in oak wood and wine: mostly ellagic acid (over 200 ppm), but also (in order of decreasing concentration, and less than 50 ppm) protocatechuic acid, vanillic acid, gallic acid, vanillin, scopoletin, caffeic acid, coniferaldehyde, ferulic acid, protocatechuic aldehyde, aesculetin, and sinapaldehyde. Have

reported a high variability in composition, which could be attributed to the age of the tree and to the distance of the samples from the base of the tree. No significant difference in extract concentration has been found between natural cork stoppers and agglomerated cork stoppers. Moreover, during the stages of cork production, the concentration of these compounds tends to decrease. Ellagic and gallic acids concentrations, in particular, are affected strongly by the boiling step in processing, which suggests hot water extraction, and by beaching with H_2O_2. These low molecular weight phenolic compounds found in cork may be formed by the breakdown of lignin and suberin, caused either physically or chemically by the manufacturing process, or by microorganism biodegradation. These compounds can have a direct influence on the organoleptic characteristics of the wine, and, subsequently, either positive or negative effects on wine quality. The washing and disinfection steps of cork processing can affect wine by affecting the sorption properties of cork. For instance, the effect of cleaning treatment products namely, aqueous solutions of chlorine-based compounds or hydrogen peroxide. A positive oxidative effect for corks with peroxide residues, but no significant effect with chlorine residues. This difference could be due to the basic pH used for the peroxide treatment, which may lead to suberin saponification and penetration of the peroxide residues into the cork, while chlorine residues remain at the surface of the material. Other less contaminating treatments, such as ozone disinfection techniques, are now considered. A more widely studied aspect of the release of organic compounds from cork closures is the transfer of those volatiles implicated in cork taint, and particular chloroanisoles (mainly 2,4,6-trichloroanisole) and chlorophenols (Karbowiak et al., 2010).

Some technical agglomerated cork stoppers are treated to protect against these compounds using supercritical fluid extraction with carbon dioxide, which decontaminates cork stoppers and also significantly reduces the aromatic compounds present in cork giving it a neutral organoleptic profile. In addition, most corks undergo surface treatment with silicone or paraffin, these hydrophobic compounds could also enhance the retention of non-polar taint compounds. Contrariwise, sorption properties of cork must also be considered. As a function of the concentration gradient between cork and wine, mass transfer can indeed occur from the cork to the wine as well as from the wine to the cork. A lot of other chemical species can also be sorbed by cork. In addition to water and ethanol, also all compounds present in wine having an affinity to cork also may be sorbed by the closure. Although less studied, this aspect should be considered in relation to long-term interactions between wine and cork during wine aging in bottle. Cork stoppers may also sorb compounds from the environment: 2,4,6-trichloroanisole, for example, has been shown to be easily sorbed by cork in the vapor phase, but sorption is mainly confined in the outer 2 mm of the cork cylinder with some slight migration towards the interior after 24 hours of exposure to the contaminant (Karbowiak et al., 2010).

Moreover, permeation of this compound through cork seems to be a very slow process, confined to the outer portion of the closure after three years. More recently, the understanding of sorption properties of cork has mostly been studied with a view to use cork powder waste as a potential biosorbent of pollutants, as it can easily be incinerated afterwards. The removal of heavy metals from aqueous solutions via biosorption on cork powder has been particularly studied for chromium, copper, nickel and zinc. The adsorption of metal ions generally showed a pH-dependent profile, revealing the important role of the carboxylic groups in binding through ion exchange mechanism. Cork has also been tested for the removal of biphentrin, a pyrethroid,

and even uranium. The sorption isotherms can, in most of these cases, be described by the non-competitive Langmuir adsorption model (Karbowiak et al., 2010).

Under standard conditions of temperature and pressure, cork contains 7% water on average. Heating at 100°C leads to a water mass loss of 4%: the 3% remaining is eliminated at a lower rate between 100 and about 200°C. Up to 250°C, it is interesting to note that no irreversible changes in cork composition occur. The water desorption process requires an activation energy of about 58 $kJ \cdot mol^{-1}$. It gives an endothermic peak close to the peak corresponding to the melting of waxes at 75°C, as measured by differential scanning calorimetry. Desorption of water molecules from the cork structure, associated with a possible anti-plasticization effect, gives rise to a modification of the dielectric properties and mechanical properties of cork, as these two relaxation processes are related to molecular mobility in the system (Karbowiak et al., 2010).

At bottling, cork stoppers are compressed horizontally, in the radial-tangential plane. The diameter is reduced by about 25%, from 24 to 18.5 mm, resulting in a 45% reduction in volume.Before closing, the ideal compression diameter is estimated to range between 15.5 and 16 mm, to avoid either too much cell damages or a strong piston effect. It is interesting to note that the mechanical characteristics of cork are roughly isotropic in the plane perpendicular to the radial axis, as dictated by its special shape and cell structure. It is, however, anisotropic in the two other planes, as revealed by compression studies. As a consequence of this material anisotropy, the best seals for mechanical properties would the oretically be obtained by punching out stoppers radially in the isotropic plane. Unfortunately, lenticels also run in the radial direction, and act as preferential pathways for liquids and gases, cork's elastic properties are characterized by a low Young modulus ($\sim$20 $MN \cdot m^{-2}$, roughly two times greater along the radial axis than along the other two directions) but also a low bulk modulus, this leads to high deformability, which could be explained in terms of cell-wall deformation recovery through bending or buckling. Furthermore, due to the existence of lenticels, the deformation of cork is not uniform and mainly occurs near these lenticular channels, which are irregularly dispersed within the material and cause local variability in mechanical properties. Despite these irregularities, cork is assumed to retain some degree of resilience for 5 to 10 years (Karbowiak et al., 2010).

1.7. Wine oxidation

Wine is a complex system capable of undergoing many different compositional changes during storage. While bottle storage is important for the improvement of red wine quality, for white wine, it can contribute to quality defects such as color alteration (browning) and eventually deterioration of the overall quality and marketability. However, some white wines may derive short-term benefits from the development of a characteristic bottle bouquet (Kallithraka et al 2009).

If wine is considered from a macroscopic point of view, the first two important sensory impressions are the color and the aroma. Browning, caused mainly by oxidation, can be perceived either as a positive aspect, in the case of sherries or sweet fortified wines such as white Ports or Rivesaltes, or as a negative aspect for dry white wines. Browning, as the name suggests, is characterized by a brown-yellow color that progressively replaces the initial (generally pale-yellow) color through the influence of oxygen, and which can be globally characterized by the absorbance at 420 nm. On one hand, oxygen seems to have a positive effect during alcoholic

fermentation or micro-oxygenation of wines. On the other hand, oxygen appears to play a negative role when sensory drifts are observed in a tank or bottle, with a loss of freshness and fruitiness, and the development of an unpleasant oxidized character. Indeed, before an easily observable chromatic change, such an oxidative aging first gives rise to typical flavors, which are generally described as “rancio” in sweet fortified wines and as non-desirable flavors of “honey-like,” “boiled-potato,” “cooked vegetable,” “farm-feed,” “hay,” and “woody-like” in dry white wines (Karbowiak et al., 2010).

The oxidative spoilage of both white and red wines is characterized by the transformation of aroma compounds, leading to a loss of characteristic aromas of wines, and subsequently to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration. Several wine compounds, such as esters and terpenes, are transformed during wine storage, and the loss of wine aroma may occur (Roussis and Sergianitis, 2008).

1.7.1. Mechanism of chemical oxidation in wine

The primary substrates for oxidation in wines are the phenolic constituents of the wine itself, which act as antioxidants. The first aspect which must be considered regarding the oxidation of phenolic compounds in wine is the equilibrium which exists between the phenol and the phenolate anion form (loss of a proton) as a function of pH. Due to high pKa values (9 to 10), the protonated form is favored under wine acidic conditions. Above this pH, the phenolate ion form is favored, and oxidation is much easier than with the protonated form. It is also very rapid, taking only 30 minutes to reach completion in model wine pH 11 at room temperature (23.5°C) under pure oxygen gas phase. However, direct oxidation of phenolate ions with oxygen cannot be responsible for white wine browning, even if a small fraction of phenols remains deprotonated and thus susceptible to react. The major hydrogen-donating antioxidants are monohydroxy or polyhydroxy phenolic compounds with various ring substitutions, phenolic acids having, in general, lower antioxidant activities. Oxidation of these phenols leads to the formation of semiquinone free radicals and quinones. The oxidation of phenolic compounds is either assumed to be catalyzed by transition metal ions, or to be autocatalytic. The corresponding reaction schemes related to these two hypotheses are shown in Fig. 1. (Waterhouse, 2006).

In the first hypothesis, the oxidation of phenols is directly mediated by a transition metal such as ferric ions, yielding the formation of a semi-quinone radical, which is further oxidized to the corresponding quinone (Fig. 1). In a cyclic chain of radical reactions, the parallel, successive monovalent reductions involve ferrous ions oxidation to form three reactive species from the triplet oxygen: hydroperoxide radical, hydrogen peroxide, and hydroxyl radical. This last oxygen specie is very unstable and reacts very quickly. It is thus considered as a non-selective oxidation reaction, not onlywith phenolic compounds but also with all oxidizable wine substances, the more concentrated substances being then the more probable substrates to be oxidized. Numerous products can be formed through this oxidation mechanism (such as quinone from phenol, or dehydroascorbic from ascorbic acid). Because of its high concentration in wine, ethanol can then be oxidized by hydroxyl radicals which are then reduced to water. In a second step, the carbon radical formed from ethanol can react with an oxygen molecule to form acetaldehyde and a new hydroperoxide radical. The regeneration of such a radical perpetuates the oxidation of phenolic compounds into their respective quinone forms (Karbowiak et al., 2010).

Phenolic oxidation can also result from the reduction of oxygen to the hydroperoxide radical (involving Fe^{2+} oxidation to Fe^{3+}) which can then oxidize a phenol into a semiquinone radical (Fig. 1). Phenolics are good hydrogen donors, and consequently enable hydroperoxide radicals to abstract protons from hydroxyl groups. The hydroperoxide radical thus becomes reduced into hydrogen peroxide through acceptance of the hydrogen radical. It can then be reduced to the highly reactive specie of hydroxyl radical through the participation of a transition metal ion, in the same manner, for example, as previously described in ethanol oxidation. The hydrogen peroxide effect is suspected to be the coupled product of phenolic compound oxidation, leading to further oxidation reactions. With about 2 moles of hydrogen peroxide reacting with each mole of gallic acid, the oxidation in highly alkaline solution leads to the consumption of 4.9 atoms of oxygen per molecule of gallic acid oxidized. The hydroxyl radical appears to be of great importance in wine oxidation, as suggested by the two hypotheses supporting the reaction mechanisms detailed in Fig. 1. It can, in particular, lead to the formation of various aldehydes and ketones via this oxidative pathway from alcohols or organic acids (Danilewicz, 2003).

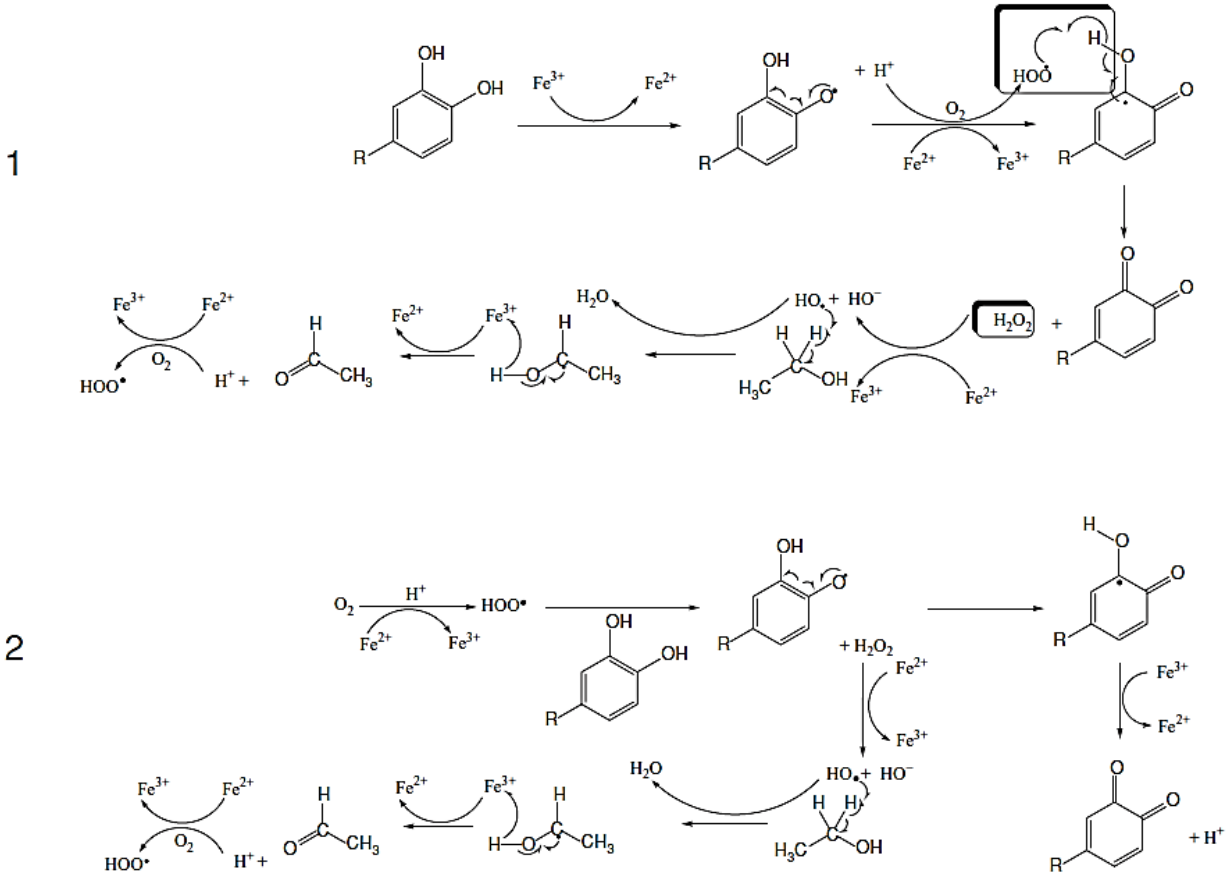


Figure 1. General scheme for oxidation of phenolic compounds involving reduction of oxygen and oxidation of ethanol. The first reaction process is based on the hypothesis of a direct role of the iron redox couple, whereas the second one assumed a two-step phenomenon with firstly the formation of an oxygen reactive specie, iron mediated, and secondly the oxidation of phenols.

As shown by these mechanisms of oxidation, the antioxidant properties of wine are clearly dependant on the phenolic content. The products of the reaction, semiquinones, display resonance stabilization of the delocalized electrons in the ortho- and para-positions of the aromatic ring, which make them susceptible to participation in other radical reactions. In this way, two semiquinone free radicals can form a covalent bond by sharing the two unpaired electrons, giving rise to a new, oxidizable dimer which can further react with oxygen. Trimer, tetramer, or even larger molecules can also be generated by such an association between two semiquinones, or by reaction between a quinone and a phenol. This process is the so-called regenerative polymerization. In addition, the brown color given by quinone molecules increases as long as polymerization occurs (Karbowiak et al., 2010).

Acetaldehyde, produced by ethanol oxidation (Fig. 1), also plays an important role in the structural modification involving wine phenolics and oxygen during the ageing (Atanasova et al., 2002). In particular, it can favor the reactions between anthocyanins and flavanols which form new polymeric phenols. Glyoxylic acid, produced from the oxidation of tartaric acid, can also participate into these polymerization reactions as a bridging molecule between phenolic compounds. Such condensation reactions, with anthocyanins and tannins in particular, contribute to the formation of stable polymeric pigments in solution, which, in turn, tend to stabilize color in red wines. The lack of polymeric phenols in white wines made by the red vinification method, in which prolonged skin contact during fermentation occurs, has been explained by the lack of anthocyanins to complex with the tannins. The subsequently lower amount of such complexes of increased solubility leads to a deficiency in tannins and astringency. The higher concentration of proteins in white wines could also play a role in polymer adsorption and precipitation. In white wines exposed to increased amounts of oxygen, a significant decrease in total phenols occurs, in which the flavonoid fraction remains stable and only the nonflavonoid fraction decreases. In this case the oxygen consumption is evaluated at 4 mL of oxygen per 10 mg of Gallic Acid Equivalent under standard temperature and pressure. The chemical structures of wine phenolics, such as flavonoids, confer varying antioxidant activities as peroxyl radical scavengers. Oxidative browning in wine displays a particularly good correlation with some flavanols, mainly catechin and epicatechin, with cinnamate derivatives also playing a minor role. Oxidation reactions involving mainly catechin, one of the most common grape flavanols, and a procyanidins constituent, lead to colorless and yellow pigments. Indeed, from studies on wine model solutions, identified the formation of two types of yellow pigments showing visible absorption maxima at 440 and 460 nm, respectively xanthylium salt pigments and ethylester of xanthylium salts, both derived from flavanol oxidation and polymerization.With an absorption maximum in the region of 400–500 nm, these pigments directly contribute to white wine browning during ageing. This reaction, and thus the extent of browning, is accelerated in model wine solution with the addition of iron and copper, which probably act as catalysts to form intermediate oxidation products. For example, the oxidation of tartaric acid to produce glyoxylic acid can further link two catechin units and lead to the formation of xanthylium cations. Manganese is also found to catalyze these reactions, and has been found to act in synergy with iron to change susceptibility of sherry wines to browning. The presence of copper may result from the use of vineyard treatments and from the use of copper sulphate in wine to remove hydrogen sulphide and other sulphide compounds. It is difficult not only to clearly identify intermediate reaction products, but also to determine the sensory modification related to the

formation of new, pigmented oligomers or larger polymers during wine ageing. Further characterization of these compounds should be pursued (Karbowiak et al., 2010) .

Other factors, both intrinsic and environmental are key in determining the extent of browning oxidation in white wine. In addition to the effect of grape variety, the region of origin and degree of maturity at time of harvest, showed that increasing temperature, oxygen content or pH (between 3 and 4) increase the browning rate (as measured by the change in optical density at a wavelength of 425 nm). An excess of ultraviolet and visible radiation also produces significant oxidative changes in the volatile and polyphenolic content during storage, with a higher visual browning (as measured by absorbance at 420 nm). For white wines, found the color stability is more dependent upon light exposure than upon oxygen concentration at 20°C, whereas at 45°C their respective effects become equal. High pH and high temperature are also found to affect a pronounced increase in browning. The increase in pH makes the concentration of the phenolate ions increase relative to the phenol form, thus increasing oxidation rates by about nine times between pH 3 and 4. However, it should be noted that the different factors implied in oxidation of white wines during storage (temperature, oxygen, pH, light) act as a whole on wine oxidation rate, and the isolated effect of each parameter remains very difficult to study (Karbowiak et al., 2010).

1.8. Aroma compounds

Wine flavor presents an extremely complex chemical pattern in both qualitative and quantitative terms. Over 1000 volatile compounds have been identified, with a wide concentration range varying between hundreds of mg/l down to ng/l. Moreover, wine aroma is generated by several classes of compounds, such as hydrocarbons, alcohols, terpene alcohols, esters, aldehydes, ketones, acids, ethers, lactones, sulphur and nitrogen compounds. Aroma production is influenced by several factors: environment (soil, climate), grape variety, ripeness, fermentation conditions and biological factors (i.e. yeast strain and other components of the oenological microflora), winemaking processes and aging. Most of the volatile compounds may play a role in the aromatic profile of each wine type depending on their concentration. In some cases it has been possible to isolate a few key compounds, mostly representing the typical flavor of a wine, while in the majority of wines several compounds seem to cooperate, with specific ratios between them. A better understanding of the key aroma compounds helps to control quality and may have an impact on the viticulture and wine technological processes. Because of the complexity of the wine aroma and the great variety of aroma compounds responsible, it is a far from simple task for researchers to quantify the volatiles and measure the wine aroma intensity. The great number of the volatile components and the fact that they have different chemical natures covering a wide range of polarity, solubility, volatility and pH explains the difficulty of this undertaking. An important number of those components in wine can only be found at very low concentrations, therefore, the samples need to be highly concentrated in order to be accurately quantified. Moreover, many of the aromatic components are unstable. They may be easily oxidized in contact with air or degraded by heat or extreme pH, giving rise to the appearance of analytical artefacts. One of the main problems that researchers face when studying the compounds responsible for wine aroma is the choice of a suitable isolation procedure, obtaining a representative extract similar to that of the wine aroma. Several methods

have been developed in an attempt to achieve that goal, each with advantages and disadvantages (Symeou et al., 2007).

Young white wines should be consumed within a short time after bottling to avoid loss of their fresh, fruity attributes and the formation of undesirable compounds. Shelf-life of white wines can be extended if they are stored under suitable conditions of light and temperature prior to consumption. The fruity character of young white wines depends on the contents of terpenes present in the grape, together with acetates and mono- and dicarboxylic acid ethyl esters which appear during the fermentation process. White wines made from aromatic varieties like Muscat loss the floral aromas produced by the monoterpenes with ageing in the bottle. Hydrolysis of acetates and esters with storage time is another important factor resulting in the loss of the fruity character of young white wines. This effect is accelerated by the high temperature and low pH. When wines are stored at 20 ^{o}C the monoterpenes contents decrease as compared to those stored at 10 ^{o}C. Acetate levels remain constant during storage of wines at 0 ^{o}C, decrease during storage at 10 ^{o}C, and decrease still further during storage at 30 ^{o}C (Perez-Coello et al., 2003).

1.8.1. Alcohols

Alcohol detected in wines are in significant quantities. Approximately 50% of aromatic compounds, excluding ethanol contrary to the esters according contribute negative in the aroma and flavor of wine (Jackson et al., 2000) .

Alcohols are distinguished in the mono-alcohols and polyols. The major mono-alcohols having C_3 (propane-1 isopropanol), C_4 (1-butanol, isobutanol), C_5 (isoamyl, methyl-2- butanol-1, pentanol-1), C_6 (1-hexanol) and C_8 (2-phenyl ethanol) The main polyalcohols are glycerol and 2,3-butanediol.

The technological interest of monoalcohols is their participation in the composition the organoleptic characteristics of wines. When these compounds are contained in small quantities have a favorable impact on the flavor of the wine. But the same when these substances are present in quantities greater than 500 - 600 mg / L.

The propanol seems to be a big impact on the flavor of the wine, because they have a neutral odor. The amyl alcohols also seems not to have favorable impact on the organoleptic characteristics of wines. The hexanol-1, which is derived from grapes, wine gives grassy smell and taste. Numerically, the most significant mono-alcohols are propanol, 2-methyl propanol (isobutanol), the amyl alcohols (3-methyl-2-methyl- butanol) and 2-phenylethanol. Most researchers believe course that contribute more to the intensity of the flavor of the wine than the quality, which is significantly reduced if more than 400 mg / L (Σουφλερός, 1997).

The exception is the 2-phenylethanol, whose concentration in wines. It has been associated positively with their quality. This compound has fragrance rose and is a key component of volatile Muscadine wines. Although contained in small quantities in wine, however is perceived to low levels. Recent research showed that phenylethanol, characterized by the rose smell or pungent (spicy) or honey or flowers. Alcohols are mainly from the alcoholic fermentation of the must, while only hexanol, the hex-3-enol and octanol present in significant amounts in grapes (Gurbuz et al., 2006).

1.8.2. Esters

Esters are formed when an alcohol function reacts with an acid function and a water molecule is eliminated (Fig. 2). It is a reversible reaction, limited by the inverted reaction of hydrolysis of the ester. When the system is in balance, there is a constant correlation between the concentrations of the substances present, governed by the mass action law. There are a large number of different alcohols and acids in wine, so the number of possible esters is also very large. Ethyl acetates are the most common for kinetic reasons, i.e. the large quantities of ethanol present and the fact that primary alcohols are the most reactive. Very few esters are present in grapes. Odoriferous molecules such as methyl anthranilate are responsible for the foxy odor in *Vitis labrusca* grapes and wines made from them. There are also methoxyl groups in pectins that release methanol by hydrolysis (Handbook of Enology).

Esters in wine have two distinct origins: enzymic esterification during the fermentation process and chemical esterification during long-term aging. The same esters may be synthesized in either way.

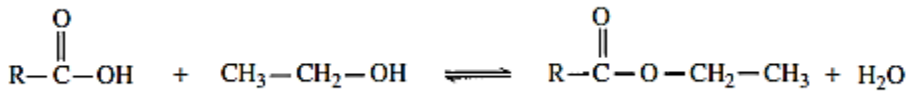


Figure 2. Esterification balance of an alcohol

1.8.3. Ethyl Acetate

The most prevalent ester in wine is certainly ethyl acetate. A small quantity is formed by yeast during fermentation, but larger amounts result from the activity of aerobic acetic bacteria, especially during aging in oak barrels. Apparently, lactic bacteria are not capable of synthesizing this ester. Ethyl acetate is responsible for the olfactory characteristics in wines affected by 'acescence'—a suffocating, vinegary odor. These wines also have high volatile acidity, but acetic acid is not responsible for acescence. In a simple solution, ethyl acetate is perceptible at concentrations approximately 200 times lower than the perception threshold of acetic acid.

The olfactory perception threshold of ethyl acetate is approximately 160 mg/l. Even below this value, while it may not be identifiable, it may spoil the bouquet with an unpleasant, pungent tang. It is, however, possible that at very low doses (50–80 mg/l) ethyl acetate contributes to a wine's olfactory complexity and thus has a positive impact on quality.

Furthermore, ethyl acetate affects wine flavor. At relatively high concentrations (above 120 mg/l) that are still below the olfactory perception threshold, it gives red wines a hot flavor which reinforces the impression of bitterness on the aftertaste. Ethyl acetate contributes to harshness and hardness in red wines. An acetic acid concentration of at least 0.90 g/l (a volatile acidity of 0.95 g/l expressed in H_2SO_4) is required to produce a noticeable bitter, sour aftertaste. Even at these high levels, however, it does not have a strong odor, whereas ethyl acetate is perceptible at much lower concentrations (Handbook of Enology).

1.8.4. Fatty Acids in the Aliphatic Series

This series is shown in Table 1. The most important of these compounds is acetic acid, the essential component of volatile acidity. Its concentration, limited by legislation, indicates the extent of bacterial (lactic or acetic) activity and the resulting spoilage of the wine. As yeast forms a small amount of acetic acid, there is some volatile acidity in all wines. Other C_3 (propionic acid) and C4 acids (butyric acids) are also associated with bacterial spoilage.

The C_6, C_8 and C_{10} fatty acids are formed by yeast. As they are fermentation inhibitors at concentrations of only a few mg/l, they may be responsible for stuck fermentations. Unsaturated long-chain fatty acids (C_{18}, C_{20}) are related to the sterol family. These compounds are fermentation activators, mainly under anaerobic conditions. The most important of these are oleic (C_{18} with one double bond) and linoleic acids (C_{18} with two double bonds). They are active in trace amounts and come from the waxy cuticle of grape skins. (Handbook of Enology).

Formula	Name	Boiling point (°C)	Concentration (g/l)	Comments
H–COOH	Formic	101	0.05	
CH_3–COOH	Acetic	118	0.5	
CH_3–CH_2–COOH	Propionic	141	Traces	
CH_3–CH_2–CH_2–COOH	Butyric	163	Traces	
$CH_3-CH(CH_3)-COOH$	Isobutyric	154	Traces	Methyl-2-propionic acid
CH_3–CH_2–CH_2–CH_2–COOH	Valerianic	186	Traces	
$CH_3-CH(CH_3)-CH_2-COOH$	Isovalerianic	177	?	Methyl-3-butyric acid
$CH_3-CH_2-CH(CH_3)-COOH$	Methyl-2-butyric		?	
$CH_3-(CH_2)_4-COOH$	Caproic	205	Traces	Hexanoic acid
$CH_3-(CH_2)_5-COOH$	Oenanthic	223	Traces	Heptanoic acid
$CH_3-(CH_2)_6-COOH$	Caprylic		Traces	Octanoic acid
$CH_3-(CH_2)_7-COOH$	Pelargonic	253	?	Nonanoic acid
$CH_3-(CH_2)_8-COOH$	Capric	270	Traces	Decanoic acid

Table 1. Fatty acids in the aliphatic series among the volatile components in wine (Ribéreau-Gayon *et al*., 1982).

1.8.5. Ethyl Acetates of Fatty Acids and Acetic Esters of Higher Alcohols

Ethyl acetates of fatty acids, mainly ethyl caproate and caprylate, are produced by yeast during alcoholic fermentation. They are synthesized from forms of the acids activated by the coenzyme A (HS-CoA), acyl-S-CoA. Acetyl-S-CoA, from pyruvic acid, may be involved in a Claisen reaction with malonyl-S-CoA, producing a new acyl-S-CoA with two additional carbon atoms (Figure 2.9). Acetyl-S-CoA thus produces butyryl-S-CoA, then hexanyl-S-CoA, etc. Specific enzymes then catalyze the alcoholysis of acyl-S-CoA into ethyl acetates of fatty acids. At the same time, the coenzyme A is regenerated. Ethyl acetates of fatty acids have very pleasant odors of wax and honey which contribute to the aromatic finesse of white wines. They are present at total concentrations of a few mg/l.

Acetic esters of higher alcohols (isoamyl acetate and phenylethyl acetate) should also be included among the fermentation esters. These compounds are present in moderate quantities, but have intense, rather unusual odors (banana, acid drops and apple). They contribute to the aromatic complexity of naturally neutral wines, but may mask some varietal aromas. The formation of all these esters is promoted when fermentation is slow and difficult, due to absence of oxygen, low temperatures and larified must (Handbook of Enology).

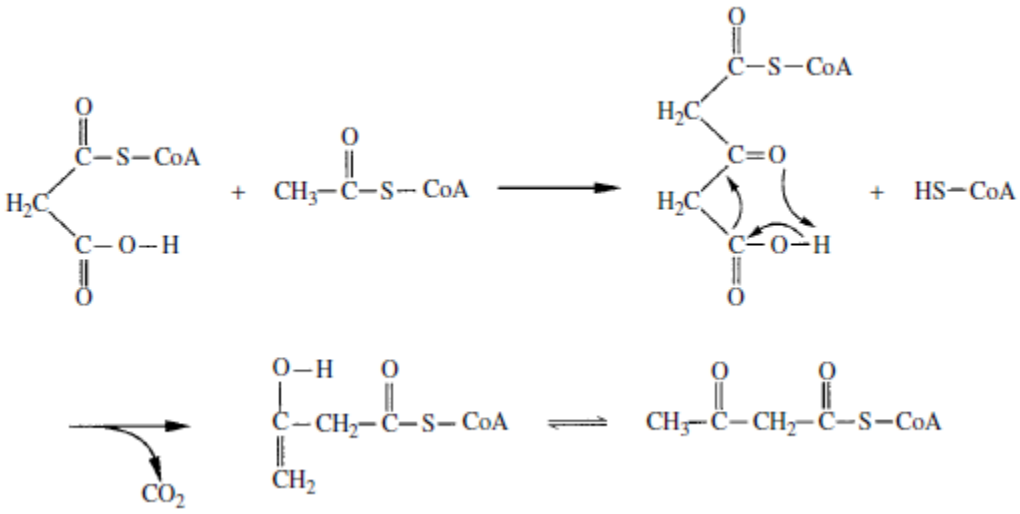


Figure 3. Biosynthesis mechanism of fatty acids.

1.8.6. Aldehydes and Ketones

Ethanal is the most important of these compounds. The many ways it can be produced and its high reactivity (the CHO radical has extensive chemical affinities), as well as its rapid combination with sulfur dioxide at low temperatures and its organoleptic properties, make ethanal a very important component of wine. The presence of ethanal, produced by the oxidation of ethanol, is closely linked to oxidation–reduction phenomena.

In wine preserved with regular, light sulfuring, the sulfite combination of ethanal (CH_3–CHOH– SO_3H), stable in an acid medium, is the most prevalent form. When grapes have been heavily sulfured, the ethanal concentration increases and may exceed 100 mg/l, also combined with sulfite. This sulfite combination of ethanal protects yeast from the antiseptic effects of SO_2.

Wines containing excess ethanal as compared to the quantity of SO_2, i.e. free (non-combined) ethanal, are described as ‘flat’. A slight trace of free ethanal is sufficient to produce a characteristic odor, reminiscent of freshly cut apple. This problem disappears rapidly if a little SO_2 is added, as it combines with the free ethanal.

A few other aldehydes are present in wine in trace amounts (Table 3). Higher aldehydes contribute to the bouquets of some wines. The neutralizing effect of sulfur dioxide on the fruitiness of certain white wines is due to the fact that it combines with the aldehyde fraction in the bouquet. Aldehydes in the aromatic series are also present in wine. The most significant of

these is vanillin, associated with barrel aging, which has a distinctive vanilla aroma. Grapes apparently contain few aldehydes. Several molecules with ketone functions have been identified, including propanone, butanone and pentanone. As previously mentioned, the most important of these are acetylmethyl carbinol and diacetyl. Finally, a mercaptopentanone has been identified among the specific components of Sauvignon Blanc aroma (Handbook of Enology).

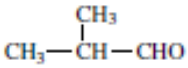
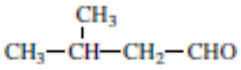
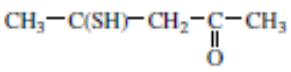
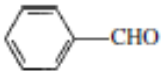
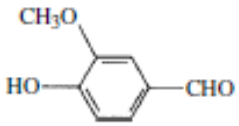
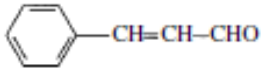
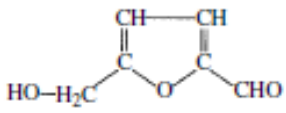
Formula	Name	Boiling point (°C)	Concentration (g/l)	Comments
H–CHO	Methanal	21	?	Formic aldehyde
CH_3–CHO	Ethanal	21	0.1	In combined state with SO_2. Only oxidized wines (Rancio, Sherry, etc.) contain free ethanal
CH_3–CH_2–CHO	Propanal	49	Traces	
CH_3–CH_2–CH_2–CHO	Butanal	76	?	Valerianic aldehyde
	Methyl-2-propanal	92	Traces	Isovalerianic aldehyde
CH_3–CH_2–CH_2–CH_2–CHO	Pentanal	102	?	Valerianic aldehyde
	Methyl-3-butanal	92	Traces	Isovalerianic aldehyde
CH_3–CH_2–CH_2–CH_2–CH_2–CHO	Hexanal	128	Traces	Caproic aldehyde
CH_3–CH_2–CH_2–CH=CH–CHO	Hexene-2-al		?	Only present in grapes
CH_3–$(CH_2)_5$–CHO	Heptanal	155	Traces	Oenanthic aldehyde
CH_3–$(CH_2)_6$–CHO	Octanal	167	?	Caprylic aldehyde
CH_3–$(CH_2)_7$–CHO	Nonanal	185	?	Pelargonic aldehyde
CH_3–$(CH_2)_8$–CHO	Decanal	208	?	Capric aldehyde
CH_3–$(CH_2)_{10}$–CHO	Dodecanal		?	Lauric aldehyde
CH_3–CO–CH_3	Propanone	56	Traces	Acetone
CH_3–CH_2–CO–CH_3	Butanone	80	?	Methylethyl ketone
CH_3–CH_2–CH_2–CO–CH_3	Pentanone-2	102	?	
CH_3–CHOH–CO–CH_3	Acetylmethyl carbinol	143	0.01	Acetoin
CH_3–CO–CO–CH_3	Diacetyl	87	Traces	
	Mercaptopentanone			Sauvignon Blanc aroma
	Benzoic aldehyde	178	?	
	Vanillin	285	?	
	Cinnamic aldehyde	253	?	
	Hydroxymethyl furfural			Grape juice or wine subjected to heat treatment

Table 2. Aldehydes and ketones in wine.

1.8.7. Phenolic compounds

Among the various constituents of wine, the diverse classes of phenolic compounds present are of significant technological and nutritional importance. Their type and levels in the end-product,which may be influenced by the grape variety, as well as various abiotic factors (climate, soil type, winemaking technique), may contribute to wine sensory characteristics and play an essential role in its oxidative stability and ageing process. Furthermore, phenolics seem to be responsible forvarious health benefits associated with the moderate consumption of wine such as protection from cardiovascular diseases and cancer. The latter has been mainly related to the antioxidant activity of phenolics and particularly the scavenging of harmful free radicals formed in vivo.Owing to all these properties the examination of phenolic content and composition of wines has been the subject of several research works, the majority of which have been coupled with athorough study of the product's antioxidant activity. To this direction many analytical techniques i.e. capillary electrophoresis, HPLC, GC/MS, have been applied to separate, identify and quantify individual phenolic compounds. However, the radical scavenging has been studied usingvarious in vitro assays with most common ones the $ABTS^{\cdot}$ and $DPPH^{\cdot}$, probably due to their simplicity, low cost of application and reproducibility, despite some shortcomings (e.g. radical reduction by ascorbic acid, other non phenolic reducing compounds) that have been extensively described in review articles (Tortoglou et al., 2014).

1.9. Analysis of volatile aroma compounds

Wine aroma is one of the most important factors that influence perceived wine quality and consumer acceptance. Volatile compounds play a significant role to wine aroma and the presence, absence or different proportions of volatile compounds can be greatly influenced by both viticultural (climate, soil, cultivar, grape-growing practices) and enological (condition of grapes, fermentation, postfermentation treatments) factors. Hundreds of volatile compounds have been identified in wines. However, not all compounds present in wine contribute to aroma. The influence of a volatile compound to the final aroma depends on its concentration in wine and on the perception threshold of this specific compound. The threshold of olfactory perception is defined as the lowest concentration capable of producing an olfactory sensation and that can be detected by human nose for at least 50% of the judges of a panel of sensory evaluation (Welke et al., 2014).

Wine improvement is an active field of research and screenings are constantly carried out to find new conditions or treatments for flavor improvement. Testing different temperatures, starter cultures or mixtures of juices of different grape varieties are examples of these screenings. MicroVinification platforms offer many advantages to significantly speed up screening and quality control compared to traditional lab scale fermentations. In these systems, fermentations are carried out employing only 5 ml of grape must, allowing the screening of several conditions at the same time, saving time and resources. However, to take advantage of this high-throughput sample capacity requires a high-throughput flavor analysis technique that allows fast detection of high numbers of aroma compounds in low sample volume (Gamero et al., 2013).

Gas chromatography coupled with mass spectrometry (GC–MS) is the most widely used technique for analysis of volatile aroma compounds. Especially in combination with cryogenic refocusing (CT) of the most volatile compounds at the beginning of the column, compounds can be analyzed with a high separation efficiency and sensitivity. Depending on the food matrix and

the aroma compounds to be determined, different aroma extraction methods can be applied in combination with GC–MS. Furthermore, criteria such as accuracy, precision (repeatability and reproducibility), sensitivity, speed and high-throughput possibilities have to be taken into consideration (Gamero et al., 2013).

Several classical analytical methods such as liquid–liquid extraction (LLE), liquid–liquid microextraction (LLME), simultaneous distillation-solvent extraction, solidphase extraction (SPE), supercritical fluid extraction, microwaves extraction and ultrasound extraction, among others, have been developed for the analysis of the minor volatile compounds in wines. These classical analytical methods have some drawbacks such as the relatively low reproducibility, required and insufficient selectivity. SPE and LLME are rapid and inexpensive, but to achieve the required limits of detection, a concentration step (solvent evaporation) is required, which increases the sample preparation step and may also cause loss of volatile analytes during the evaporation (Camara, et al., 2006).

In the beginning of 90 decade, a new variation of adsorption technique called solid-phase microextraction (SPME) has been developed. Compared to traditional techniques this new technique offers many advantages such as high sensitivity and reproducibility, does not require solvent and combines extraction and pre-concentration in a single step without pre-treatment of samples. Moreover it is fast, inexpensive, requires low sample volumes and can be easily automated. This technique has been successfully been used in wine samples to characterise a wide range of aroma cpmpounds, possibility of contamination with solvents, the length of time (Camara et al., 2006).

On the other hand, direct immersion solid-phase microextraction (DI-SPME), stir bar sorptive extraction (SBSE) and monolithic material sorptive extraction (MMSE) are solvent free but constitute a more invasive way of sampling than headspace techniques. Except MMSE, these extraction techniques have also been used in wine aroma analysis. MMSE is the most novel extraction technique and as far we know has not previously been applied to the analysis of wines. On the contrary, it has been successfully used in the analysis of organic compounds in other food products such as water or milk (Gamero et al., 2013).

Nowadays, the trend is to develop sophisticated methods as an improvement of common methods to detect certain minor wine compounds or families of compounds. In some cases, these new techniques are time-consuming or involve the use high sample volumes, which make them unsuitable for high-throughput purposes employing micro-scale fermentations (Gamero et al., 2013).

1.10. Prevention of oxidation in wine

1.10.1. Use of antioxidants

Current research has confirmed that food rich in antioxidants plays an essential role in the prevention of several diseases. On the other hand, oxidation of lipids in foods is a major cause of chemical spoilage and its products are potentially toxic. Antioxidants are widely used in many foods to prevent fat rancidity. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are widely used because they are effective, and cheaper than natural ones. However, the safety and toxicity of synthetic antioxidants have raised important concerns. Hence, considerable interest has been given to the use of natural antioxidants which may also have nutritional properties. (Roussis et al., 2008).

Many phenolic antioxidants are present in wines. Wine phenolics are considered to scavenge reactive oxygen species (ROS), to inhibit oxidation of oil systems, and to inhibit human low-density lipoprotein (LDL). Wine phenolics originate from grape juice and especially skins, and also from barrels used in winemaking. Red and white wines differ in their phenolic composition due to differences in phenolic composition of red and white grapes and in the winemaking procedures. Red winemaking includes the procedure of maceration while white winemaking does not. This is thought to be the main reason for the relative low polyphenol content and for the lower antioxidant activity of white wine in comparison to red wine. Red wines are good dietary source of various phenolics, including benzoic and cinnamic acid derivatives, flavanols, flavonols and anthocyanins. White wines contained mainly hydroxycinnamates, and benzoic acids (Roussis et al., 2008).

In order to protect musts and wines against oxidation, sulfur dioxide is used from pressing to bottling, especially for white wines. Its empiric use began in the 18th century. In addition to antiseptic properties, sulfur dioxide acts as an inhibitor of enzymatic and chemical oxidation and therefore has a positive effect in decreasing the browning rate (Sioumis et al., 2005). Sulfur dioxide is highly soluble in water and ethanol as compared to oxygen or other gases solubilities are high and increases with decreasing temperature. Sulfur dioxide is also highly volatile, with a solubility coefficient of 1.2×10^{-2} $mol\cdot m^{-3}\cdot Pa^{-1}$. Concentrations of added Sulphur dioxide to wine generally vary from 50 to 200 $mg\cdot L^{-1}$, and are of greater importance for sweet wines. In wine, there is an equilibrium between the molecular and ionic forms of sulfur dioxide. At wine pH, it can exist in the molecular form, SO2, but is more commonly present (94 to 99% at wine pH) or in the ionic form as the bisulfite ion, HSO^{-3} (SO_2+ $H_2O \rightarrow H^+$+ HSO^{-3}, pKa = 1.91). The sulfite ion, $SO2^{-3}$, only appears at a higher pH (pKa = 6.91), and is thus present at very low concentrations at wine pH. Once in solution in wine medium, sulfur dioxide may bind with several wine constituents such as acetaldehyde, anthocyanins, pyruvic acid, glutaric acid, glucose, or certain phenolic compounds; of which ethanal, pyruvic acid, and 2-oxoglutaric acid appear to react with particular efficiency. Some binding agents, such as aldehydes, quinones, or keto acids, may derive from oxidation reactions. Thus, these two fractions of SO_2 present in wine are respectively referred to as "free SO_2," referring to HSO^{-3} and SO_2, and "bound SO_2," indicating sulfur dioxide bound mainly to unsaturated compounds. Only free SO_2 is active against oxidation however, below 10 $mg\cdot L^{-1}$ of free SO_2 in wine, this protective effect is no longer efficient. SO_2 in wine plays an important role against oxidation, not in direct oxygen scavenging, but by reacting with hydrogen peroxide, which subsequently decreases the oxidation potential (Karbowiak et al., 2010).

The reaction involves a nucleophilic displacement of HSO_{-3} by H_2O_2 to form sulfuric acid, HSO^{-4}, as an end product. In this way, sulfur dioxide can inhibit the aldehyde forming reaction by competing for hydrogen peroxide. However, the considerably larger concentration of ethanol, compared to that of sulfur dioxide, makes its oxidation possible (to ethanal) even in the presence of SO_2. It is generally thought that a concentration at or above approximately 10 $mg\cdot L^{-1}$ of free SO_2 is necessary to ensure acceptable protection against oxidation. SO_2 is also thought to play an important role in reducing quinones, formed during the oxidation process product, back to their phenol form (Waterhouse et al., 2006).

Ascorbic acid, the L-enantiomer of which is commonly known as vitamin C, is used widely in the food industry as an antioxidant and could be applicable in wine production. This water

soluble organic acid is a 6-carbon lactone ring structure with a 2,3-enediol functional group that confers antioxidant properties. It is, indeed, a good electron donor, as it is easily converted into semi-dehydroascorbic acid, and then into dehydroascorbic acid, via the donation of a hydrogen atom and an electron in each step of the oxidation process. The reaction rate can be very rapid for the electron transfer to reactive oxygen species. As with SO_2, it is also assumed that ascorbic acid reduces the oxidized phenolic compound, quinone back to its original form (phenol), in addition to acting as an oxygen scavenger. However, the effect of ascorbic acid used in combination with sulfur dioxide to protect white wines against oxidation is not clearly evident, especially for long storage. In particular, observed no synergistic effect between these two antioxidants for the quantities currently employed in wine-making. The reduction in browning measured by absorbance at 420 nm is also not evident when ascorbic acid is used in combination with SO_2 after disgorgement for sparkling wines (Karbowiak et al., 2010).

CHAPTER II
EXPERIMENTAL PART

1. Materials and methods

1.1. Wine samples

Wine samples were provided from Alpha Estate, Estate Argyros, Domaine Biblia Chora, Domaine Costa Lazaridi, Domaine Porto Karras and Papargyriou Estate.

Three different Greek dry white wines of 2014 vintage (Assyrtiko, Malagouzia and Sauvignon blanc) were used in the analyses. All the samples were in 750-ml glass bottles. The bottles were sealed using two type corks: DIAM P015= 0.0008 cm^3/day , DIAM P035 = 0.0015 cm^3/day (different oxygen flux rate) (http://www.diam-closures.com/) and stored in a dark room at 20°C and 30°C. After 0, 90, and 210 days of storage, 2 bottles were taken and each was analyzed in two replicates.

1.2. SO_2 Analysis

Determination of sulfur dioxide based on redox reaction of sulfur dioxide by iodine as follows:

$HSO_3^- + I_3^- + H_2O \longrightarrow SO_4^= + 3H^+ + 3I^-$

The oxidation is done in a strongly acidic environment, otherwise the iodine reacts with polyphenols, sugars, aldehydes and other reducing agents. The end of the reaction is controlled by the appearance of blue color when the excess iodine color gives the presence of starch. So the free sulfur dioxide is determined.

By varying the pH of the wine in a strongly alkaline by addition of KOH freeing the anhydride of the compounds of the acetaldehyde permitting determination of the bound form. The sum of free and bound gives the total sulfur dioxide.

The determination of free SO_2 must be performed immediately after opening the bottle because the anhydride is oxidized by air. In a conical 250 mL flask transfer 25 mL wine, 2.5 ml solution H_2SO_4 25%, 0.5 mL starch indicator and stir. Titrate with standard iodine solution 0.02 N until a bluish tinge and remain stable for 20-30 sec. Let A be the ml of I_2 consumed.

About total SO_2, in a conical 250 mL flask transfer 25 mL of wine and 12.5 mL of 1N KOH solution. The mixture was shaken and allowed to react for 10 min. Then add 5 mL solution of 25% H_2SO_4, 0.5 mL of starch indicator band stir. Titrate with standard iodine solution 0.02 N until a bluish tinge and remain stable for 20-30 sec. Let B be the mL of the I_2 consumed. (Κοτσερίδης και Προξενιά, 2012).

1.3. Color intensity

The color of wine is important for wine quality factor. Mainly due to anthocyanins, the tannins and other phenolic compounds. The absorbance is calculated by measuring the optical density by a spectrophotometer according to the official method of OIV (http://www.oiv.int/oiv/info/enmethodesinternationalesvin). The assessment of the color of the white wine is made by measuring the absorbance at 420 nm, measured in yellow. The absorption of a white wine at 420 nm (generally in the range 400- 440 nm) is proportional to the degree of oxidation.

1.4. Accelerated browning test

The model used to assess browning development by (Sioumis, Kallithraka, Makris, and Kefalas, 2006). Wine lots of 30 mL were filtered through pharmaceutical cotton and placed in a 45-mL, screw-cap glass vial (9.5 cm length, 2.5 cm internal diameter). Samples were subjected to heating at a constant temperature of 55.0 ± 0.2^{o}C in a water bath, in obscurity. Aliquots were withdrawn at 24-h intervals over a period of 12 days, and browning (A420) was measured. The samples were then immediately returned to the vials to maintain the initial headspace volume.

Figure 4. Wine samples after 12 days in water bath.

1.5. Acetaldehyde

Acetaldehyde in wine is generated through the oxidation of ethanol via hydrogen peroxide. Its concentration is considered to be an indication of the oxidative status of the wine.

The concentration of acetaldehyde determined according to the official method of OIV (http://www.oiv.int/oiv/info/enmethodesinternationalesvin). In 25 mL of wine added 2 g of activated charcoal. The flask shacked vigorously for a few seconds, allowed to stand for 2 minutes and filtered through a fluted slow filter to obtain a clear filtrate. Then in 2 mL of the clear filtrate added, 5 mL of the sodium nitroferricyanide solution and 5 mL of the piperidine solution. The mixture immediately placed into a 1 cm optical cell. The coloration produced, which varies from green to violet, is measured with reference to air at a wavelength of 570 nm. This color change increases then decreases rapidly; measure immediately and record the maximum value of the absorbance that is obtained after about 50 seconds. The concentration of acetaldehyde in the liquid analyzed is obtained using a calibration curve.

1.6. Antioxidant capacity

DPPH method is performed to measure the antioxidant capacity. The method used to assess antioxidant activity was a modification of that described by Brand-Williams and coworkers, 1995, based on the absorption of the radical 1,1-diphenyl-2-pikrylhydrazyl (DPPH) (Figure 5.A.). When the solution of a substance added with antioxidant activity then the DPPH radical with reduced intake of a hydrogen atom (or an e-) and converted to 1,1-diphenyl-2-picrylhydrazyl (Figure 5.B.), which has a yellow color, resulting to decrease the optical absorption. The absorption measurement is performed at 515nm.

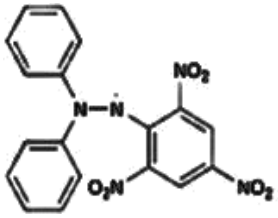
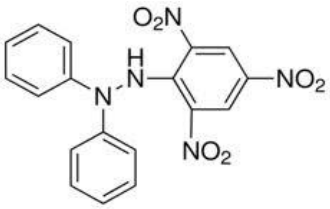
A.  B. 

Figure 5. Chemical structure of the radical 1,1-diphenyl-2-picrylhydrazyl (A) and 1,1-diphenyl-2-picrylhydrazyl (B).

DPPH decolorization was measured after the reaction of sample with the free stable radical DPPH·. Fresh methanol solution 1950 µL DPPH and 50 µL of wine were transferred into plastic cuvettes, after stirring with a hand stirrer in cuvettes and absorbance at a wavelength of λ = 515 nm was measured. Absorbance at time t_0 ranged between 0.200 and 1.000 depending on the nature of the sample assayed. The reaction mixture was left to stand for 30 min. The absorbance was again measured and the percent of inactivation calculated from the decrease of absorbance according to the relationship:

$$\% \Delta A_{(515)} = [A_{(t0)} - A_{(t30)} / A_{(t0)}] \times 100$$

The calibration curves of Trolox expressed antioxidant capacity in mg/L Trolox.

1.7. GC Analysis

1.7.1. SPME extraction and analysis

The SPME holder, for manual sampling, and fiber 50/30-µm divinylbenzene – carboxen on poly(dimethylsiloxane) (DVB–CAR–PDMS) used in the analyses were purchased from Supelco (Aldrich, Bornem, Belgium).

Figure 6. Sampling of aroma compounds**.**

The SPME fiber was conditioned as recommended by the manufacturer at some degrees below each fiber's maximum temperature before it was used for the first time. Before the first daily analysis, the fiber was conditioned for 5 min at 220°C in the GC injector. For the following analyses, 5 min of desorption after each extraction was used as conditioning time.

The fiber were immersed in the headspace of the samples. For sampling an aliquot of 7 ml of wine, 3ml distilled water, 3g/10 ml for saturation NaCl and 10µl 3-octanol as internal standard were transferred into a screwcap glass vial with a Teflon rubber septum. The vial was placed in a thermostated bath 35^{o}C and stirred for 10 min at 400 rpm, and a constant length of fiber was then exposed to the headspace for another 30 min under the same conditions.

1.7.2. Gas chromatography

All samples were analyzed with a Hewlett - Packard 5890 gas chromatograph equipped with a flame ionization detector (FID). Compounds were separated on a polar column DB-WAX (30m length × 0.320mm I.D.) coated with a 0.25µm film of stationary phase. The FID temperature was 250 ^{o}C. Helium was used as a carrier gas with a column flow rate of 1 ml*min^{-1}. The GC oven temperature was programmed from 40^{o}C (held for 5 min) at 3^{o}C*min^{-1} to 220^{o}C (held for 5 min). Selected aroma compounds were identified using known standards (ethyl isobutyrate, ethyl butyrate, ethyl-2methyl butyrate, isoamyl acetate, isoamyl alcohol, ethyl caproate, hexyl acetate, ethyl caprylate, linalool, ethyl decanoate, 2-phenylethyl acetate, ethyl dodecanoate, phenethyl alcohol) and the quantification was performed using the internal standard for which the response coefficient of each compound was determined. A calibration curve was created for each volatile compound. Since all of the volatile compounds are naturally present in the wine sample, the calibration was corrected by subtracting the blank ratios (peak area of analyte/peak area of internal standard). The ratios of the peak area of analyte to peak area of internal standard were plotted against the corresponding volatile compound concentration (linear regression). The calibration equations that were obtained were used to quantify the volatiles in each of the wines.

1.8. Sensory analysis

We used two paired comparison test. A panel of 25 trained persons performed the organoleptic examination of all samples in order to provide the quality limits. We used two paired comparison test to find the differences between two types of corks.

1.9. Statistical analysis

All determinations were run in duplicate and values were averaged. The standard deviation (SD) was also calculated. Correlations between P0.15 and P0.35 closures were established using one way analysis and comparisons for each pair using Student's t. Also comparisons for all pairs using Tuckey – Kramer HSD. All statistical analyses were performed by JMP (10.0.0)

Values with ***** show pairs of means that are significantly different. Levels not connected by same letter are significant different.

2. RESULTS AND DISCUSSION

Following text contains the analytical results in the order of appearance, free SO_2, total SO_2, color intensity, accelerated browning test, acetaldehyde, antioxidant capacity, aroma compounds and sensory evaluation, each one performed for the three wine varieties Assyrtiko, Malagouzia and Sauvignon blanc, closed with two types of corks (P0.15 and P0.35), when stored at 20°C and 30°C for up to 7 months period.

2.1. Free SO_2

The free SO_2 concentration in mg/L (± standard deviation of two replicates per sampling time), is following for the Assyrtiko variety (Fig. 7), Malagouzia variety (Fig. 8) and Sauvignon blanc variety (Fig. 9) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the relevant figure index. Statistical significant differences are indicated with different letters, while capital letters refer to the one cork and lower letters for the other.

2.1.1. Assyrtiko

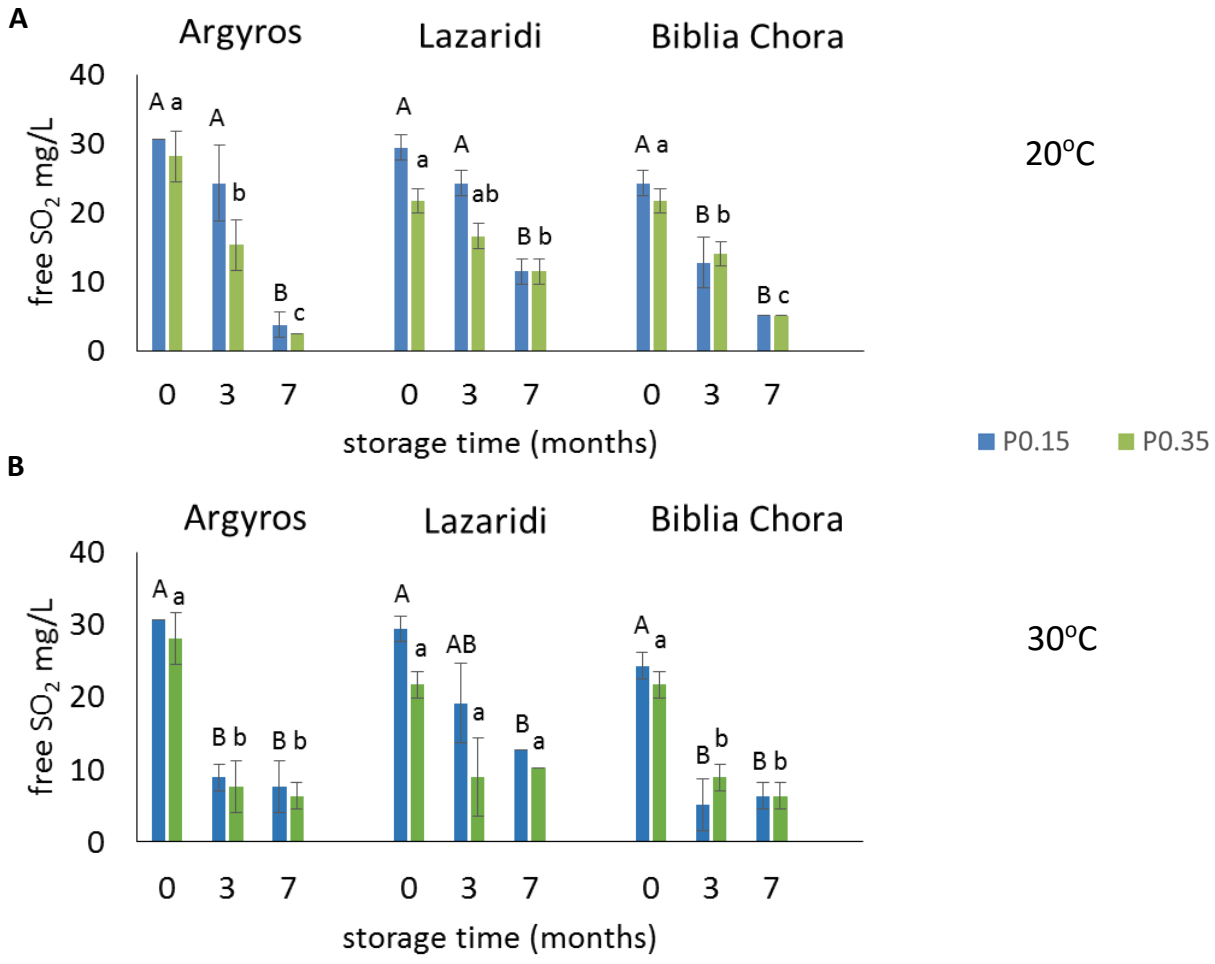


Figure 7. Concentration of free SO_2 during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

We are observing lower concentrations of free sulfur dioxide at 20°C, with a statistically significant difference between the 3th and 7th month of storage regarding the Argyros and Lazaridi samples, but not in the case of the Biblia Chora samples.

In comparison, lower concentration of free SO_2 during storage time at 30°C was observed with statistical significant differences between the 0 and 3rd month of storage. Between the two types of corks, P0.15 and P0.35, there is no significantly statistical difference at either storage temperature.

2.1.2. Malagouzia

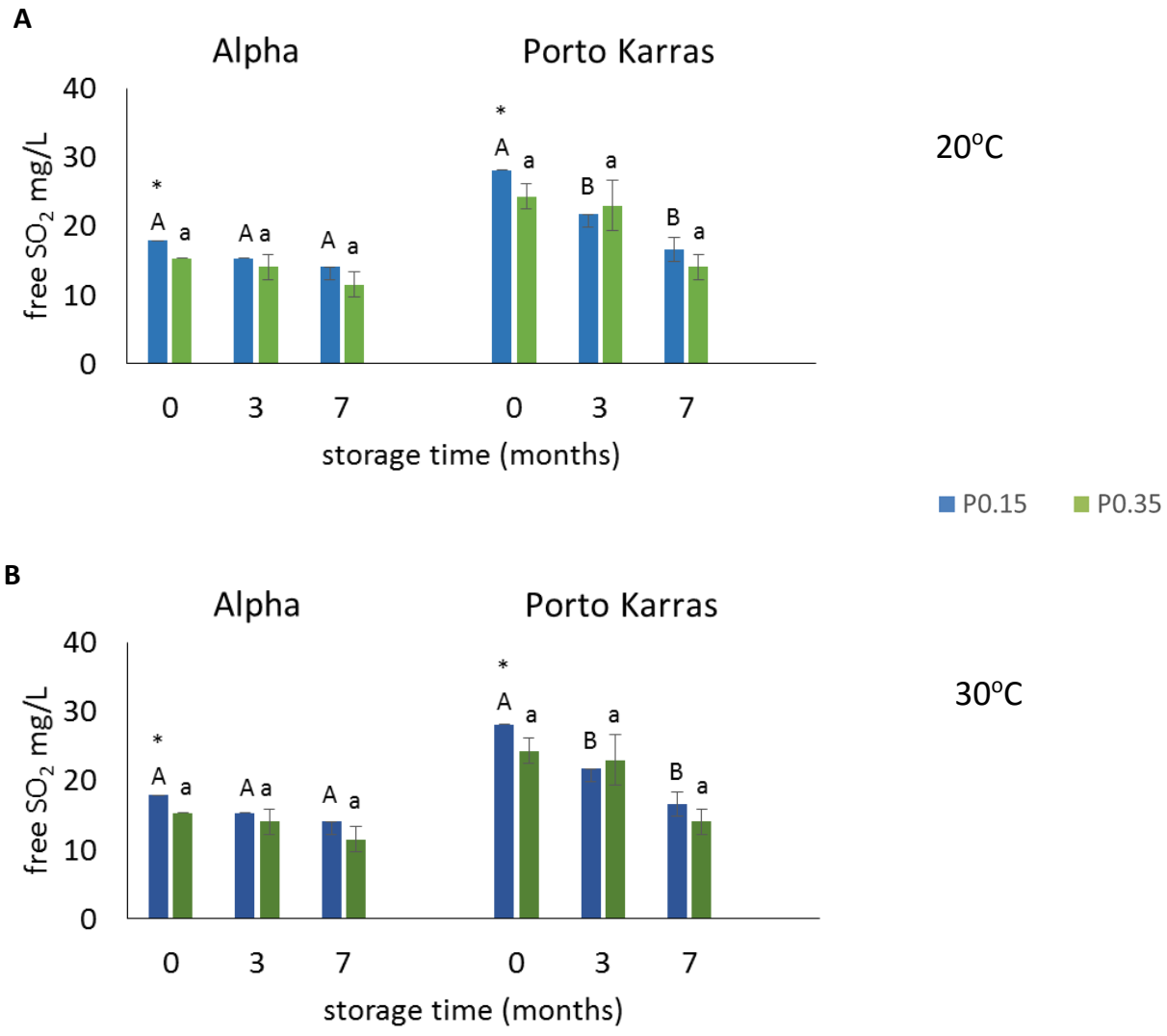


Figure 8. Concentration of free SO_2 during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

We are observing lower concentrations of free sulfur dioxide at 20°C, with a statistically significant difference between the 0 and 3rd month about Porto Karras samples but not in the case of Alpha samples.

In comparison, lower concentration of free SO_2 during storage time at 30°C was observed with statistical significant differences between the 0 and 3rd month of storage about Porto Karras samples. Between the two types of corks, P0.15 and P0.35, there is no significantly statistical difference at either storage temperature.

2.1.3. Sauvignon blanc

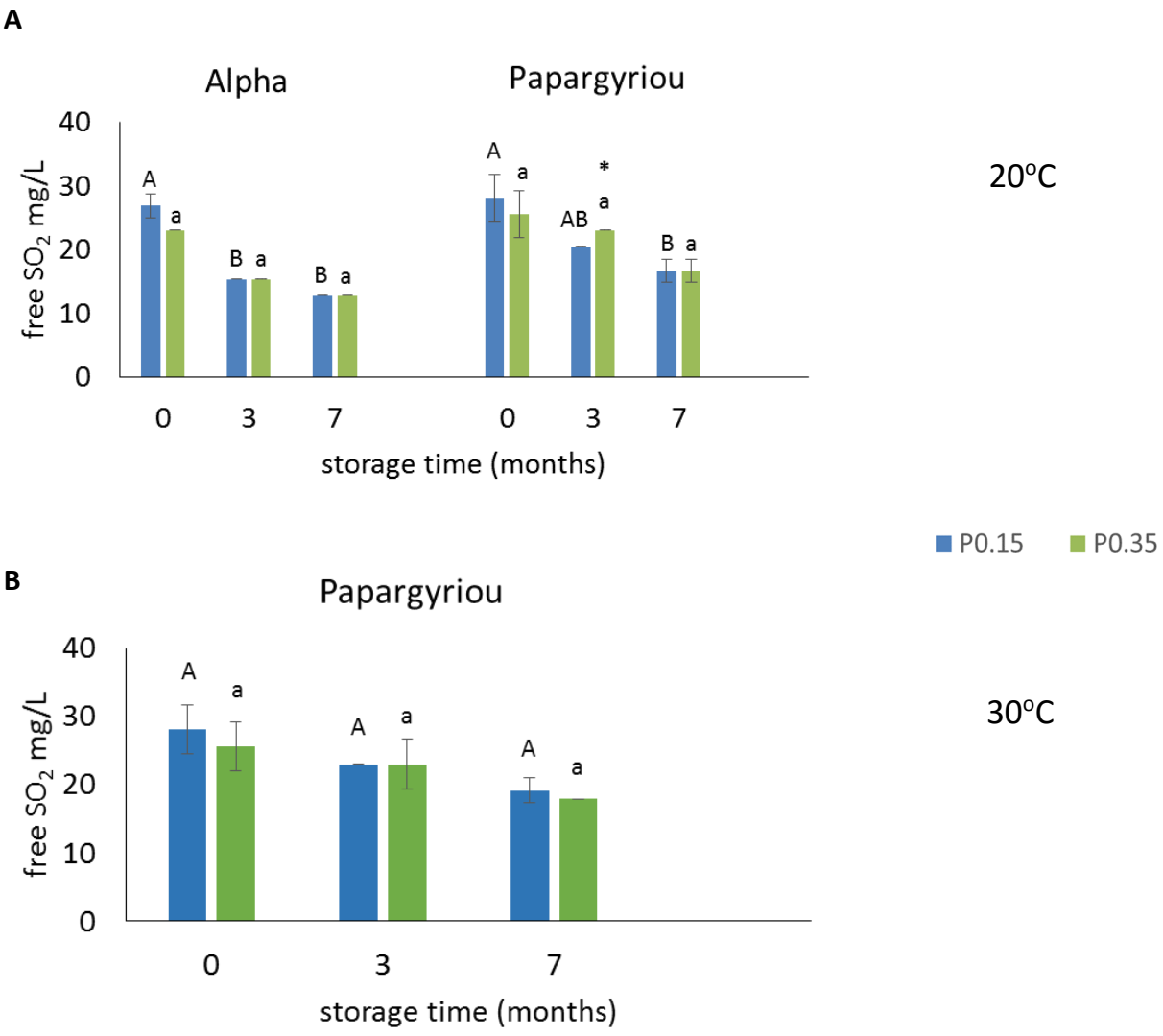


Figure 9. Concentration of free SO_2 during storage time about Sauvignon blanc variety at 20°C (A) and 30°C (B).

We are observing lower concentrations of free sulfur dioxide at 20°C, with a statistically significant difference between the 0 and 3rd month about Alpha samples but not in the case of Papargyrioy samples. Between the two types of corks, P0.15 and P0.35, there is no significantly statistical differences, except for the Papargyriou samples at 3 months of storage which P0.35 cork maintain more free SO_2, but this trend was not confirmed in 7 months of storage. In comparison, at 30°C was observed no significantly statistical differences during storage time.

In a study aiming in correlating the oxidative alterations of wine compounds to the oxygen availability through permeation, Garde-Cerdán and Ancín-Azpilicueta (2007) demonstrated that wine stored for 6 months in bottle with SO_2 showed a higher concentration of the majority of the flavor compounds studied in comparison to wines aged in bottle without SO_2. If the free SO_2 content drops below 10 mg*L^{-1}, white wine will be subjected to increasing oxidation (Li, Guo, & Wang, 2008). The values determined for free SO_2 in the various packaging materials were low as a potential result from sulphites acting reductively by producing oxidations products (combined SO_2). In fact, sulphur dioxide is the most important and widely used chemical to prevent wine from browning. Besides antioxidant activities, SO_2 also has antimicrobial properties and other important functions. However, its excessive use can drastically compromise the quality of wine and excessive quantities of SO_2 can actually give the wine unpleasing flavors and aromas or may favor the wine to turn cloudy during its keeping (Li et al., 2008). The decrease of the SO_2 content in a very short period confirmed the higher oxygen transfer rate.

As expected, a decrease in SO_2 occurred in all the packaging configurations was not limited for the lower temperatures neither the low permeability cork. The final SO_2 content in the other configurations was below 20 mg*L^{-1}, which is rather low value for the 7 months storage. Apparently, oxygen that diffuses in the wine causes a SO_2 depletion similar in all the wine samples. Therefore the insignificant detected differences were likely due to the fact that within the 7 months-time corks did not determine the permeation of further oxygen and, as a consequence, a similar SO_2 oxidation occurred in the packed wine. (Mentana et al., 2009).

According to Godden et al. (2001), the loss of SO_2 was in general highly correlated with an increase in wine browning (OD_{420}) and the concentration of SO_2 in the wine at six months was a strong predictor of future browning in the wine, particularly after eighteen months. Neither the concentration of dissolved oxygen at bottling (0.6–3.1 mg/L), nor the physical closure measures were predictors of future browning. For several closures upright storage tended to accelerate loss of SO_2 from the wine, but in many cases this effect was marginal.

However, the direct reaction of sulfur dioxide with oxygen under wine conditions is very slow and essentially irrelevant. Thus, the sulfur dioxide probably reacted with hydrogen peroxide, aldehydes and ketones (Lopez et al., 2009).

2.2. Total SO_2

The total SO_2 concentration in mg/L (± standard deviation of two replicates per sampling time), is following for the Assyrtiko variety (Fig. 10), Malagouzia variety (Fig. 11) and Sauvignon blanc variety (Fig. 12) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the relevant figure index. Statistical significant differences are indicated with different letters, while capital letters refer to the one cork and lower letters for the other.

2.2.1. Assyrtiko

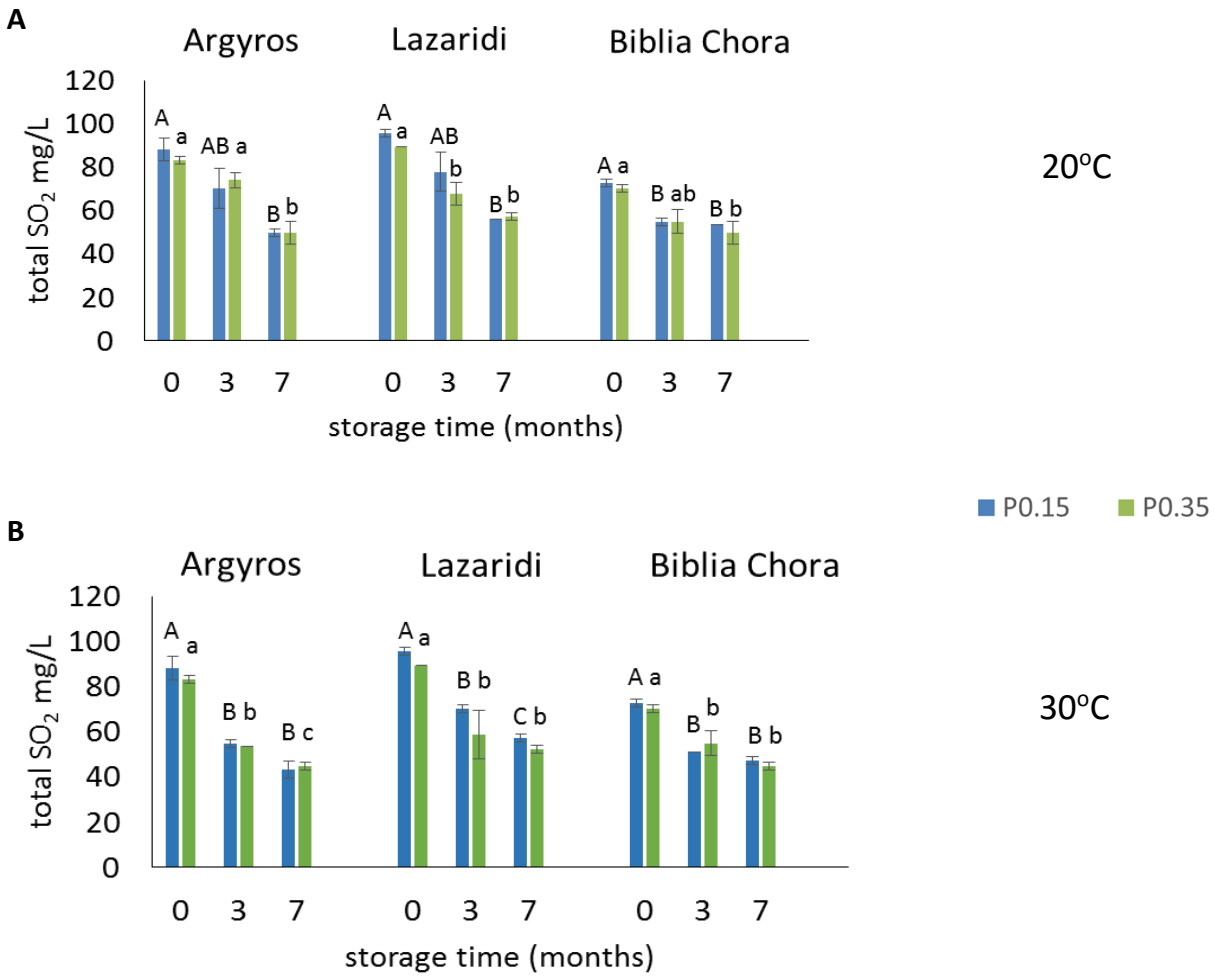


Figure 10. Concentration of total SO_2 during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

We are observing lower concentrations of free sulfur dioxide at 20°C, with a statistically significant difference between the 3rd and 7th about all samples.

In comparison, lower concentration of free SO_2 during storage time at 30°C was observed with statistical significant differences among all the sampling times. Between the two types of

corks, P0.15 and P0.35, there is no significantly statistical difference at either storage temperature.

2.2.2. Malagouzia

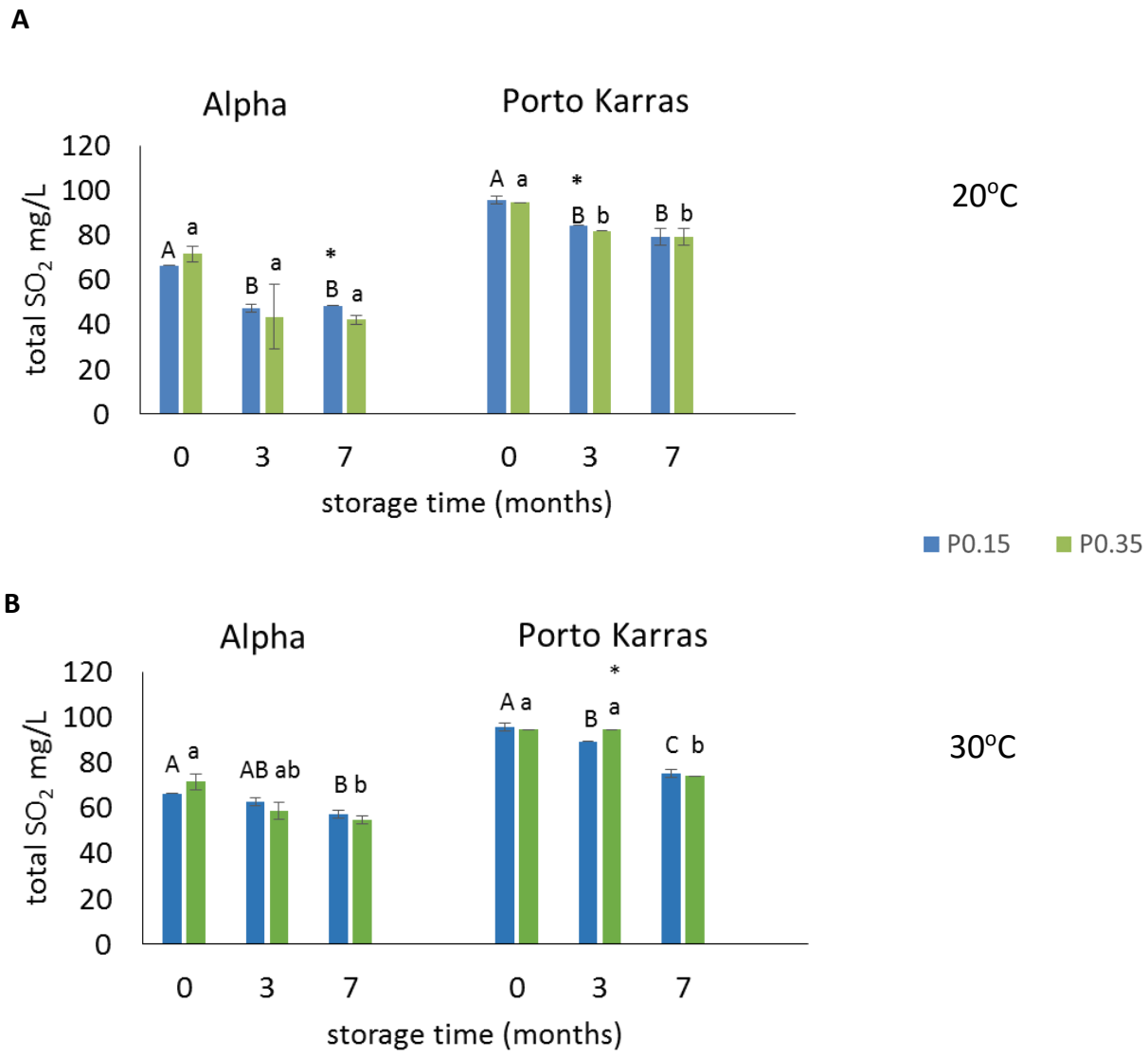


Figure 11. Concentration of total SO_2 during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

There was statistically significant difference between the 0 and 3rd month of storage at 20°C. Moreover, at 3 months of storage, P0.15 cork of Alpha samples maintain more total SO_2 than P0.35 cork and at 7 months of storage P0.15 cork of Porto Karras samples maintain more total SO_2 than P0.35 cork.

In comparison, at 30°C was observed with statistical significant differences between among all the sampling time. Between the two types of corks, P0.15 and P0.35, there is no significantly statistical difference at either storage temperature except in the case of Porto Karras cork P0.35 which keep more total SO_2 than cork P0.15.

2.2.3. Sauvignon blanc

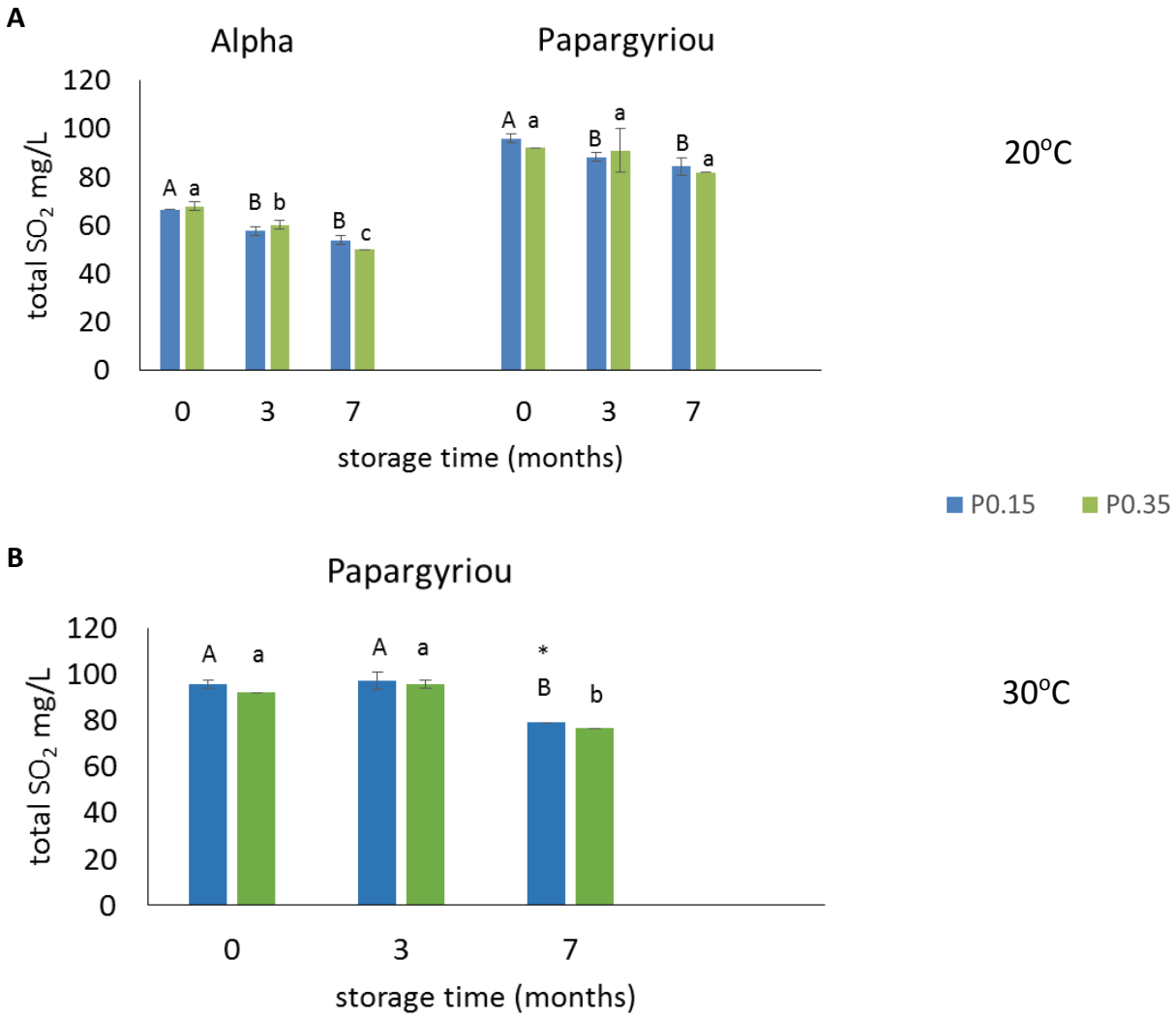


Figure 12. Concentration of free SO_2 during storage time about Sauvignon blanc variety at 20°C (A) and 30°C (B).

There was statistically significant difference between the 0 and 3rd month of storage at 20°C.In comparison, at 30°C was observed with statistical significant differences between 3rd and 7th month of storage. Between the two types of corks, P0.15 and P0.35, there is no significantly statistical difference at either storage temperature except in the case of Papargyriou sample with cork P0.15 at 30°C which keep more total SO_2 than cork P0.15 than cork P0.35.

2.3. Color intensity

The wine absorbance at 420 nm (A420nm) is a measure of the level of yellow/brown color of white wine, being considered as a useful indicator of wine development and degree of oxidation. The values of A420nm for the wines during the storage period are given. The absorbance at 420 nm (± standard deviation of two replicates per sampling time), is following for the Assyrtiko variety (Fig. 13), Malagouzia variety (Fig. 14) and Sauvignon blanc variety (Fig. 15) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the relevant figure index. Statistical significant differences are indicated with different letters, while capital letters refer to the one cork and lower letters for the other.

2.3.1. Assyrtiko

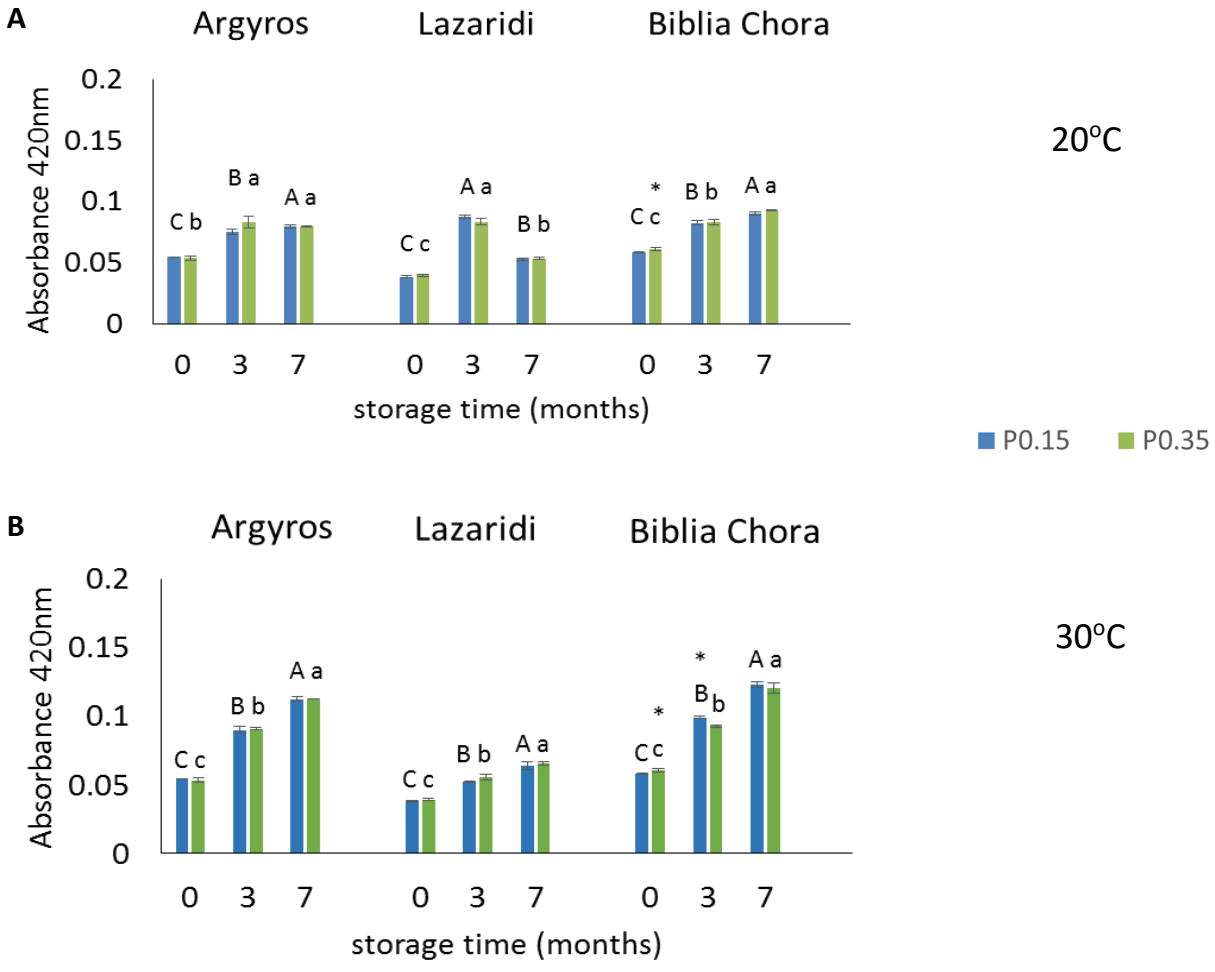


Figure 13. Absorbance 420 nm during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

We are observing an increasing trend in absorbance at 420 nm as expected at 20°C, except in the case of Lazaridi sample 7 month of storage. Between the two types of corks, P0.15 and P0.35 there is no significantly statistical difference.

In comparison, higher absorbance during storage time at 30°C was observed with statistical significant differences among all the sampling times. Biblia Chora sample with cork P0.15 has higher absorption at 420 nm at 3 months of storage.

2.3.2. Malagouzia

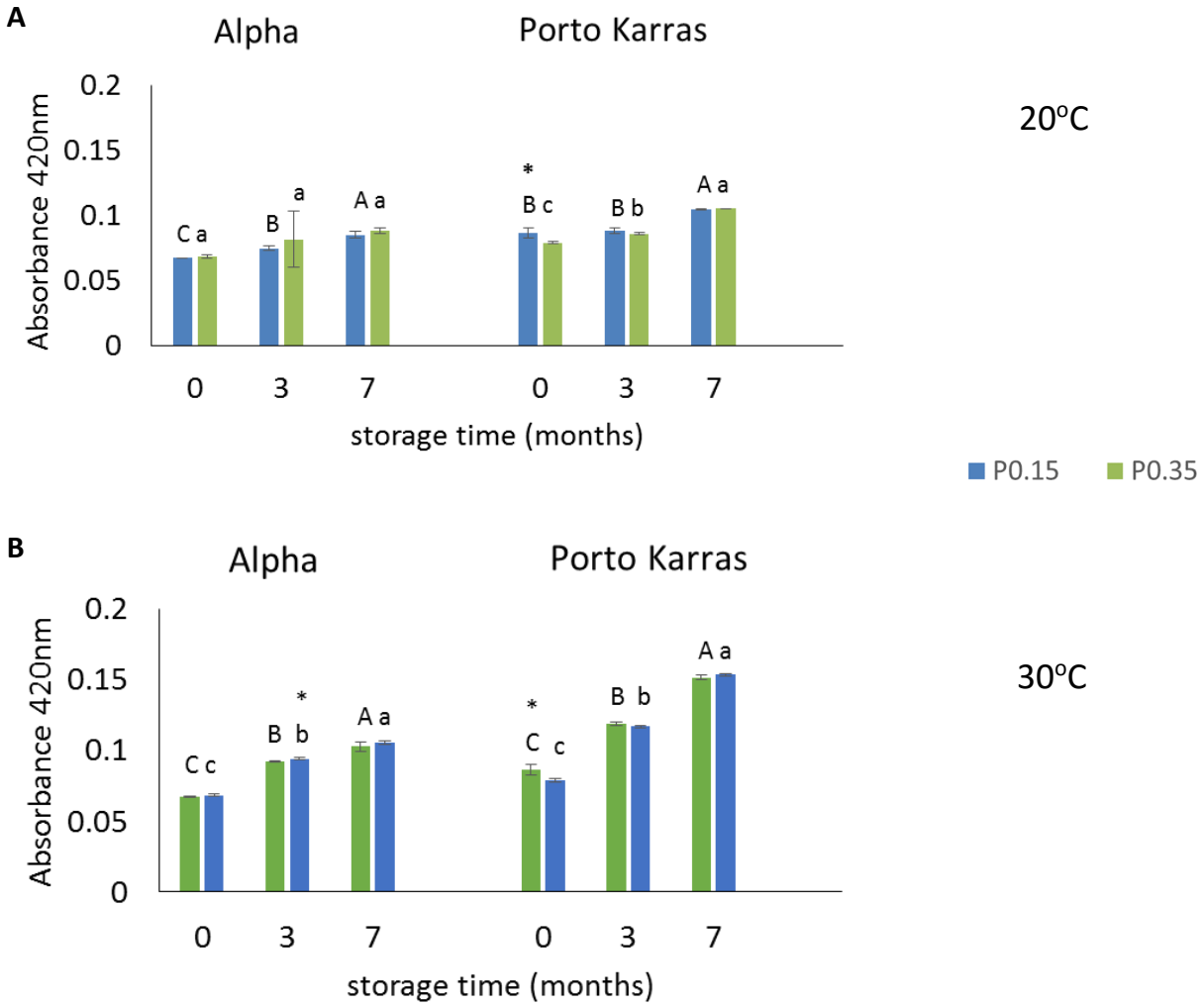


Figure 14. Absorbance 420 nm during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

We are observing an increasing trend in absorbance at 420 nm as expected at 20°C. Between the two types of corks, P0.15 and P0.35 there is no significantly statistical difference.

In comparison, higher absorbance during storage time at 30°C was observed with statistical significant differences among all the sampling times. Alpha sample with cork P0.35 has higher absorption at 420 nm at 3 months of storage.

2.3.3. Sauvignon blanc

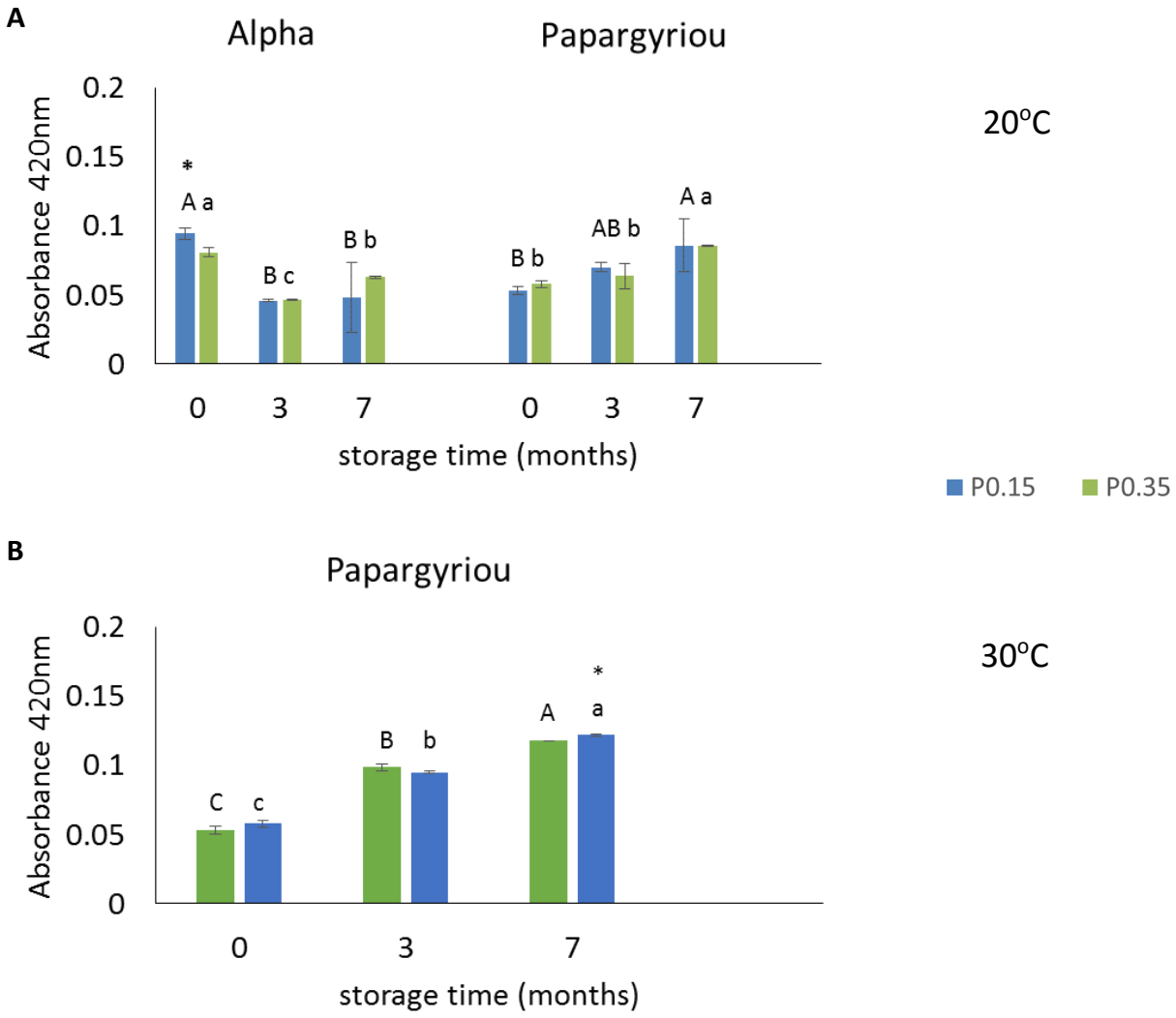


Figure 15. Absorbance 420 nm during storage time about Sauvignon blanc variety at 20°C (A) and 30°C (B).

We are observing an increasing trend in absorbance at 420 nm as expected at Papargyriou samples but Alpha samples has a peculiar decreasing trend during storage time at 20°C at the 3rd moth, which could be a miss-calculated result. Between the two types of corks, P0.15 and P0.35 there is no significantly statistical difference for the same temperature.

In comparison, higher absorbance during storage time at 30°C was observed with statistical significant differences among all the sampling times. Papargyriou sample with cork P0.35 has higher absorption at 420 nm at the end of storage time.

These findings indicate that wine color changed throughout storage, being rather distinctive at 7 months with a further decreasing trend. It was reported by Lopez et al, (2009) that when the levels of ascorbic acid and sulfur dioxide were almost depleted, the color change was significant. Comparatively, these researchers showed that under anaerobic environment (minimum oxygen), the wine color changes were residual when compared with other wines exposed to higher oxygen levels.

A decrease in SO_2 was shown to accelerate the oxidation of wine and the change of hue. Browning is an oxidative process involving sugars, lipids, amino acids or phenols. It is one of the main problems encountered during the vinification of wine as it on one hand, adversely affects the sensory properties of wine (loss of color, flavor and aroma and increase of astringency), (Ghidossi et al., 2012). Therefore, color development after bottling depends on the contact of wine with oxygen throughout storage.

Furthermore, the chromatic changes during wine browning were well documented regarding the aromatic deterioration occurring prior to the color change (Escudero et al., 2002; Silva Ferreira et al., 2002). At the same time, flavor degradation during wine browning has received attention on the relationship between the changes of flavor and color in wine (Ferreira et al., 1997; Silva Ferreira, Oliveira, Hogg, & Guedes de Pinho, 2003).

Timberlake and Bridle (1976) first proposed one of the mechanisms that acetaldehyde could contribute to the formation of dimer and trimer between flavanols (tannins), and later it was confirmed by other researchers (Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999; Fulcrand, Doco, Es-Safi, Cheynier, & Moutounet, 1996; Saucier, Guerra, Pianet, Laguerre, & Glories, 1997). The outcome of this increases the color of the yellow spectral region as it does the condensation degree (Lopez-Toledano, Villano-Valencia, Mayen, Merida, & Medina, 2004).

As shown in model systems and red wines, direct condensation would be achieved between anthocyanins and tannins or catechins to form anthocyanin–tannin and tannin–anthocyanin adducts, but the reaction is very slow, and the eventual products are yellow xanthylium salts, which always changes the color of red wine into orange (Atanasova et al., 2002).

Compared to direct condensation between anthocyanins and tannins or catechins, rapid polymerization between them mediated by acetaldehyde occurs with increased wine color intensity and stability, but further polymerization with flavanols gives rise to instability, precipitation and decreased color (Es-Safi et al., 2002, 2003b; Liu & Pilone, 2000).

In addition, acetaldehyde might form new types of pigments such as Vitisin B and other proanthocyanidins that are more stable to SO_2 bleaching and the pH effect than free anthocyanins in model systems, which may be important in stabilizing wine colour (Morata, Calderon, Gonzalez, Gomez-Cordoves, & Suarez, 2007). The evolution of acetaldehyde will be discussed further below.

Although the oxygen management at bottling and the choice of wine closure type is likely to have a considerable impact on the wine color after bottling, that impact could not be detected in this study for the wines in storage up to 7 months at either temperature (Lopez et al., 2009).

2.4. Accelerated browning test

Oxidation rate calculated from the slope of the regression lines, obtained after plotting A_{420} as a function of time (± standard deviation of three replicates per sampling time), is following for the Assyrtiko variety (Fig. 16), Malagouzia variety (Fig. 17) and Sauvignon blanc variety (Fig. 18) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the relevant figure index. Statistical significant differences are indicated with *.

2.4.1. Assyrtiko

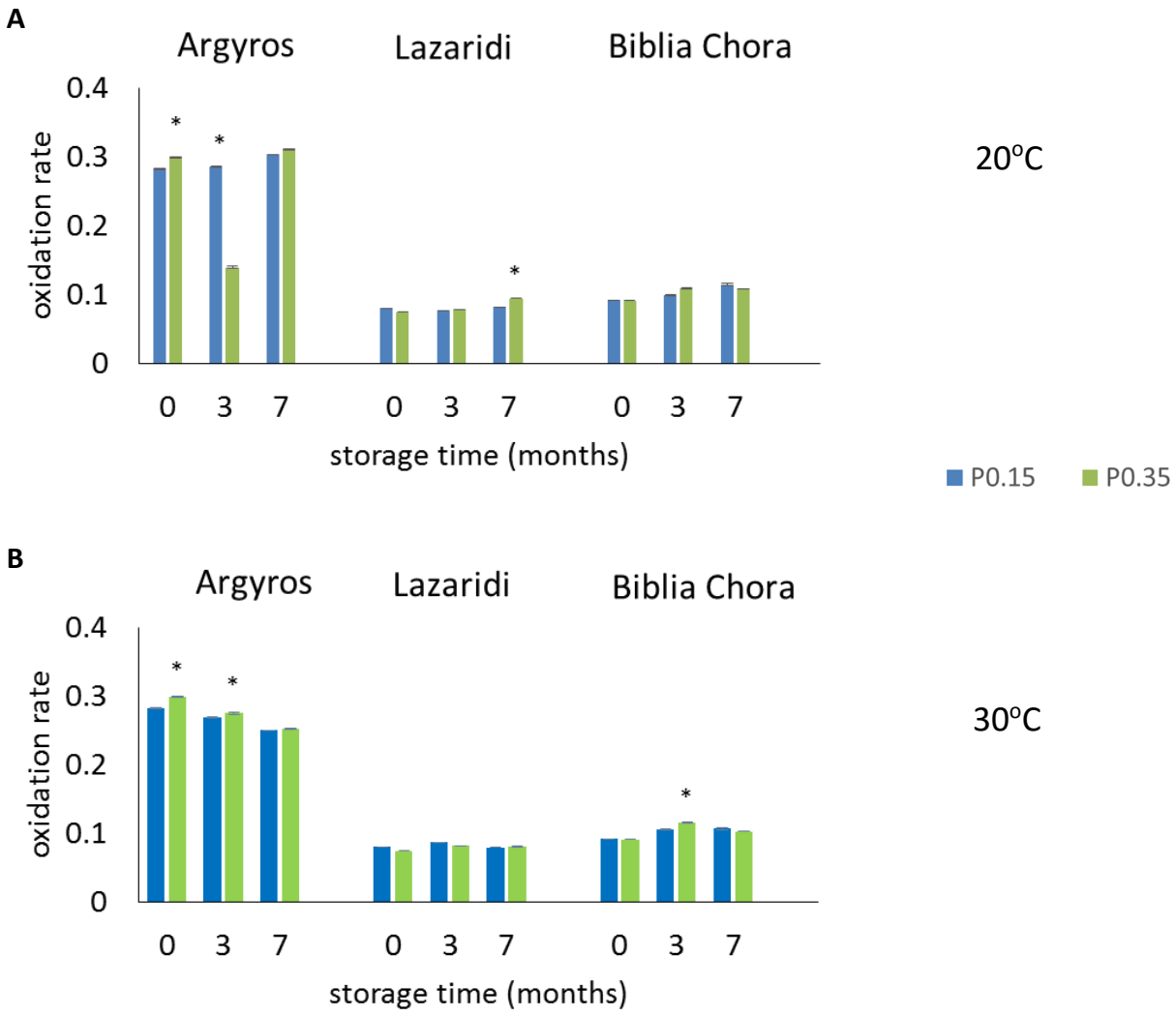


Figure 16. Oxidation rate during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

It is interesting the comparison between the two types of corks individually in each time and not during the storage time.

So oxidation rate is the same about Biblia Chora samples but not in the case of Argyros which at 0 months P0.35 cork seems to oxidize faster than cork P0.15 but unexpected, it happens the opposite at time 3 months at 20°C. Moreover, between the two types of corks, P0.15 and P0.35 there is a significantly statistical difference at time 7 months about Lazaridi samples.

In comparison to samples at 30°C, we are observing that Argyros samples with cork P0.35 oxidize faster than cork P0.15 at 0 and 3 months of storage. Between the two types of corks, P0.15 and P0.35 there is a significantly statistical difference at time 3 months about Biblia Chora samples.

2.4.2. Malagouzia

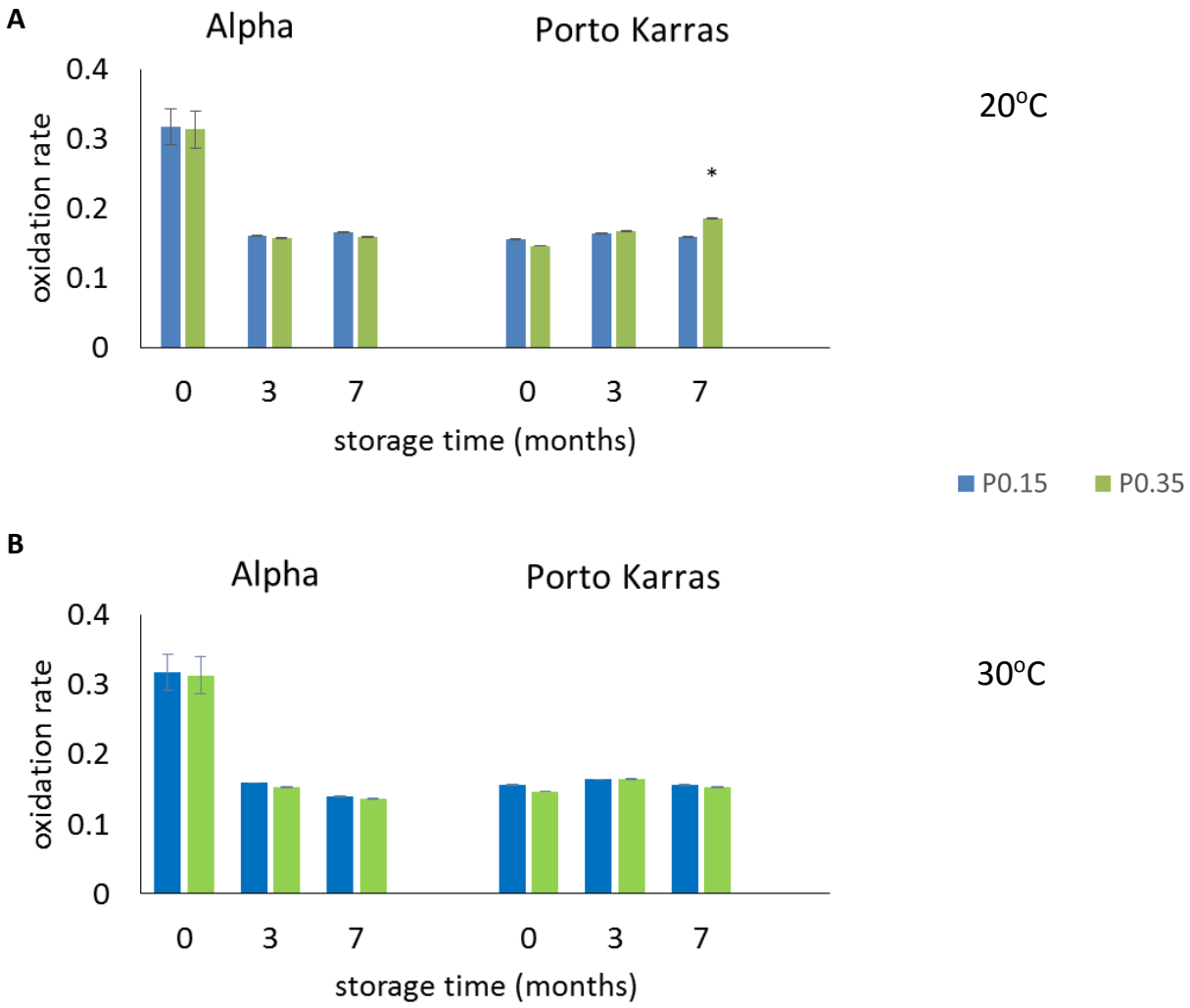


Figure 17. Oxidation rate during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

Comparing two types of corks P0.15 and P0.35 there is no significantly statistical differences either temperatures, except in the case of Porto Karras where cork P0.35 seems to oxidize faster than cork P0.15.

2.4.3. Sauvignon blanc

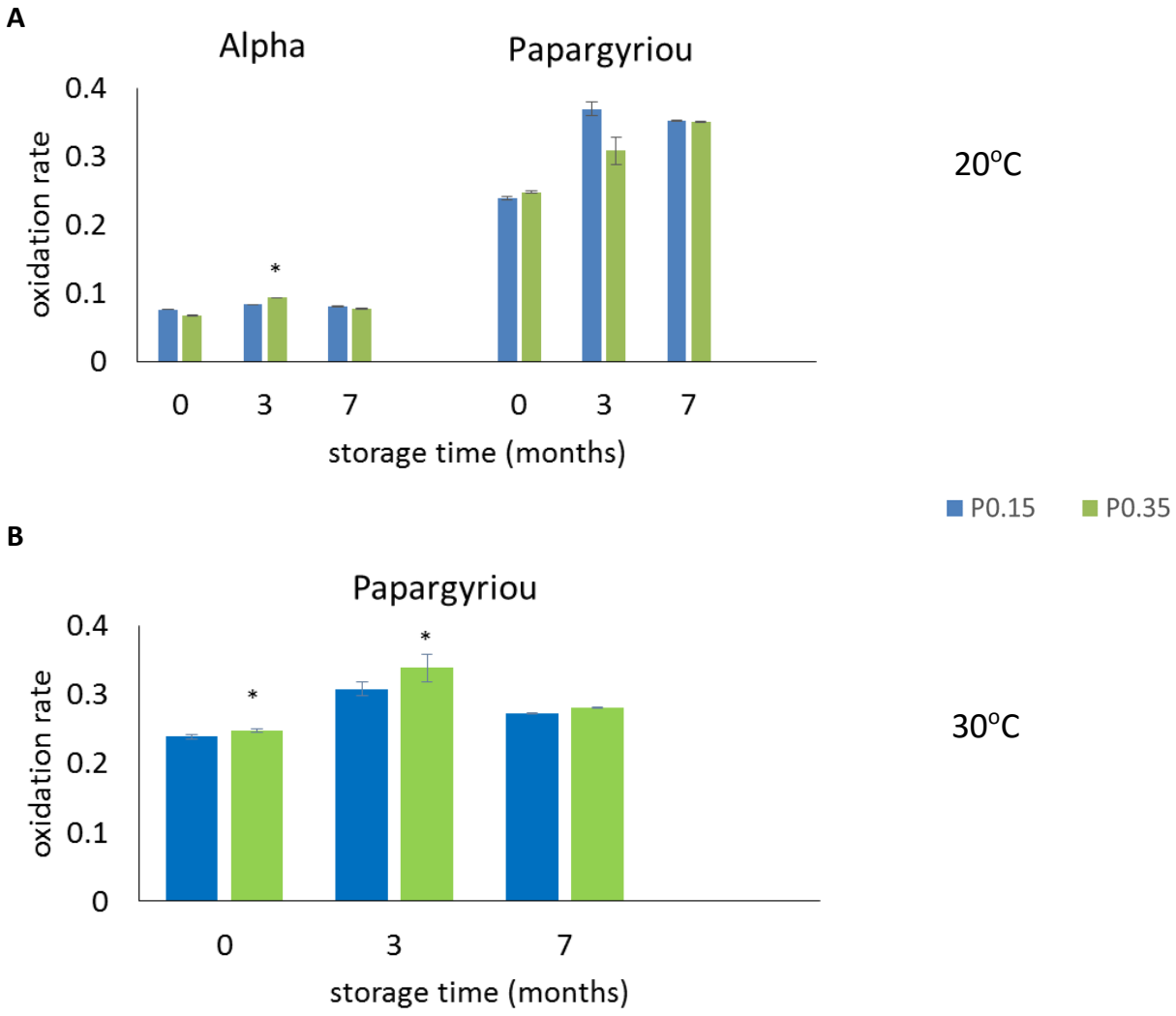


Figure 18. Oxidation rate during storage time about Sauvignon blanc variety at 20°C (A) and 30°C (B).

Oxidation rate is the same among the samples at 20°C except in the case of Alpha sample where cork P0.35 oxidize faster than P0.15 at 3 months of storage.

On the other hand, cork P035 of Papargyriou samples oxidize faster than cork P0.15 at 0 and 3 months of storage at 30°C.

2.5. Acetaldehyde

The acetaldehyde concentration in mg/L (± standard deviation of two replicates per sampling time), is following for the Assyrtiko variety (Fig. 19), Malagouzia variety (Fig. 20) and Sauvignon blanc variety (Fig. 21) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the

relevant figure index. Statistical significant differences are indicated with different letters, while capital letters refer to the one cork and lower letters for the other.

2.5.1. Assyrtiko

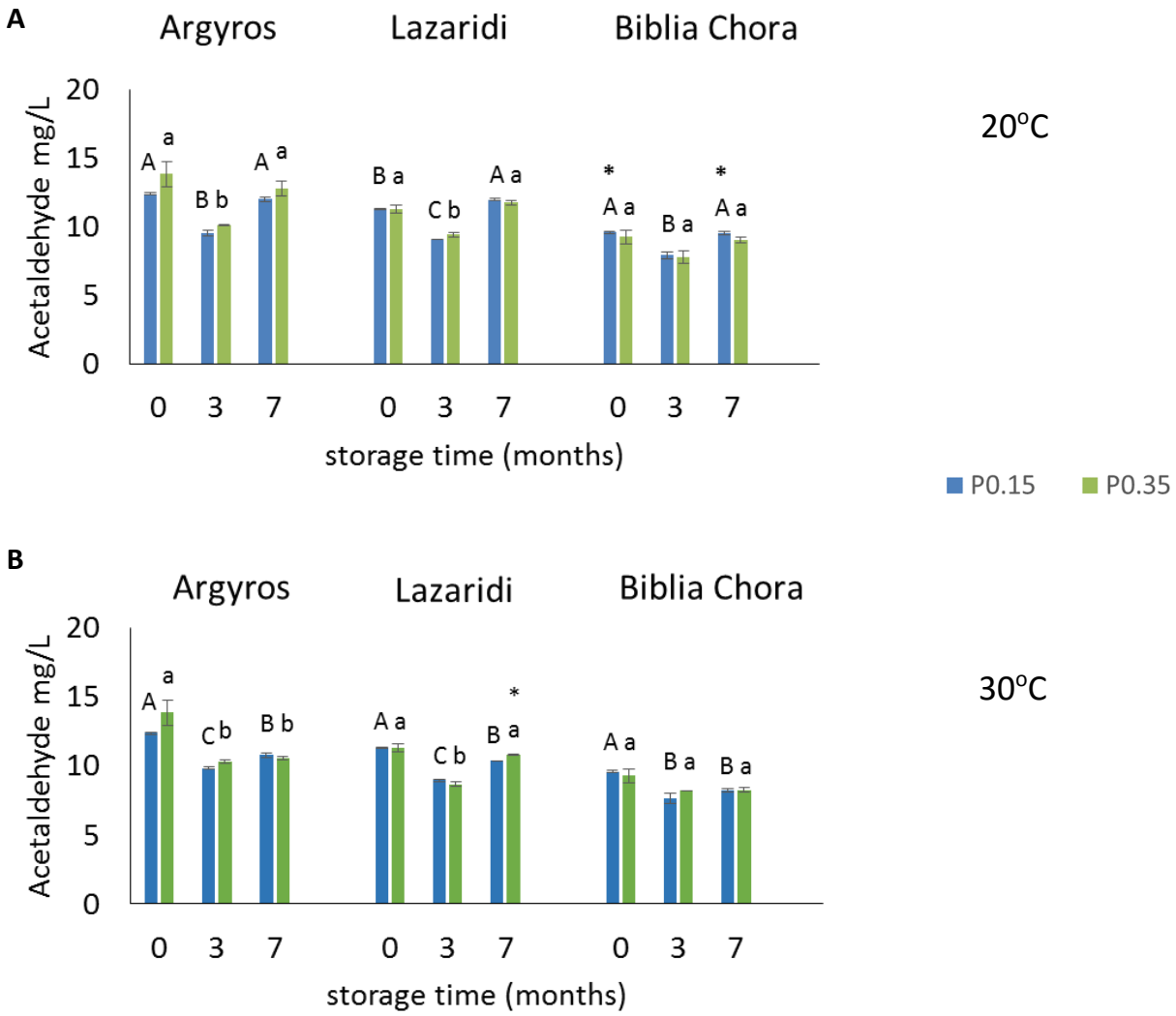


Figure 19. Concentration of acetaldehyde during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

Firstly, we are observing a decreasing trend of concentration due to acetaldehyde bound of SO_2 at 20°C. After 3 months we can see an increasing trend as expecting. Between two types of corks, P0.15 and P0.35, there is a significantly statistical difference at Vivlia Chora sample with cork P0.15 (lower permeability) shows higher values compared to cork P0.35 (higher permeability).

In comparison, there is no significantly statistical differences at 30°C. But in this case Lazaridi sample with cork P0.35 has higher values compared to cork P0.15.

2.5.2. Malagouzia

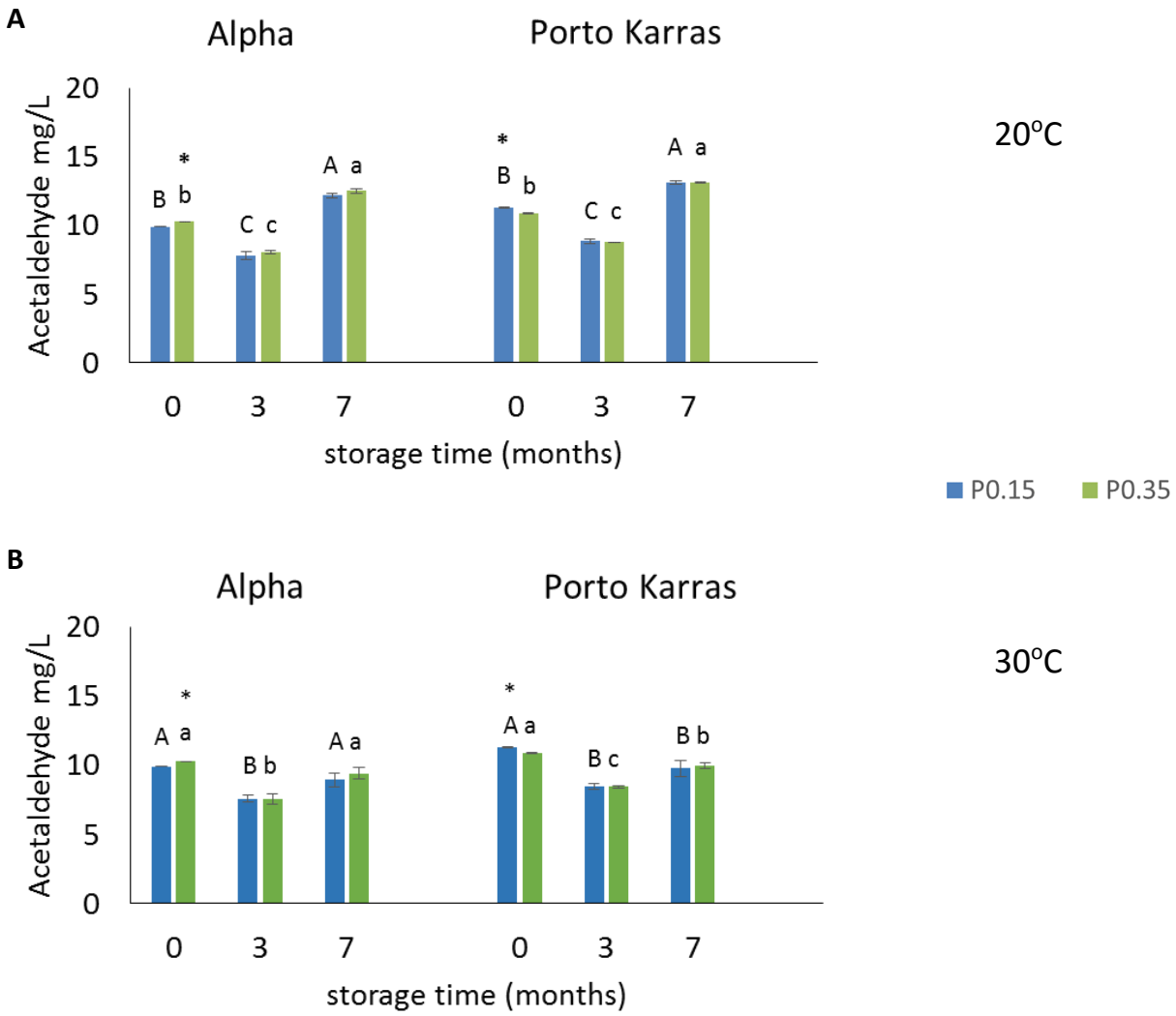


Figure 20. Concentration of acetaldehyde during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

We are observing a similar situation such as Assyrtiko variety either storage temperatures. Also between the two types of corks, P0.15 and P0.35 there is no significantly statistical difference in the end of storage time.

2.5.3. Sauvignon blanc

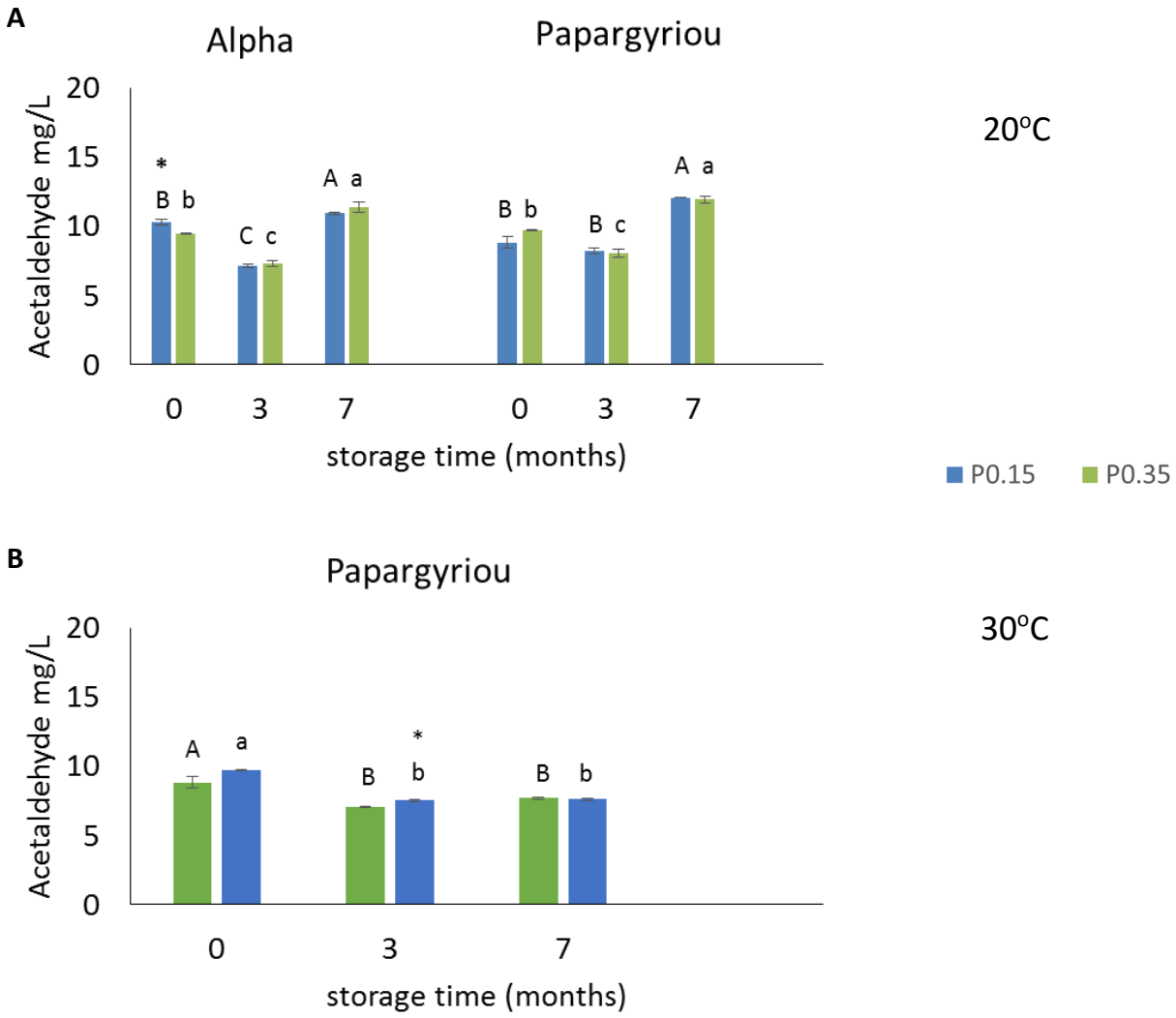


Figure 21. Concentration of acetaldehyde during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

It is also observed a similar fluctuation in concentration of acetaldehyde such as the two above cases of Assyrtiko and Malagouzia either storage temperatures. Between the two types of corks, P0.15 and P0.35 there is no significantly statistical differences, except in the case of Papargyriou sample at 3th month of storage at 30°C which cork P0.35 has higher value of acetaldehyde.

Traditionally, acetaldehyde is considered to possess an offensive odor and taste, which brings bitterness and oxidized flavor to wine, and if its level exceeds 50 mg/L in a table wine, it means that the wine has been oxidized (Zhai et al., 2001). However, acetaldehyde appears to be the typical substances of flavor like ripen nuts in some dry sherry wines subjected to biological or oxidative aging (Ferreira et al., 1997).

Acetaldehyde present in wine is derived from the yeast metabolism during fermentation or eventual biological aging and from the oxidation of ethanol catalyzed by transition metals or through coupled oxidation of phenols. The first route in which acetaldehyde is produced mostly takes place in certain wines such as sherry wines, and the second route is the most important in most wines.

2.6. Antioxidant capacity

The antioxidant capacity expressed in mM Trolox (± standard deviation of two replicates per sampling time), is following for the Assyrtiko variety (Fig. 22), Malagouzia variety (Fig. 23) and Sauvignon blanc variety (Fig. 24) wines for 0, 3 and 7 months of storage. At every sampling time the results for the two different corks are presented in different color columns as given in the relevant figure index. Statistical significant differences are indicated with different letters, while capital letters refer to the one cork and lower letters for the other.

2.6.1. Assyrtiko

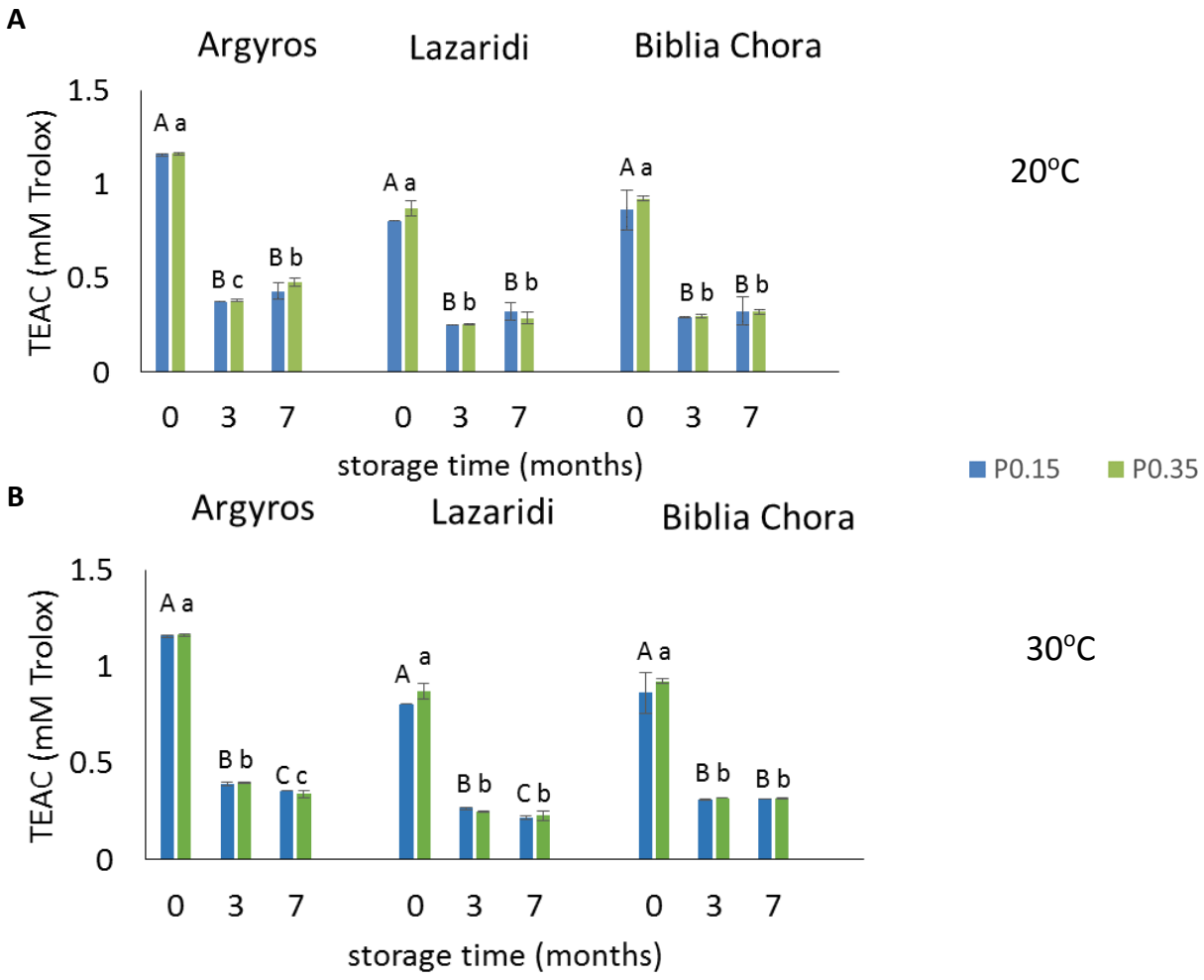


Figure 22. Concentration of TROLOX during storage time about Assyrtiko variety at 20°C (A) and 30°C (B).

During the 7 months of storage antioxidant capacity reduced at either temperatures among all the samples. But between two types of corks P0.15 and P0.35 there is no significantly statistical differences either temperatures.

2.6.2. Malagouzia

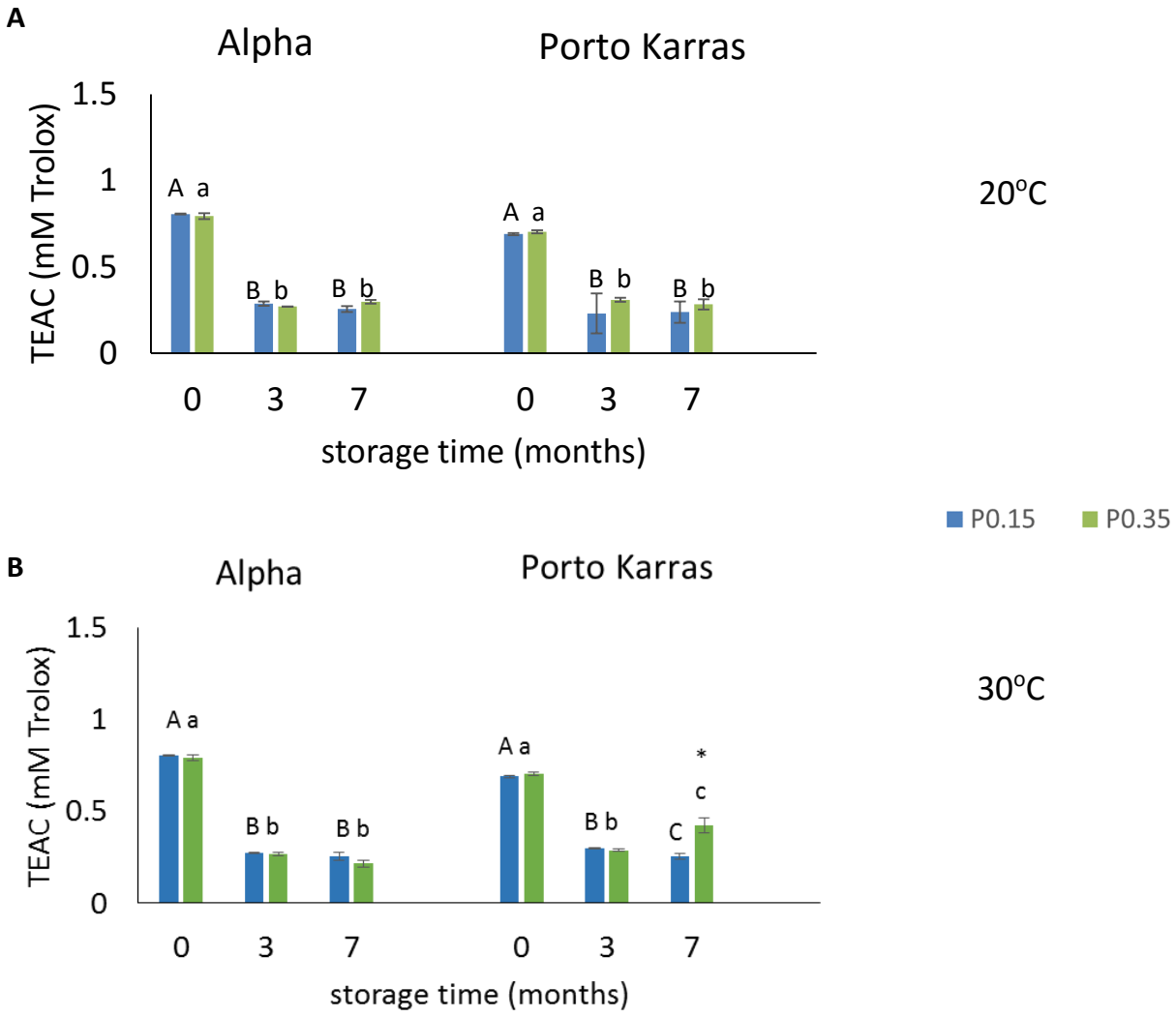


Figure 23. Concentration of TROLOX during storage time about Malagouzia variety at 20°C (A) and 30°C (B).

Antioxidant capacity seems to follow similar reducing trend with Assyrtiko samples. Either temperatures. Unexpected in the end of storage Porto Karras cork P0.35 keeps more antioxidant capacity than cork P0.15 at 30°C.

2.6.3. Sauvignon blanc

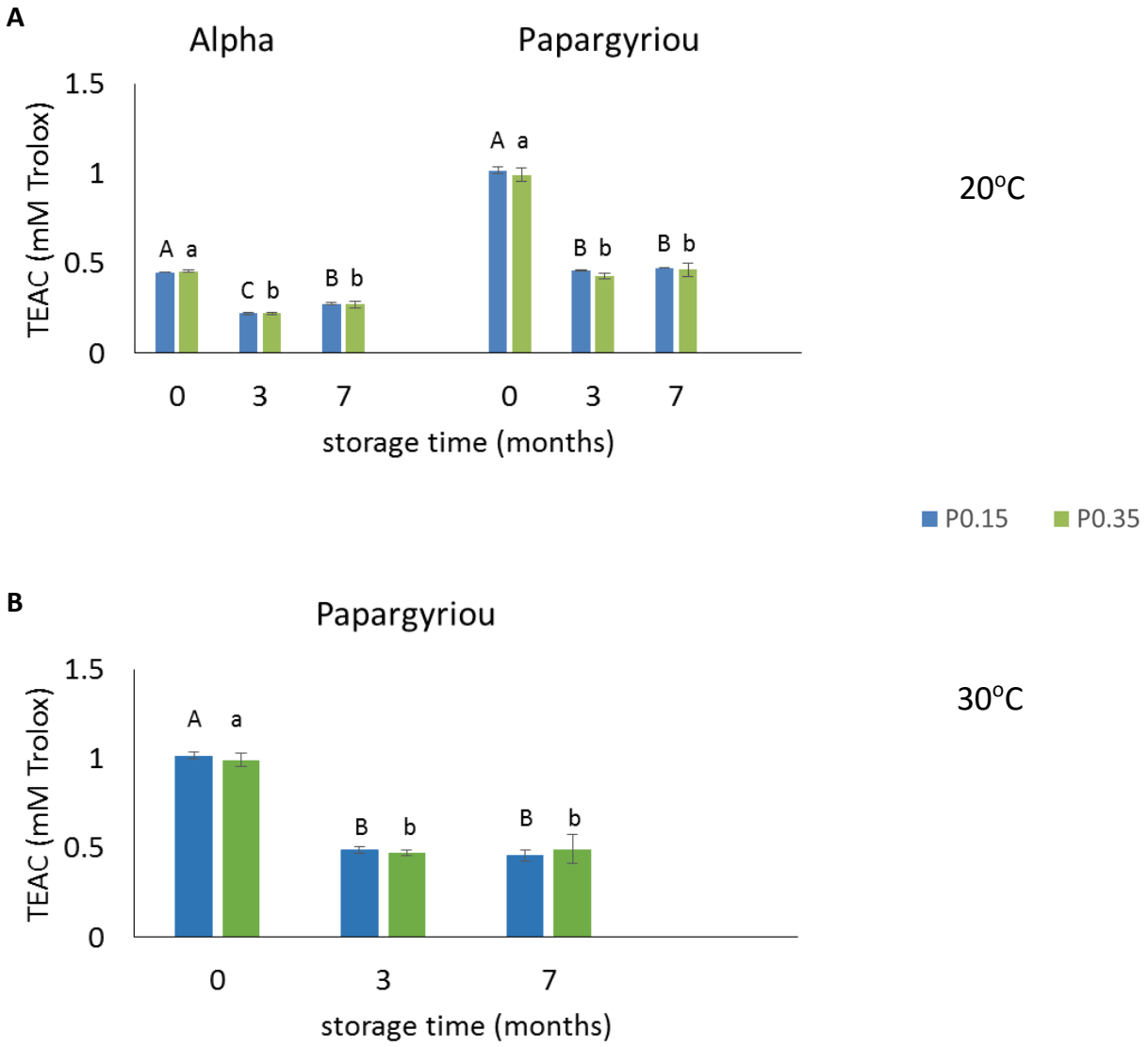


Figure 24. Concentration of TROLOX during storage time about Sauvignon blanc variety at 20°C (A) and 30°C (B).

All samples have lower antioxidant capacity during storage time also between two types of corks there is no significantly statistical differences at either temperatures.

2.7. Aroma compounds

A summary of the identified aroma compounds along with the ANOVA analysis outcome are presented in Figures 25 to 31. Significant differences were established across the two different corks and the two different storage conditions for characteristic compounds per wine variety, while for the majority of the compounds in the three varieties indicated that the

interaction effect between these two factors was estimated and found to be non-significant for most of the compounds, meaning that the effect of cork and storage temperature can therefore be considered independently of each other. The compounds with significant differences will be further discussed in the relevant sections.

2.7.1. Assytiko

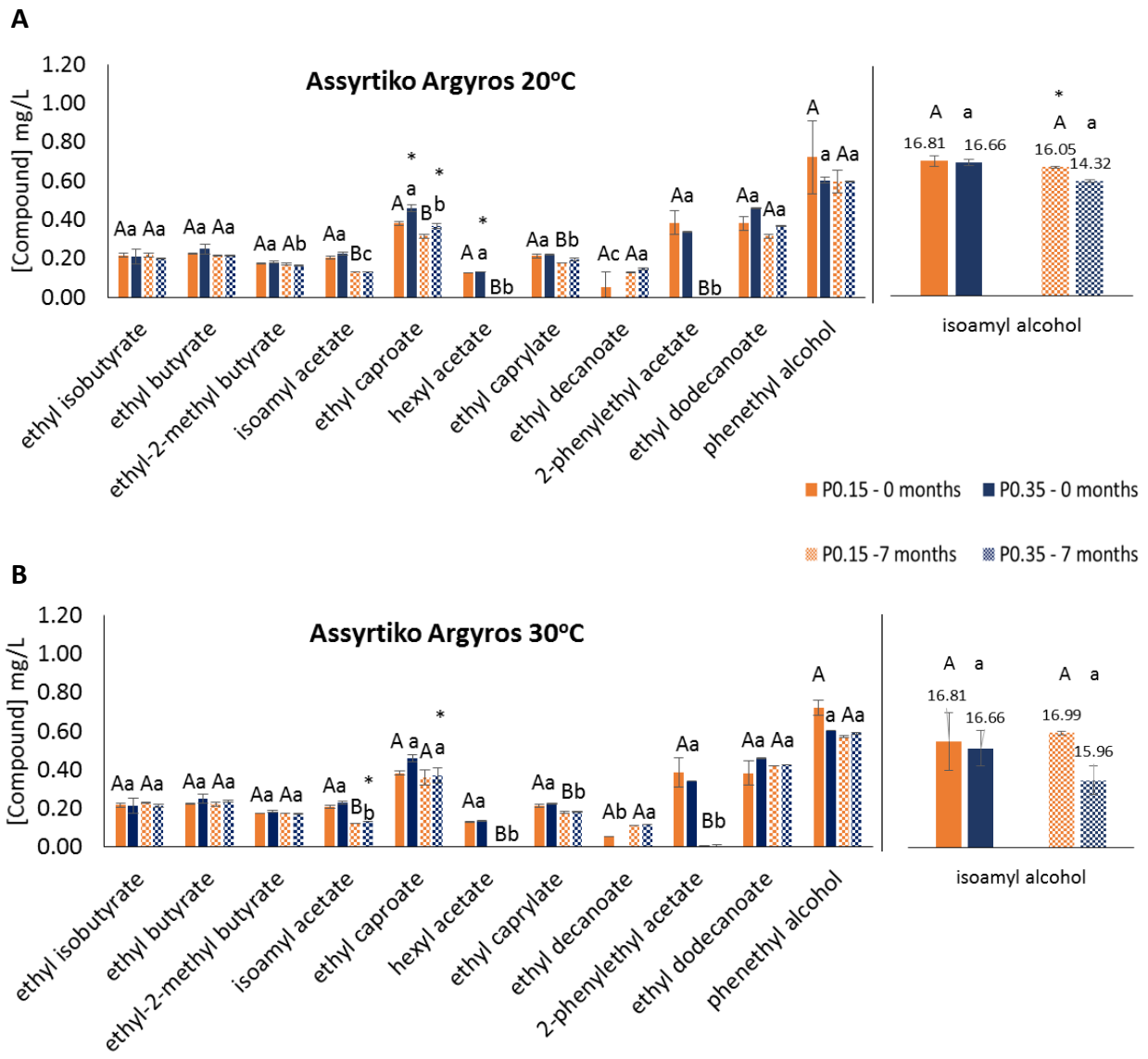


Figure 25. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Assyrtiko Argyros at 20ºC (A) and 30ºC (B).

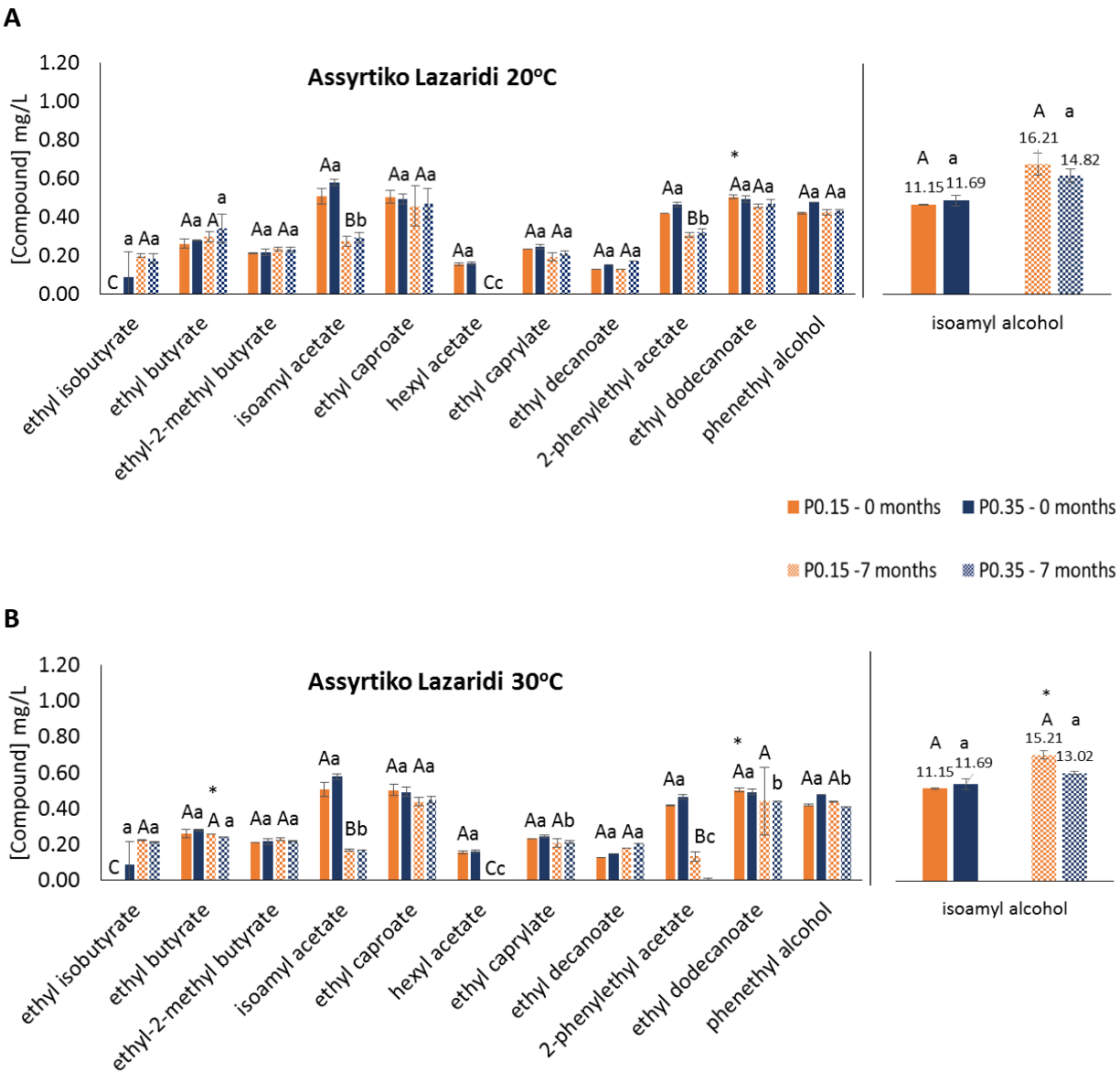


Figure 26. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Assyrtiko Lazaridi at 20°C (A) and 30°C (B).

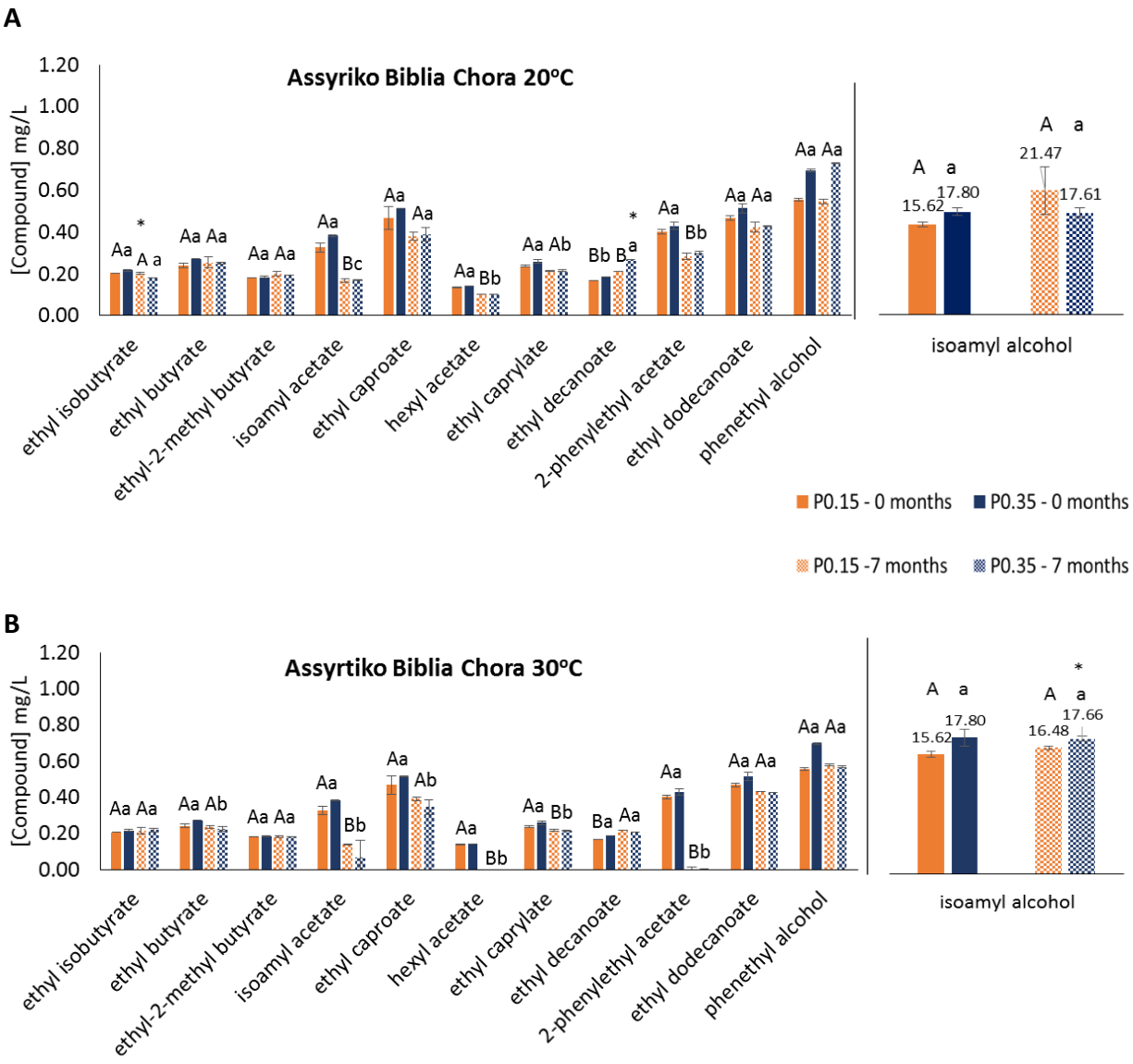


Figure 27. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Assyrtiko Biblia Chora at 20°C (A) and 30°C (B).

2.7.2. Malagouzia

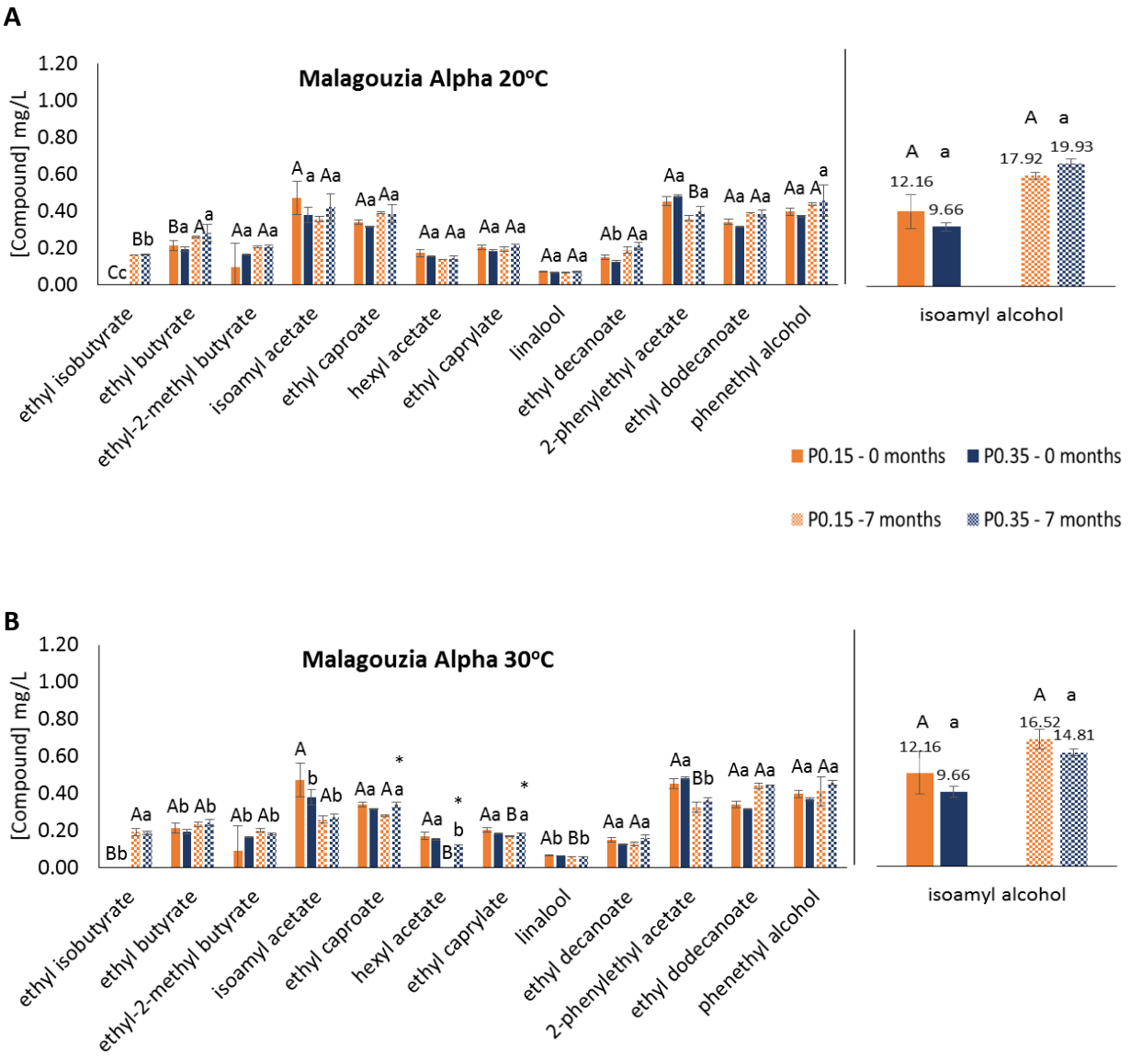


Figure 28. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Malagouzia Alpha at 20ºC (A) and 30ºC (B).

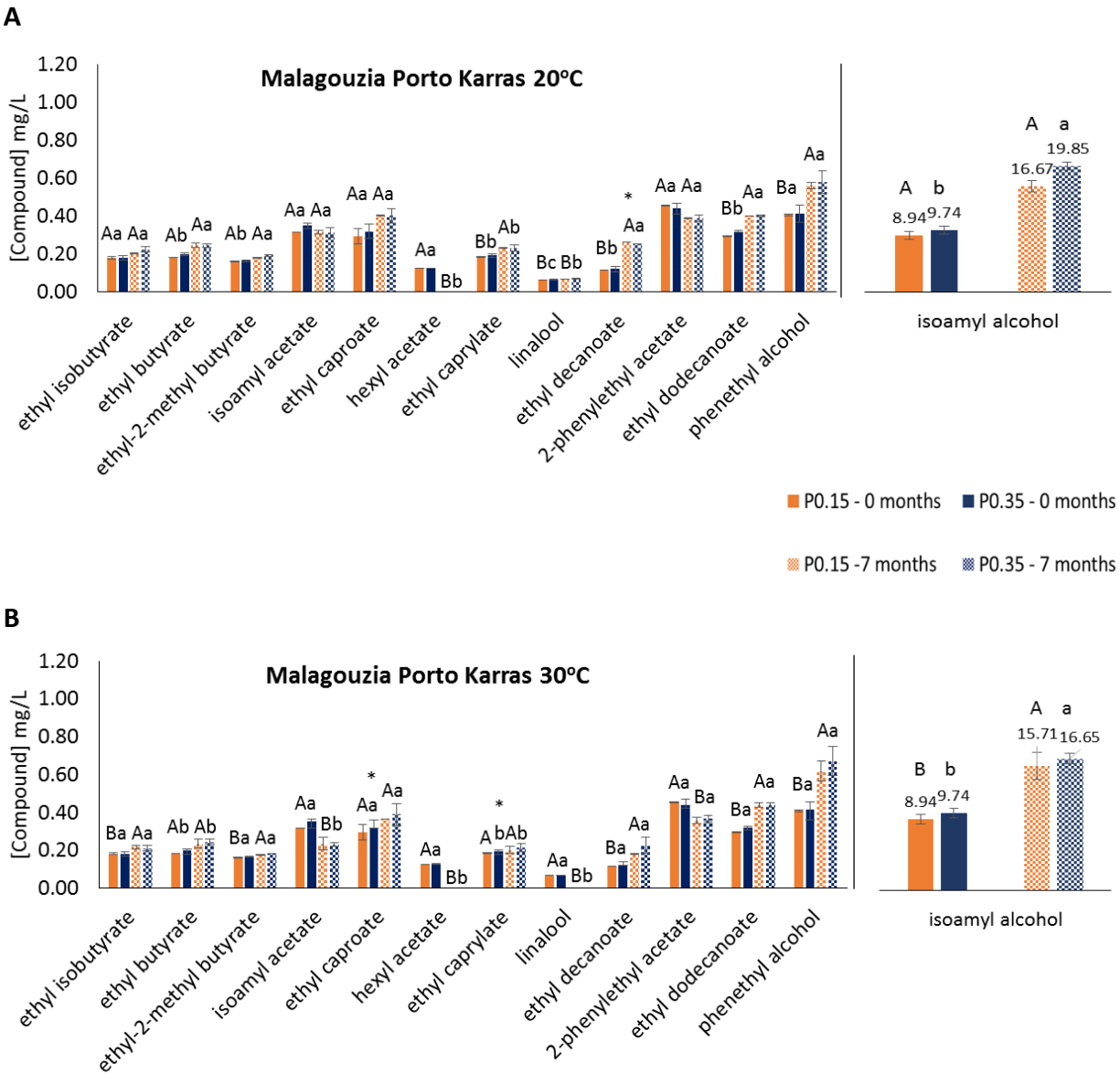


Figure 29. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Malagouzia Porto Karras at 20ºC (A) and 30ºC (B).

2.7.3. Sauvignon blanc

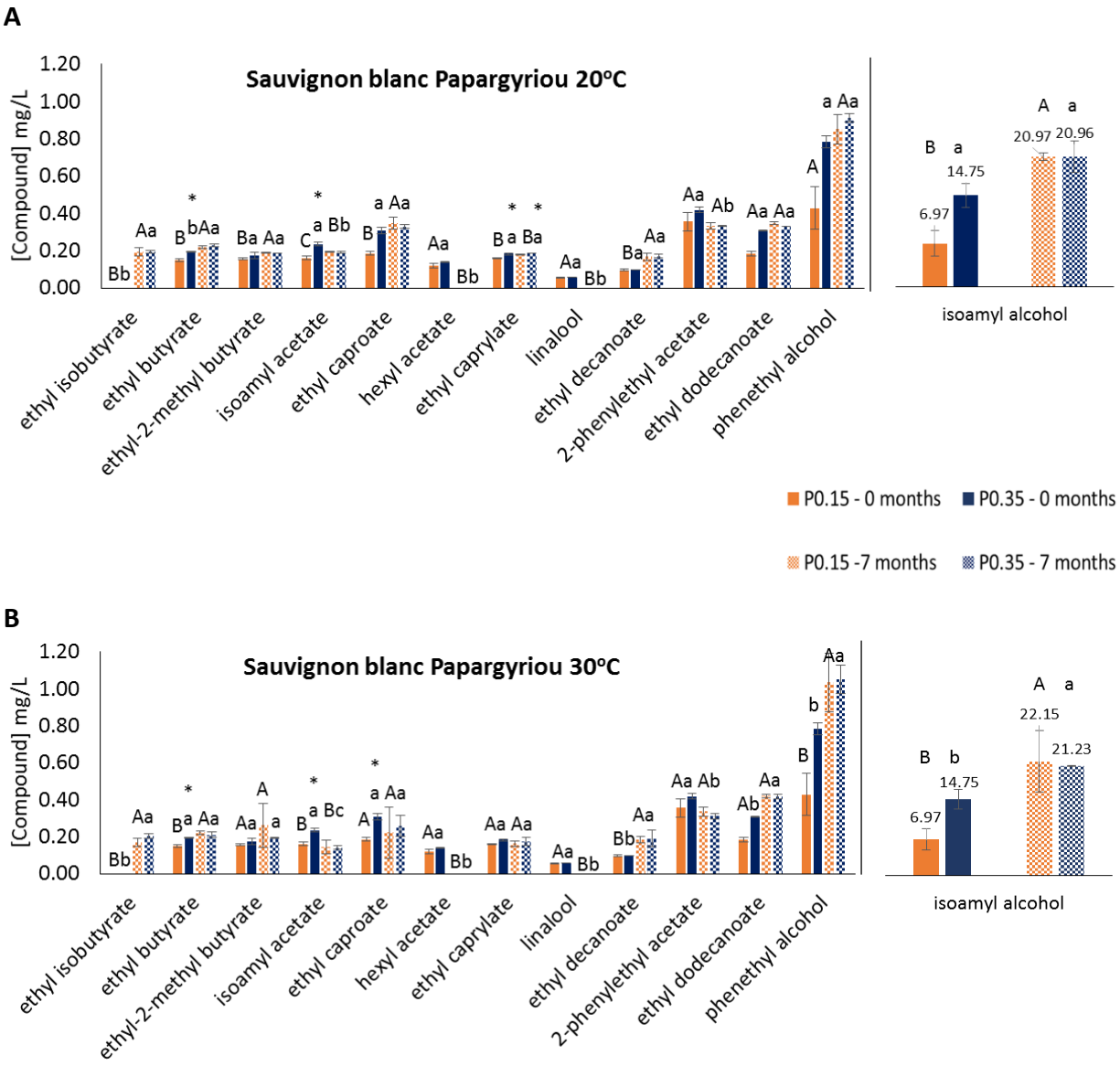


Figure 30. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Sauvignon blanc Papargyriou at 20°C (A) and 30°C (B).

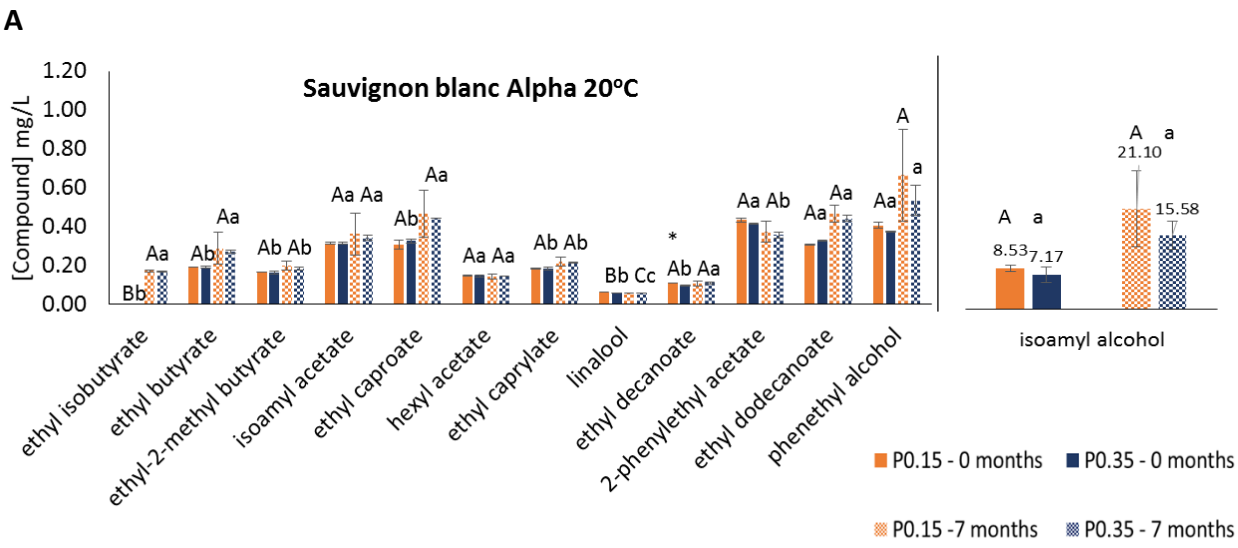


Figure 31. Sums of relative concentrations of aroma compounds at 0 and 7 months of storage about Sauvignon blanc Alpha at 20°C.

The effect of two storage temperatures (20 and 30°C) and two different types of corks on the aroma composition of Assyrtiko, Malagouzia and Sauvignon blanc wines were monitored during 7 months of storage.

As it is well known (Makhotkina and Kilmartin, 2012), wines lose their fresh, fruity characters over time in the bottle. Such changes have been associated with oxidation reactions occurring in white wines.

Garde-Cerdán and Ancín-Azpilicueta (2007) concluded that the SO_2 concentration of has an influence on the evolution of the alcohols and the esters in wine and, to a lesser extent, on the evolution of the acids during bottle aging.

The concentration of volatile acetate esters, including isoamyl acetate, hexyl acetate and 2-phenyl ethyl acetate found to decrease with time. The temperature at which the wines were stored significantly influenced the rate of acetate ester degradation: the higher the temperature the faster the rate of degradation. The process for the loss of acetate esters in wines during time is expected to be hydrolysis of the ester to acetic acid and an alcohol, which occurs readily at wine pH.

The evolution of the ethyl esters in the wines was more complex than that of the acetate esters. The concentration of particular ethyl esters, such as ethyl isobutyrate, ethyl dodecanoate, ethyl caproate and ethyl butyrate, increased at 7 months, while the concentrations of the rest of the esters (ethyl decanoate, ethyl-2-methyl butyrate) did not change significantly compared with their initial concentrations. These results could be explained by the particular hydrolysis – esterification equilibrium involved. The rate of esterification reactions depends on the initial concentration of the branched acid from which the ester is formed i.e. the more of the acid a wine contains the higher the esterification rate. (Makhotkina and Kilmartin, 2012)

Furthermore, the wine hydrolysis products such as those deriving from the hydrolysis of acetate esters are the acetic acid and the respective higher alcohols. That was confirmed via the

monitoring of the alcohols in all of the wines. An increase in the concentration were observed, for the phenethyl alcohol and isoamyl alcohol. In similar studies, an increase in the concentrations of higher alcohols in different wines was reported (Garde - Cerdan et al., 2008) while in other studies the concentration remained unchanged during storage under various conditions (Roussis et al., 2005).

During storage time observed statistically significant differences at Assyrtiko samples. Isoamyl acetate, hexyl acetate and 2-phenylethyl acetate compounds decreased, but ethyl isobutyrate and ethyl decanoate increased during storage time.

For the Malagouzia variety wines observed increase at ethyl isobutyrate, ethyl decanoate and ethyl dodecanoate also an increasing trend found at isoamyl acetate, hexyl acetate, linalool and 2-phenylethyl acetate.

In the end, for Sauvignon blanc variety isoamyl alcohol, ethyl isobutyrate and ethyl butyrate increased during storage time but hexyl acetate, linalool and 2-phenylethyl acetate compounds decreased.

Changes in the oxidation markers concentrations during aging are for the following compounds based on relevant references, the phenylacetaldehyde (Silva Ferreira, Hogg, & Guedes de Pinho, 2003), the methional (Escudero, Cacho, & Ferreira, 2000), and the sotolon (Escudero et al., 2000; Silva Ferreira et al., 2003) all of which are well known to be associated with the oxidative evolution of dry white wines stored under oxygen. Since the above researchers showed that the choice of packaging can influence the dissolved oxygen level in the bottle and consequently the redox potential of the wine, they have suggested to monitor changes in these compounds during the experimentation.

It is relatively common for the aromas of white wines aged in bottle to age abnormally rapidly and develops defects. Accordingly, the variability of this aromatic deterioration is due to considerable differences in permeability to oxygen among cork stoppers (Skouroumounis et al., 2005). Their results demonstrated that the choice of the packaging, as the choice of the closure if uncontrolled, was capable of maximizing the formation of oxidation markers in dry white wines during a short period aging.

For bottled wines stored under controlled temperature and humidity conditions were analyzed for sulfur dioxide and ascorbic acid concentration, sensory analysis of appearance and aroma attributes, and spectral measures. Wines sealed with the synthetic closure were relatively oxidised in aroma, brown in colour, and low in sulfur dioxide compared to wines held under the other closures. A *struck flint/rubber* (*reduced*) aroma was discernible in the wines sealed under the screw caps or in glass ampoules. Wines sealed under natural bark corks in this study showed negligible *reduced* characters. The bottle orientation during storage under the conditions of this study had little effect on the composition and sensory properties of the wines examined (Skouroumounis et al., 2005).

Within our experiment the outcome of the flavor compounds analysis following different rates of evolution for those particular compounds identified with significant different presence over time, are given in Tables 4-6, at the section following the sensory evaluation paragraph as a summary of the overall indicators changes studied and recorded in this work.

2.8. Sensory evaluation

In the following table the qualitative results of the 25 trained panelists verdict is given for the attributes of aroma intensity, fruity character, color intensity, hue intensity and preference score.

Storage time	t=0 months	t=3 months	t=7 months
Aroma intensity	No differences between corks	No differences between corks	Malagouzia Porto Karras **P0.15>P0.35** Assyrtiko Lazaridi **P0.35>P0.15** No other differences
Fruity character	No differences between corks	Malagouzia Porto Karras **P0.15 > P0.35** Sauvignon blanc Papargyriou **P0.35 > P0.15** No other differences	Malagouzia Porto Karras **P0.15>P0.35** Sauvignon blanc Papargyriou **P0.35 > P0.15** Assyrtiko Argyros **P0.35 > P0.15** No other differences
Color intensity	No differences between corks	No differences between corks	No differences between corks
Hue intensity	No differences between corks	No differences between corks	Sauvignon blanc Alpha **P0.35>P0.15** No other differences
Preference	No differences between corks	Sauvignon Blanc Papargyriou **P0.35>P0.15** No other differences	No differences between corks

Table 3. Results of sensory analysis at 20^{o}C.

For all the wines at 3 months of storage the panelists reported no differences between the two corks, regarding the aroma, color and hue intensity. Color intensity remained the same also at 7 months of storage. At 7 months of storage overall results were depended on the wine variety/winery.

Sensory analysis indicated large differences in wine flavor properties, with closures which tended to result in the best retention of free SO_2 having wine sensory scores for *'citrus'* that were generally high whilst scores for the attributes *'developed'/'oxidised'* were low. The situation was reversed for wine under closures that performed poorly in the retention of free SO_2. It was found that below a critical level of free SO_2 remaining in the wine, closures exhibited substantially higher *'oxidized'* aroma (Godden et al., 2001).

2.9. Summary of results

For demonstration and easy comparison purposes the studied wine-oxidation indicators as evolved within the various wines (as per one selected winery) over time, for the two storage temperatures will be provided. The evolution rate will be given via the best-fit-line slope for each of the indicators over time. A comparison of the corks/temperature impact per wine may be then derived though these values.

ASSYRTIKO	20°C		30°C	
	P0.15	P0.35	P0.15	P0.35
Free SO_2	-2.700[1]	-2.3700[1]	0.9600[2]	0.5186[2]
A_{420}	0.0044[1]	0.0044[1]	0.0091[1]	0.0084[1]
Acetaldehyde	0.1360[2]	0.1163[2]	0.1120[2]	0.0527[2]
Antioxidant capacity	0.0840[2]	0.0309[2]	0.0263[2]	0.0287[2]
Isoamyl acetate	-0.0789[1]	-0.1067[1]	-0.0941[1]	-0.1579[1]
Hexyl acetate	0.1203[2]	0.1230[2]	0.0683[2]	0.0711[2]
Ethyl caprylate	-0.0120[1]	-0.0215[1]	-0.0108[1]	-0.0225[1]
Ethyl decanoate	-0.0652[2]	-0.0382[2]	0.0186[2]	0.0176[2]
2-phenylethyl acetate	-0.0601[1]	-0.0643[1]	0.2012[2]	0.2144[2]

Table 4. The overall wine-oxidation indicators for the Assyrtiko (Biblia Chora winery) wines as evolved over time, for the two storage temperatures (20 and 30°C). The evolution rate is given via the best-fit-line slope for each of the indicators over time.

The A_{420} (increased), the isoamyl acetate (decreased) and the ethyl caprylate (decreased) showed linear evolution for either temperature or cork type. Similarly acetaldehyde, antioxidant capacity, hexyl acetate, ethyl decanoate showed square polynomial evolution, while free SO_2 and 2-phenylethyl acetate were reduced linearly in samples stored at 20°C and reduced polynomial at 30°C.

MALAGOUZIA	20°C		30°C	
	P0.15	P0.35	P0.15	P0.35
Free SO_2	-1.6086[1]	-1.2454[1]	-1.6259[1]	-1.5049[1]
A_{420}	0.0027[1]	0.0038[1]	0.0093[1]	0.0106[1]
Acetaldehyde	0.2681[2]	0.2555[2]	0.1793[2]	0.1699[2]
Antioxidant capacity	0.0178[2]	0.0222[2]	0.0170[2]	0.0245[2]
Ethyl-2methyl butyrate	0.0106[1]	0.0140[1]	0.0160[2]	-0.0209[2]
Isoamyl acetate	-0.0767[2]	-0.1486[2]	-0.0438[2]	-0.0869[2]
Hexyl acetate	-0.0622[1]	-0.0630[1]	-0.0628[2]	-0.0661[2]
Ethyl caprylate	-0.0334[2]	-0.0470[2]	-0.0438[2]	-0.0574[2]
Linalool	-0.0100[2]	-0.0142[2]	-0.0373[2]	-0.0402[2]
Ethyl decanoate	0.0341[2]	0.0343[2]	0.0325[1]	0.0801[2]
Ethyl dodecanoate	-0.0770[2]	-0.1306[2]	-0.0609[2]	-0.0574[2]

Table 5. The overall wine-oxidation indicators for the Malagouzia (Porto Karras winery) wines as evolved over time, for the two storage temperatures (20 and 30°C). The evolution rate is given via the best-fit-line slope for each of the indicators over time.

The free SO_2 (decreased), A_{420} (increased), the isoamyl acetate (decreased) and the ethyl caprylate (decreased) showed linear evolution for either temperature or cork type. Similarly acetaldehyde, antioxidant capacity, isoamyl acetate, ethyl caprylate, linalool, ethyl decanoate and ethyl dodecanoate showed square polynomial evolution, while free SO_2 and ethyl-2-methyl byturate were reduced linearly in samples stored at 20°C and reduced polynomial at 30°C.

SAUVIGNON BLANC	20°C		30°C	
	P0.15	P0.35	P0.15	P0.35
Free SO_2	-2.6984[1]	-2.3697[1]	-1.2620[2]	-1.1070[2]
A_{420}	0.0046[1]	0.0040[1]	0.0090[1]	0.0090[1]
Acetaldehyde	0.1644[2]	0.2183[2]	0.1058[2]	0.1072[2]
Antioxidant capacity	0.0269[2]	0.0283[2]	0.0239[2]	0.0255[2]
Ethyl isobutyrate	-0.1050[2]	-0.0677[2]	-0.0871[2]	-0.0689[2]
Isoamyl acetate	-0.0955[2]	-0.0217[1]	-0.0863[2]	-0.0462[2]
Hexyl acetate	0.0594[2]	0.0705[2]	0.0594[2]	0.0705[2]
Ethyl decanoate	0.0356[1]	-0.0524[2]	0.0435[1]	0.0459[1]
2-phenylethyl acetate	-0.0143[2]	0.0387[2]	0.0198[2]	0.0491[2]

Table 6. The overall wine-oxidation indicators for the Sauvignon blanc (Papargyriou winery) wines as evolved over time, for the two storage temperatures (20 and 30°C). The evolution rate is given via the best-fit-line slope for each of the indicators over time.

The A_{420} (increased) and the ethyl decanoate (increased) showed linear evolution for either temperature or cork type. Similarly acetaldehyde, antioxidant capacity, ethyl isobutyrate, isoamyl acetate, isoamyl acetate, hexyl acetate and 2-phenylethyl acetate showed square polynomial evolution while free SO_2 was reduced linearly in samples stored at 20°C and reduced polynomial at 30°C.

The characteristic graphs produced to derive the Tables 4-6, are given in Appendix 1.

Given the permeability of the two cork types, as provided by the producer, (DIAM P015= 0.0008 cm^3/day , DIAM P035 = 0.0015 cm^3/day, see Materials and Methods), we may comment that the amount of oxygen entering the bottles at 7 months-time, approximately 210 days), is respectively 0,168 cm^3 and 0,315 cm^3 per 750ml of wine, or 0,224 cm^3 and 0,420 cm^3 per liter of wine, corresponding to 0,32 and 0,6 mg, respectively.

Accordingly, each and every alteration of the oxidation indicators (increase or decrease in mg/lt, see Appendix 1), may correspond to the respective increase of the oxygen in the wine mass. Hence, for the same amount of oxygen present in the wine mass, there are certain alterations in the wine chemical, physical and sensorial properties.

Assyrtiko					
A	**Free SO_2**	**A_{420}**	**acetaldehyde**	**antioxidant capacity**	
20°C					
P0.15	-78.95	53.90	-0.94	-62.33	
P0.35	-76.47	51.28	-2.51	-65.12	
30°C					
P0.15	-73.68	111.37	-14.48	-63.54	
P0.35	-70.59	97.33	-11.04	-65.61	
B	**isoamyl acetate**	**hexyl acetate**	**ethyl caprylate**	**ethyl decanoate**	**2-phenylethyl acetate**
20°C					
P0.15	-48.37	-23.92	-10.12	25.76	-29.85
P0.35	-55.86	-26.87	-16.64	42.95	-30.00
30°C					
P0.15	-57.73	-100.00	-9.09	28.90	-100.00
P0.35	-82.65	-100.00	-17.41	9.11	-100.00

Table 7. The percentage (%) alterations of the oxidation indicators for the Assyrtiko variety (Biblia Chora winery) for the two corks at two temperatures. (A) Physical-Chemical, (B) flavor compounds.

Malagouzia							
A	**Free SO_2**	**A_{420}**	**acetaldehyde**	**antioxidant capacity**			
20°C							
P0.15	-40.91	21.17	16.10	-65.58			
P0.35	-36.84	32.95	20.65	-59.78			
30°C							
P0.15	-40.91	75.80	-13.60	-62.68			
P0.35	-42.11	94.48	-8.32	-39.62			
B	**ethyl-2methyl butyrate**	**isoamyl acetate**	**hexyl acetate**	**ethyl caprylate**	**linalool**	**ethyl decanoate**	**ethyl dodecanoate**
20°C							
P0.15	13.23	0.01	-100.00	25.68	8.12	130.33	36.03
P0.35	17.52	-10.60	-100.00	19.93	7.49	107.17	25.62
30°C							
P0.15	9.66	-26.05	-100.00	8.48	-100.00	56.67	48.28
P0.35	10.33	-34.47	-100.00	9.94	-100.00	82.72	37.20

Table 8. The percentage (%) alterations of the oxidation indicators for the Malagouzia variety (Porto Karras winery) for the two corks at two temperatures. (A) Physical-Chemical, (B) flavor compounds.

Sauvignon blanc					
A	**Free SO_2**	**A_{420}**	**acetaldehyde**	**antioxidant capacity**	
20°C					
P0.15	-78.95	61.44	37.07	-53.26	
P0.35	-76.47	47.54	22.85	-53.16	
30°C					
P0.15	-31.82	121.28	-12.74	-54.96	
P0.35	-30.00	109.91	-21.76	-50.38	
B	**ethyl isobutyrate**	**isoamyl acetate**	**hexyl acetate**	**ethyl decanoate**	**2phenyl ethyl acetate**
20°C					
P0.15	-100.00	20.65	-100.00	74.29	-5.86
P0.35	-100.00	-18.55	-100.00	74.46	-20.95
30°C					
P0.15	-100.00	-9.78	-100.00	90.98	-5.50
P0.35	-100.00	-39.53	-100.00	93.82	-25.85

Table 9. The percentage (%) alterations of the oxidation indicators for the Sauvignon blanc variety (Papargyriou winery) for the two corks at two temperatures. (A) Physical-Chemical, (B) flavor compounds.

Such a discussion can only be based on the following assumptions: i) each and every oxygen molecule entering the bottle through the cork is immediately consumed in a certain reaction, meaning that the ΔP_{O_2} remains constant, ii) the initial diluted oxygen concentration is insignificant and, if present, immediately consumed so that all the alterations are due to the oxygen permeating the corks.

3. CONCLUSIONS

The aim of this study has been defined as the investigation of the impact of oxygen permeating through the corks on the oxidation markers for various Greek white wines. A series of three characteristic verities bottled at different wineries were used in order to broaden the picture of the oxidative alterations among different samples. Apparently, a rather distinct preservation 7methodology is followed by each winery. Characteristic differences are supported by the different levels of added SO_2 concentrations in the wine. Additionally the practice of using extra antioxidants such as ascorbic acid should be considered as an extra parameter in understanding the antioxidant capacity of the wines pictures via the different oxidation markers within this study. As a consequence the evolution of color and acetaldehyde could be appreciated as characteristic event influenced by these additional antioxidants besides SO_2. Nevertheless, a certain correlation could be established for the same wine at different temperatures.

Regarding the impact of the two corks it was rather profound that in significant differences could be concluded between the two corks for wines stored at 20^{o}C compared to those wines stored at 30^{o}C. Such an observation most likely indicates a potentially solid dependency of oxidation to elevated temperatures, which allows us to recommend that wines are better protected against oxidation at low temperatures. Whether the reaction rates among the various reactions in the wines are similarly affected by temperature remains to be further investigated.

According to previously reported results and to the fact that corks are a minor oxygen permeation surface in comparison to the whole bottle surface, we may safely comment that the two corks studied in this work supported a limited oxidation acceleration with indistinctive differences. Thus, the selection of packaging materials regarding the quality of wine is heavily depended on the selection of the body materials and apparently on the initially dissolved oxygen along with the antioxidant additives and preservatives.

The combination of oxygen dissolved at bottling and the oxygen transferred through closures has a significant effect on Sauvignon Blanc development after bottling. Wines highly exposed to oxygen at bottling and those sealed with a synthetic, Closures highly permeable to oxygen, maybe relatively oxidized in aroma, brown in color, and low in antioxidants and volatile compounds compared to wines sealed with other closures.

Following these last remarks, we may ultimately conclude that it is packaging properties engineering within certain technological borderlines that may allow for a certain modification of the added chemicals and preservatives in the wine. That aims in a common collaborative approach between edible product and packaging, synergistically contributing to high quality end-products. No question that additional factors to be considered with in this engineering approach are initial wine quality, target markets, cost and packaging and wine making technology. Nevertheless, it is worth mentioning the significant role of packaging materials and storage conditions in the fine-tuning of products' quality that may satisfy the potential consumers to the maximum possible level strengthening and securing the product in a highly competitive modern environment.

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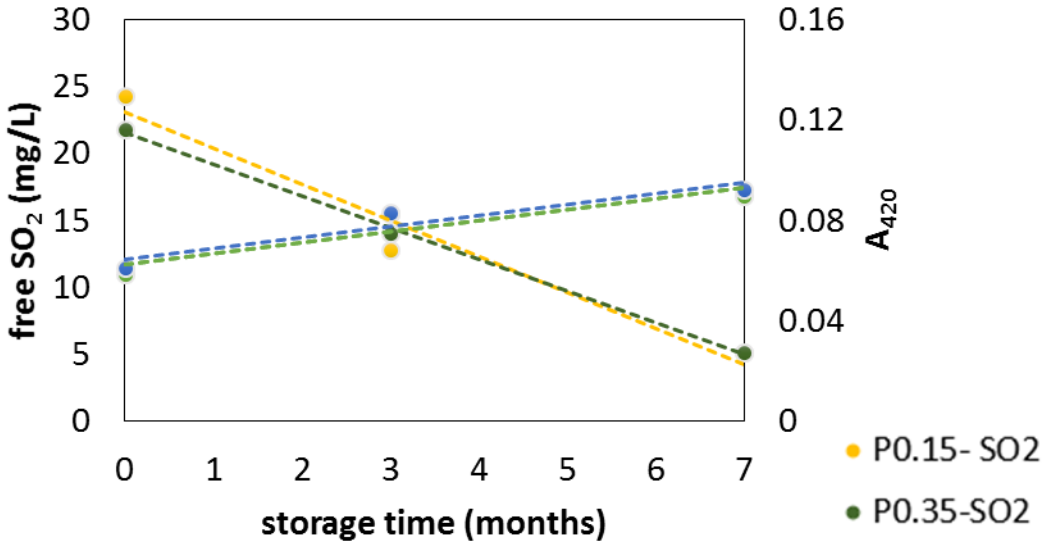
APPENDICES

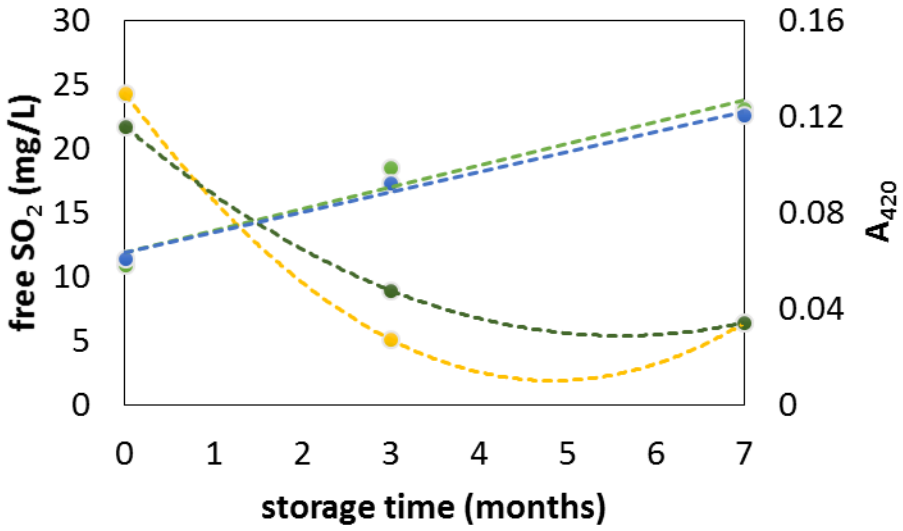
Appendix 1. A comparative evolution of pairs of wine-oxidation indicators for the Assyrtiko (Biblia Chora), Malagouzia (Porto Karras) and Sauvignon blanc (Papargyriou winery) selected wines as evolved over time, for the two storage temperatures (20 and 30°C).

1.A. Assyrtiko

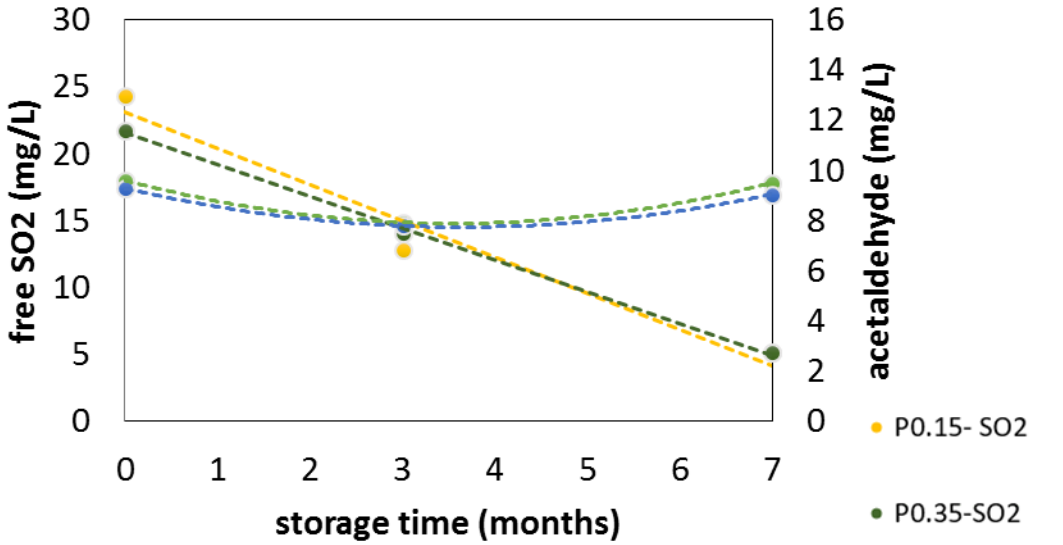
20°C



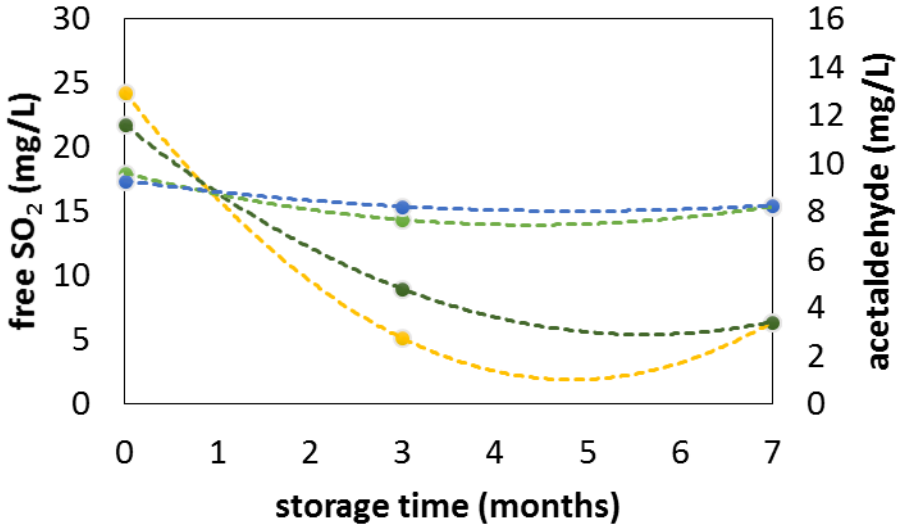
30°C



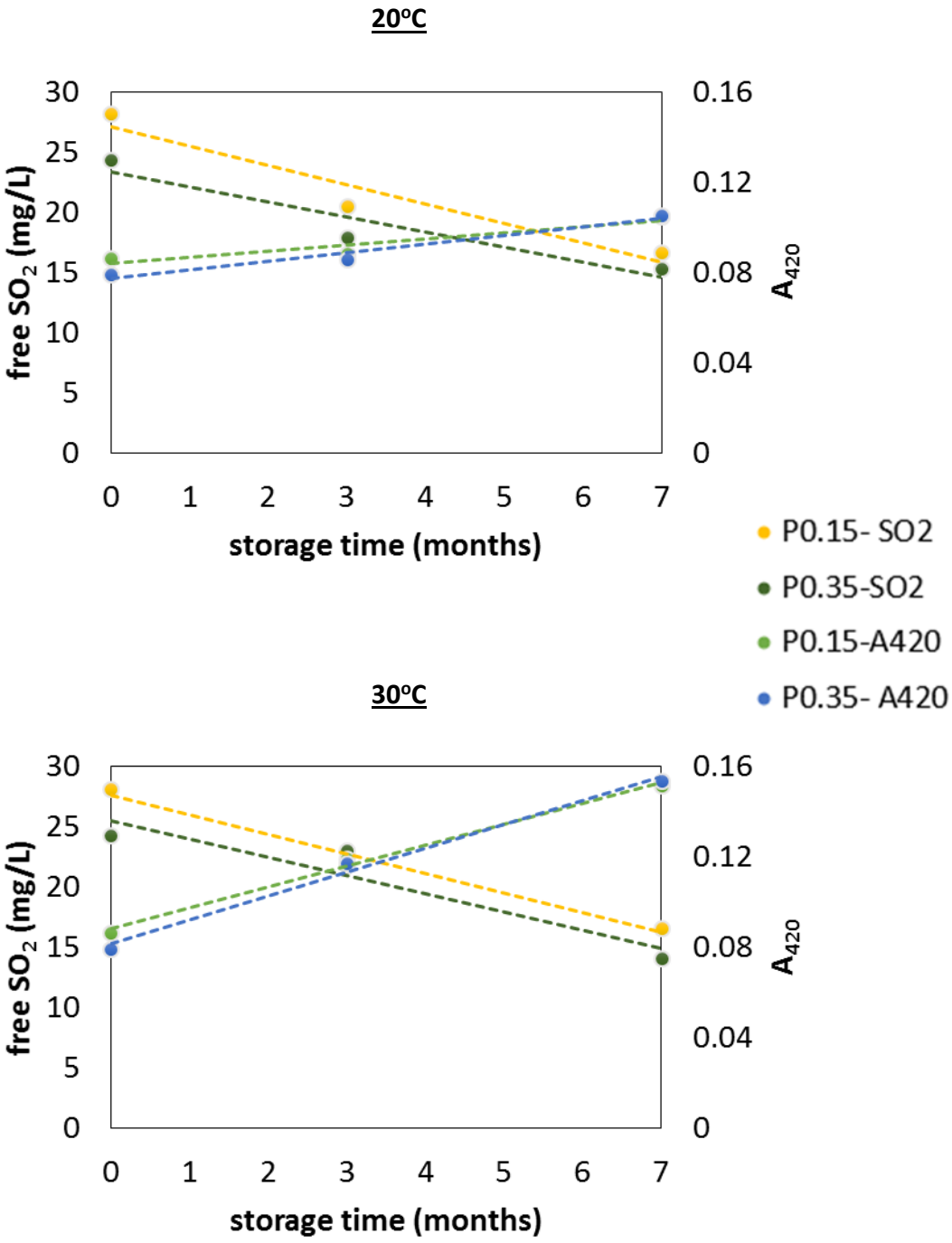
20ºC


free SO2 (mg/L)
acetaldehyde (mg/L)
storage time (months)
P0.15- SO2
P0.35-SO2
P0.15-acetaldehyde
P0.35-acetaldehyde

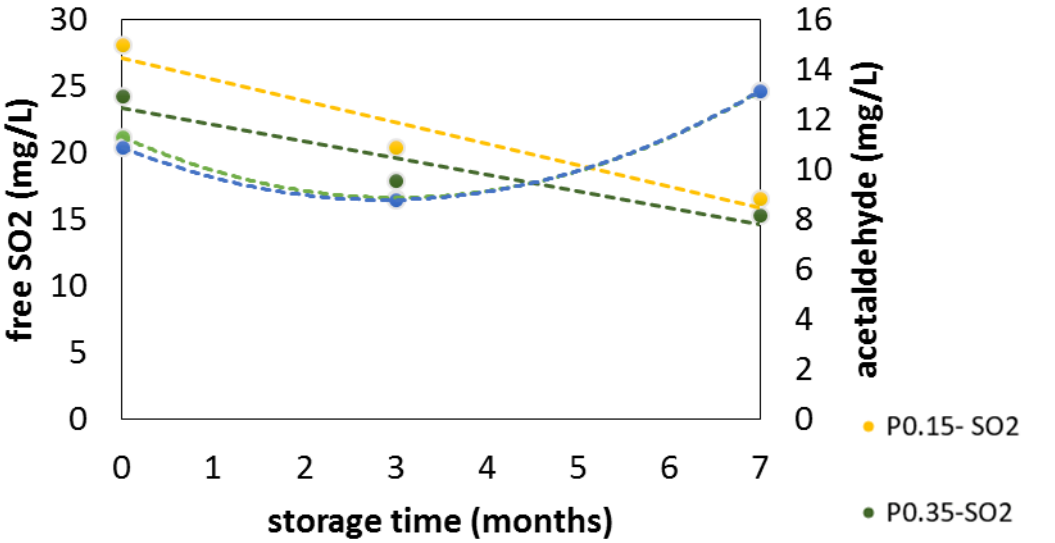
30ºC


free SO2 (mg/L)
acetaldehyde (mg/L)
storage time (months)

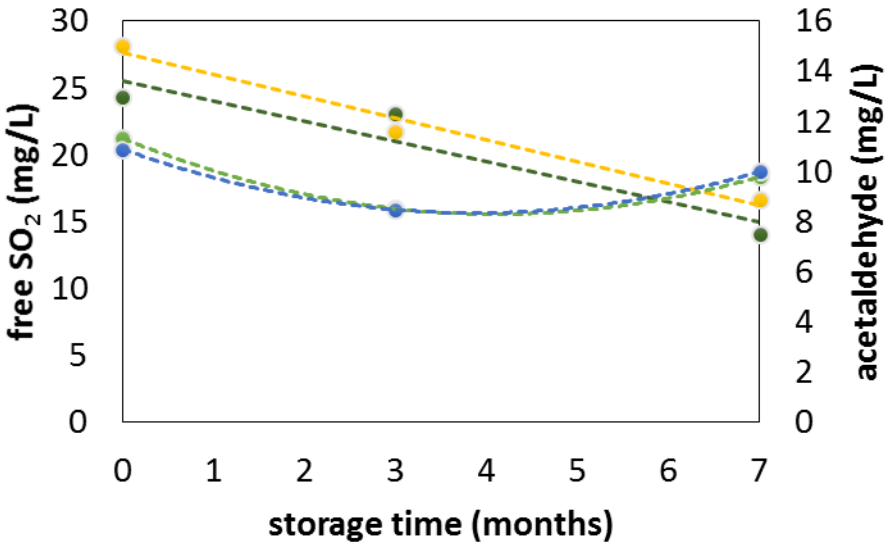
1.B. Malagouzia



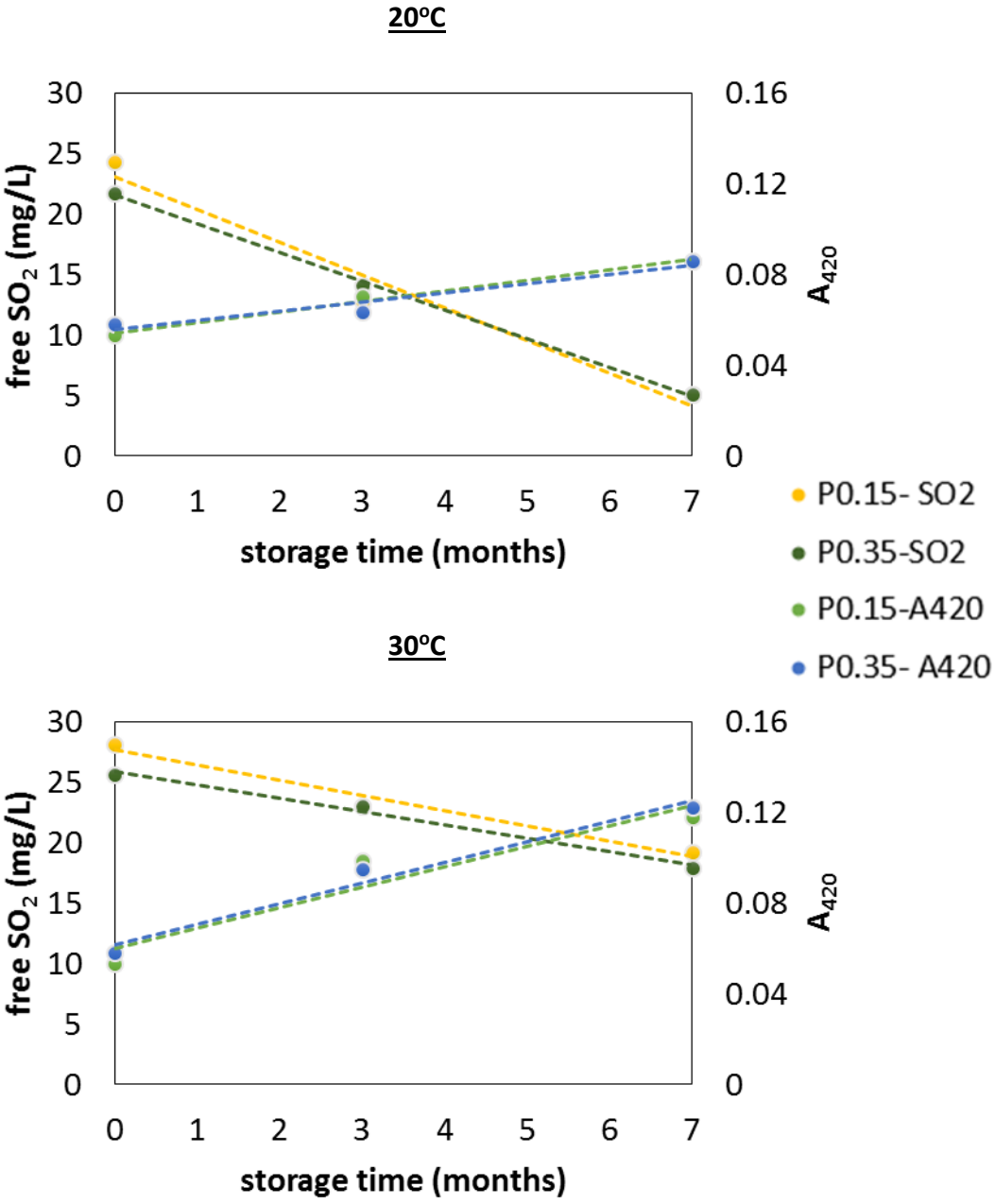
20°C


free SO2 (mg/L)
acetaldehyde (mg/L)
storage time (months)
P0.15- SO2
P0.35-SO2
P0.15-acetaldehyde
P0.35-acetaldehyde

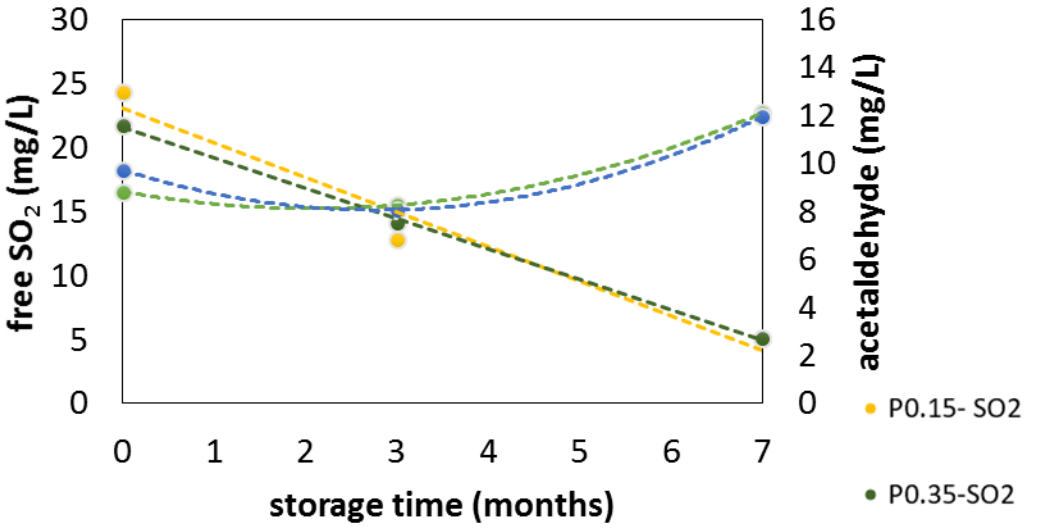
30°C


free SO2 (mg/L)
acetaldehyde (mg/L)
storage time (months)

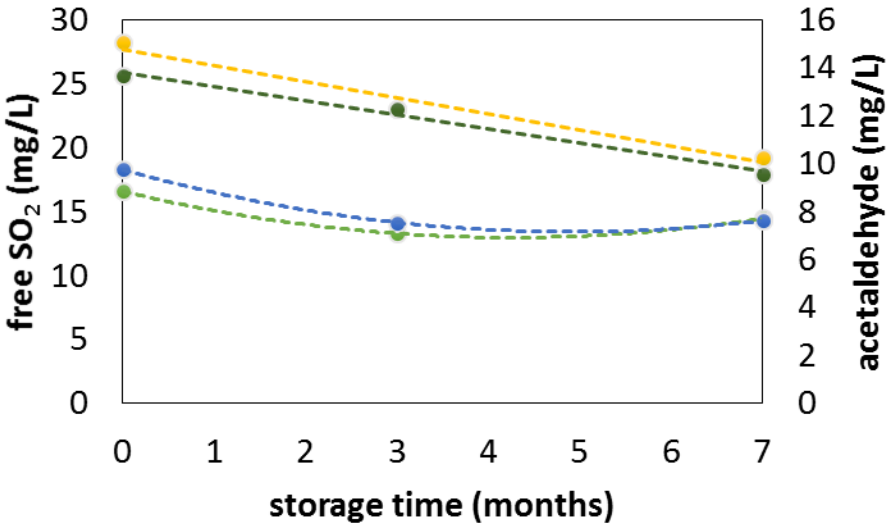
1.C. Sauvignon blanc



20°C


free SO_2 (mg/L)
acetaldehyde (mg/L)
storage time (months)
P0.15- SO2
P0.35-SO2
P0.15-acetaldehyde
P0.35-acetaldehyde

30°C


free SO_2 (mg/L)
acetaldehyde (mg/L)
storage time (months)