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Impact of Soils on the Quality of Grapes from Two Vine Varieties [Vitis Vinifera L. (Vitaceae)] Produced in Côte D'ivoire

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ABSTRACT

Production remains closely linked to its terroir (climate, soil, grape variety and human activity), which sometimes affects the organoleptic qualities of derived products such as wine. Against this backdrop, a study was carried out in Côte d'Ivoire to assess the impact of four agro-ecological zones on the quality of the grapes produced. Grapes from two grape varieties (Béquignol and Aleatico) were harvested in four different agro-ecological zones (Abidjan, Aboisso, Man and Korhogo). A number of biochemical parameters, such as sugar and total phenol content, as well as purification of these compounds, were carried out. The results showed that the total sugar and polyphenol contents of the different grape varieties were higher at the Man site.

Keyswords: Vine, grape variety, grape, wine, Ivory Coast, terroir, polyphenols

INTRODUCTION

The grapevine (Vitis vinifera L.) is one of the most important fruit species grown in the world (Houel, 2011). Global grape production is estimated at over 69 million tonnes per year (Yara, 2022), of which 27% is used for fresh consumption, 2% for raisin production and 71% for wine production (Yara, 2022). The quality of the grape, and therefore the wine that comes from it, reflects the environment in which it is produced. This relationship is sometimes so strong that

it is perceptible on the market, where countless labels proudly brandish the characteristics of their places of origin. This multitude of very different wines each offers us its own identity, with its own tastes and qualities depending on the environment (Gatti, 2012). This effect of the environment on wine quality is incorporated into the concept of "terroir".

This links the quality of the grapes and wine to the environmental conditions in which the vines are grown and the grapes are produced (van Leeuwen and Seguin, 2006). However, climate change phenomena such as frost and hail are currently causing considerable damage in many French vineyards, as well as in other countries such as England, Germany, Italy and Switzerland (Rochard et al., 2018). It is therefore necessary to find terroirs that are suitable for growing vines to produce quality grapes. The aim is to assess the impact of four agro-ecological zones on the quality of the grapes produced. This will enable us to better identify the terroirs that are best suited to quality viticulture in Côte d'Ivoire.

MATERIAL AND METHODS

Plant Material



Figure 1: Bunches of grapes from two varieties, A: bunch of Aleatico grapes; B: bunch of Béquignol grapes

The plant material consisted of mature grapes (Figure 1).

The fruit was harvested from vines grown in four agro-ecological zones of Côte d'Ivoire (Aboisso, Abidjan, Man, Korhogo).

Methods

Grape Sample Collection

Five bunches of each grape variety were collected from four different experimental plots. During collection, the different positions (top, middle, bottom) and exposures of the bunches (berries inside or outside the foliage) were taken into account. These samples were used to assess a number of biochemical properties.

Evaluation of Grape Biochemical Parameters

Approximately 50 g of grapes were freeze-dried and then used for the biochemical study (determination of sugars and polyphenols) (Zouid, 2011).

Extraction and Determination of Total Sugars

Total sugars were extracted using the method of Kouakou et al (2009). 500 mg of freeze-dried grapes were immersed in 10 mL of ethanol in test tubes and placed in the dark for 8 h. The ethanol extract was filtered through a Millipore membrane (0.45 µm). The ethanoic extract was filtered through a Millipore membrane (0.45 µm). The supernatant obtained constituted the crude sugar extract (CSE) to be analysed. The quantity of sugars in the grapes from the different experimental plots was determined spectrophotometrically using the method described by Dubois et al. (1951). A solution of concentrated sulphuric acid (H2SO4) was used to break the osidic bonds between D-glucose and D-fructose, bringing into solution all the sugars present, which were then revealed by phenol. For the assay, 0.2 mL of 5% phenol and 0.2 mL of phenolic extract (EBS) were taken and placed in a test tube. The reaction mixture was made up to 1 mL with distilled water to which 1 mL of concentrated sulphuric acid (97%) was added. The solution was incubated for 5 min in a boiling bath, then cooled in the dark for 30 min. The intensity of the colour produced by the reaction was measured using a spectrophotometer (Shimadzu Spectrophotometer UV-120-02, Kyoto, Japan) at 490 nm against a control containing no sugar. The amount of sugar was determined using a calibration curve with a glucose solution (1 mg/mL). It was expressed as (mg/g DM).

Extraction and Assay of Total Polyphenols

Phenolic compounds were extracted using the method of Kouakou et al (2009). 500 mg of freeze-dried grapes were immersed in 10 mL of ethanol contained in test tubes and placed in the dark for 8 hours. The ethanoic extract obtained was filtered through a Millipore membrane (0.45 µm). The supernatant obtained constituted the phenolic crude extract (PCE) to be analysed. Total phenols were determined using the method of Singh et al (2000). 0.5 mL of 5 N Folin-Ciocalteu reagent and 0.9 mL of distilled water were added to 0.1 mL of phenolic extract. After stirring at room temperature, 1.5 mL of a 17% sodium carbonate solution was added. After 35 min incubation, the intensity of the blue coloration of the reaction mixture (proportional to the concentration of polyphenols) was monitored using a spectrophotometer at 765 nm. During the assay, a control was carried out and the phenolic extract was replaced by distilled water. The total phenol content was determined using a standard curve ($y = 0.032x + 0.078$; $R2 = 0.999$ where y is the absorbance and x is the concentration of gallic acid) performed with different concentrations of a gallic acid stock solution (200 µg/mL). It was expressed in milligrams per gram of dry matter (mg/g DM).

Extraction and Purification of Phenolic Compounds

Extraction and purification of phenolic compounds were carried out using the method described by Kouakou et al (2009). Approximately 50 mg of freeze-dried grapes were placed in a haemolysis tube to which 10 mL of methanol was added. The mixture was placed at 4°C for 15 h and then centrifuged at 5,000 rpm for 10 min. Next, 4 mL of supernatant was removed and evaporated using a Speed Vac mini-concentrator (Savant, USA). The dry residue obtained was dissolved in 1 mL of 30% (v/v) methanol and then placed on a C18 grafted silica mini-column

(Sep pack®; Macherey-Nagel, Düren, German) in the Supelco Visiprep™ refill system. The columns were pre-equilibrated by successive washing with 100% methanol (2 mL), then 50% (2 mL) and finally distilled water (6 mL). After deposition of the methanol extract, the phenolic compounds were washed with 2 mL of distilled water and then eluted with 4 mL of 90% (v/v) ethanol. The eluate obtained was evaporated at Speed Vac, then the dry residue was taken up in 1 mL of 50% (v/v) methanol and finally filtered through a Millipore membrane with 0.45 µm porosity. The filtrate obtained represents the purified extract of phenolic compounds to be analysed.

Conditions for U-HPLC Analysis

Ultra-high performance liquid chromatography (U-HPLC) analysis was carried out using the method of Verdu (2013). The samples were analysed on two coupled U-HPLC chains; the first chain is of the Agilent LC type (1100 series, UV-Visible U-HPLC System, Germany), equipped with a degasser (G1322A), an automatic injector, a high-pressure binary pump and a thermal column compartment (G1316A). The second chain is an Agilent LC 1200 series and comprises a quaternary pump (G1311A) connected to an iodine bar detector (G1315A). The U-HPLC is controlled by a computer running a Workstation operating system and chromatogram display is managed by ORACLE VM VirtualBox chromatography software running under WinXPSP3. The entire U-HPLC system was coupled to a nuclear magnetic resonance spectrometer (Bruker Avance III) with an operating frequency of 600 MHz for one proton. Separation of phenolic compounds was carried out on a reverse-phase C18 silica column (Zorbax Eclipse XDB-C18, 150 x 4.6 mm, 5 µm, Agilent). Elution was carried out with a binary gradient composed of:

- solvent A: ultra pure water / 0.25% (99/1, v/v) trifluoroacetic acid
- solvent B: acetonitrile / 0.25% trifluoroacetic acid (99/1, v/v)

The elution gradient profile is shown in Table 1

Table 1: The elution gradient profile of phenolic compounds

Time (min)	Solvent A (%)	Solvent B (%)
0-5	95	5
5-10	80	20
10-15	70	30
15-20	60	40
20-22	90	10
22-25	95	5

TFA, trifluoroacetic acid; solvent A (0,25 % TFA in ultra-pure water); solvent B (0,25 % TFA in acetonitrile)

Separation and Identification of Phenolic Compounds

Separation of the phenolic compounds was carried out in computer-controlled U-HPLC under a pressure of 550 to 1500 bar. A 10 µL aliquot of the purified extract of phenolic compounds was injected into U-HPLC at a flow rate of 1.3 mL/min. The separated compounds (peaks) were revealed at 280 nm. The detection limit of the compounds was set at an absorbance of 30 mA. Below this value, the compound was considered not detected. The various peaks obtained were

then identified using a reference library of phenolic compounds. This library contains retention times and nuclear magnetic resonance (NMR) spectra of phenolic compounds. It was previously compiled using phenolic compounds likely to be present in grapevines, from pure isolated compounds or those obtained commercially (Belhadj et al., 2008; Faurie et al., 2009; Wei et al., 2016; Guerrero et al., 2020).

RESULTS

Total Sugar Content

The results showed that whatever the experimental plot, the total sugar content of Béquignol grapes was higher than that of Aleatico (Figure 2). However, these different levels developed differently from one growing site to another. The total sugar content of Béquignol berries was highest at the Man (974 mg/g DM) and Korhogo (917 mg/g DM) sites. These were followed by Abidjan (841 mg/g DM) and Aboisso (674 mg/g DM). Similarly, for the Aleatico grape variety, the highest (statically identical) levels of total berry sugars were recorded at the Man (436 mg/g DM), Korhogo (371 mg/g DM) and Abidjan (352 mg/g DM) sites. The lowest total berry sugar content for this variety was obtained at the Aboisso site (197 mg/g DM).

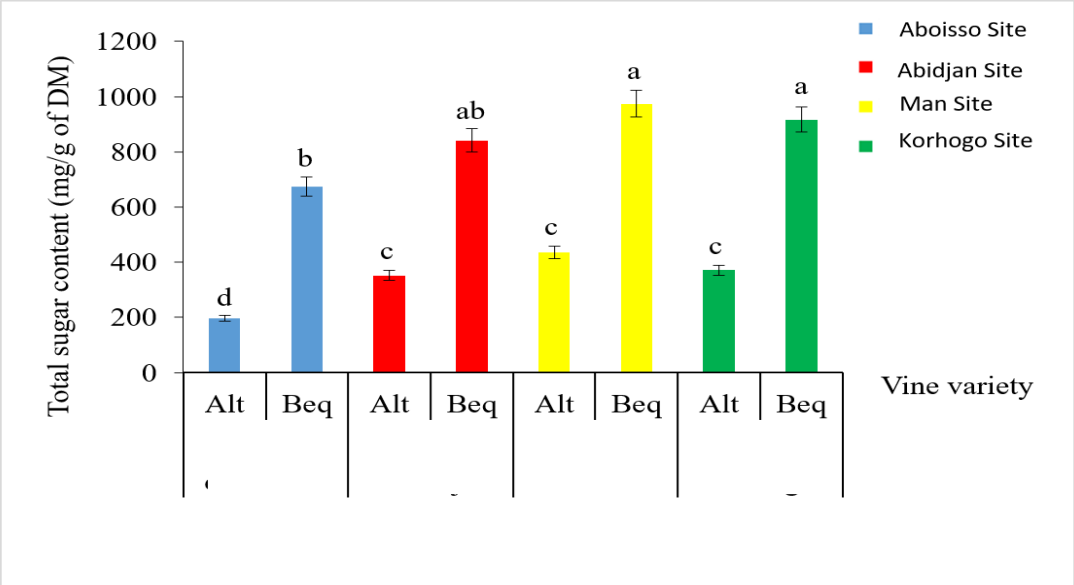


Figure 2: Changes in total sugar content in grape extracts from two grape varieties grown on different experimental sites.

Total Phenol Content

The results obtained show that the total phenol content of the grapes differs significantly from one experimental site to another (Figure 3). Generally speaking, the Aleatico grapes had a higher phenol content than the Béquignol grapes, regardless of the study site. However, grapes from the Man site had the highest phenol content (12.46 mg/g DM). This was followed by grapes from Abidjan (9.15 mg/g DM) and Korhogo (7.12 mg/g DM). The lowest total phenol content was obtained in grapes from the Aboisso plots (4.03 mg/g DM). The highest levels of total phenols in Béquignol grapes were recorded at the Man (4.62 mg/g DM) and Korhogo (4.67 mg/g DM) sites. The berries of this variety had the lowest total phenol content in the

experimental plots at Abidjan (3.22 mg/g DM) and Aboisso (3.11 mg/g DM), which were statically identical.

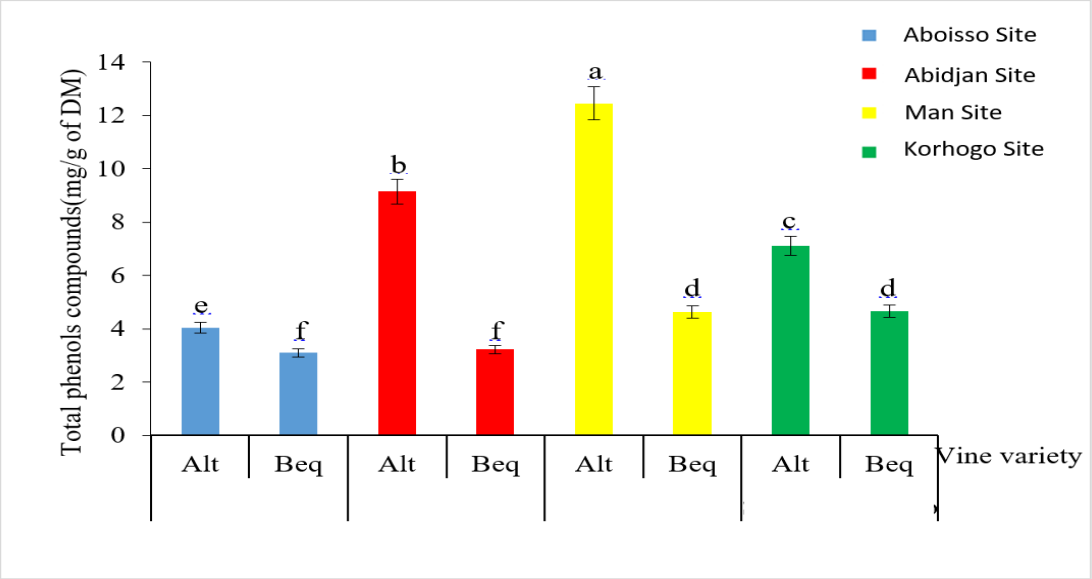


Figure 3: Total phenol content of grape extracts from two grape varieties grown on different experimental sites

Qualitative Analysis of the Phenolic Content of the Grapes Produced

Aleatico Grape Variety:

Analysis of Figure 4 shows that the chromatographic profiles of the phenolic content of Aleatico grapes from the four growing sites (Man, Abidjan, Korhogo and Aboisso) show similarities and differences. In the experimental plots in Man, Abidjan and Korhogo, the grapes synthesised 23 compounds. Compared with the grapes from these three sites, the grapes from the Aboisso plot synthesised (1) compound less. This was compound 22, which did not appear. However, the fact remains that the grapes from this variety induced the synthesis of a very high number of polyphenols on the different experimental plots. The results also showed that the phenolic content of grapes from the Man plot had the highest amplitudes of phenolic peaks. These phenolic peak amplitudes were followed by those of grapes from the Abidjan and Korhogo sites. On the other hand, the lowest phenolic peak amplitudes were recorded at the Aboisso site. In other words, the lowest levels of the various phenolic compounds. The compounds detected were all identified as polyphenols on the basis of their retention times and nuclear magnetic resonance (NMR) spectra or NMR profiles. The compounds were identified as 1: gallic acid (1.09 min); 2: protocatechic acid (1.41 min); 3: cinnamic acid (2.27 min); 4: hydroxybenzoic acid (2.66 min); 5: catechin (3.36 min); 6: ferulic acid (3.75 min); 7: epicatechin (4.76 min); 8: caftaric acid (4.99 min); 9: p-coumaric acid (6.37 min); 10: gentisic acid (7.34 min); 11: kaempferol (8.39 min); 12: myricetin (8.87 min); 13: isorhamnetin (9.76 min); 14: malvidin 3-O-glucoside (10.32 min); 15: quercetin (11.69 min); 16: resveratrol (12.74 min); 17: delphinidin 3-O-glucoside (13.55 min); 18: petunidine 3-O-glucoside (14.60 min); 19: peonidine 3-O-glucoside (15.97 min); 20: cyanidine 3-O-glucoside (16.69 min); 21: pikeid (17.26 min); 22: astringin (18.55 min); 23: pallidol (19.03 min).

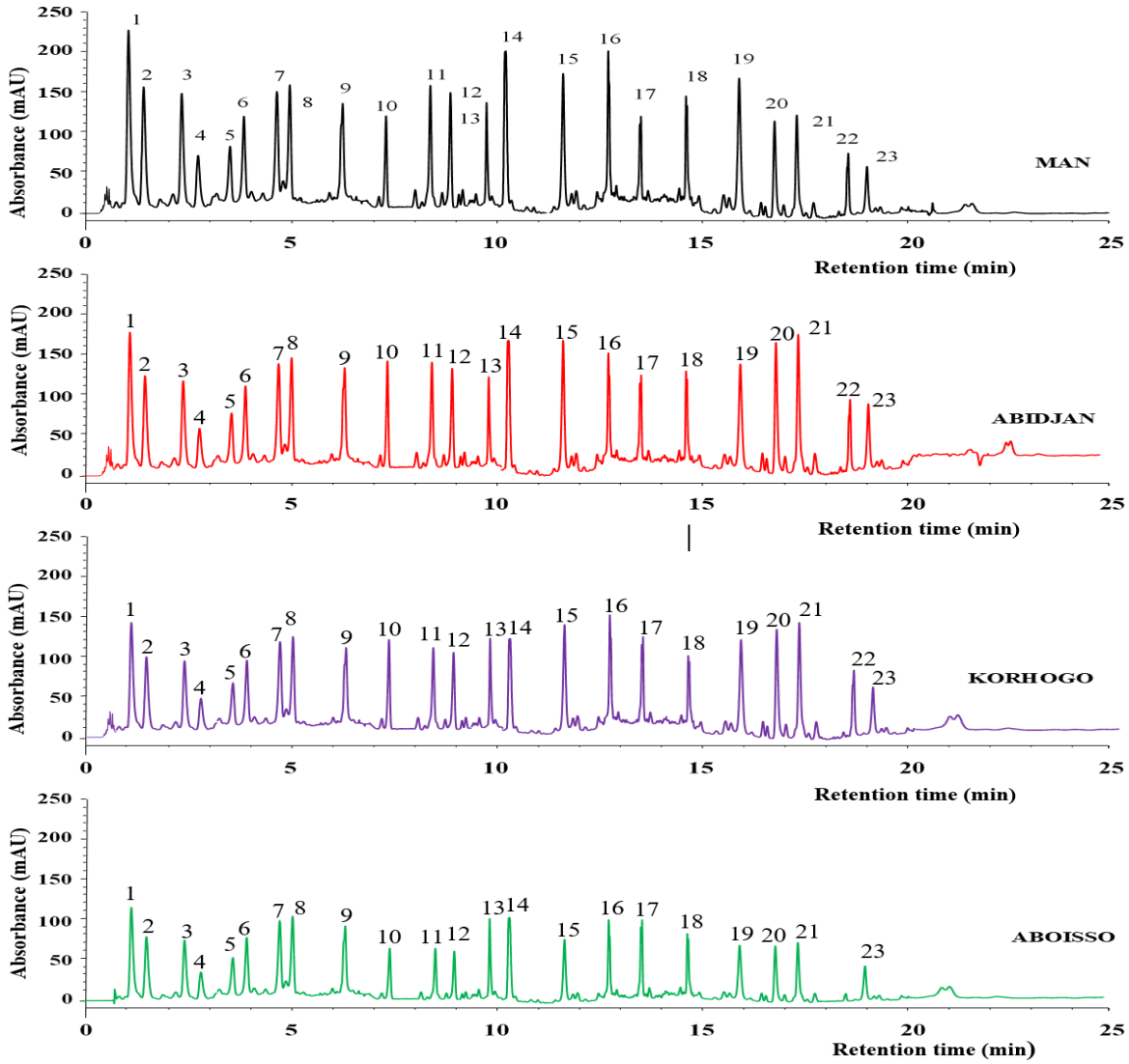


Figure 4: Chromatographic profile of the phenolic content of Altéatico grapes

Béquignol Grape Variety

Analysis of Figure 5 shows that the chromatographic profiles of the phenolic content of Béquignol grapes from the four growing sites (Man, Abidjan, Korhogo and Aboisso) show similarities and differences. Indeed, the grapes of this vine variety synthesised the same number of compounds (13), as well as the same amplitudes of phenolic peaks on these three sites. As for the Aboisso plot, the number of compounds in the grapes from this variety was 12. The grapes from this site produced (1) fewer compound. It is therefore compound (1) that did not appear. Also, the phenolic contents of the grapes from this site had the lowest amplitudes of phenolic peaks. In addition, the compounds detected were all identified as polyphenols on the basis of their retention times and their nuclear magnetic resonance (NMR) spectra or NMR profiles. The compounds were identified as 1: gallic acid (1.09 min); 2: protocatechic acid (1.41 min); 4: hydroxybenzoic acid (2.66 min); 5: catechin (3.36 min); 7: epicatechin (4.76 min); 9:

p-coumaric acid (6.37 min); 10: gentisic acid (7.34 min); 11: kaempferol (8.39 min); 14: malvidin 3-O-glucoside (10.32 min); 16: resveratrol (12.74 min); 18: petunidin 3-O-glucoside (14.60 min); 20: cyanidin 3-O-glucoside (16.69 min); 22: astringin (18.55 min).

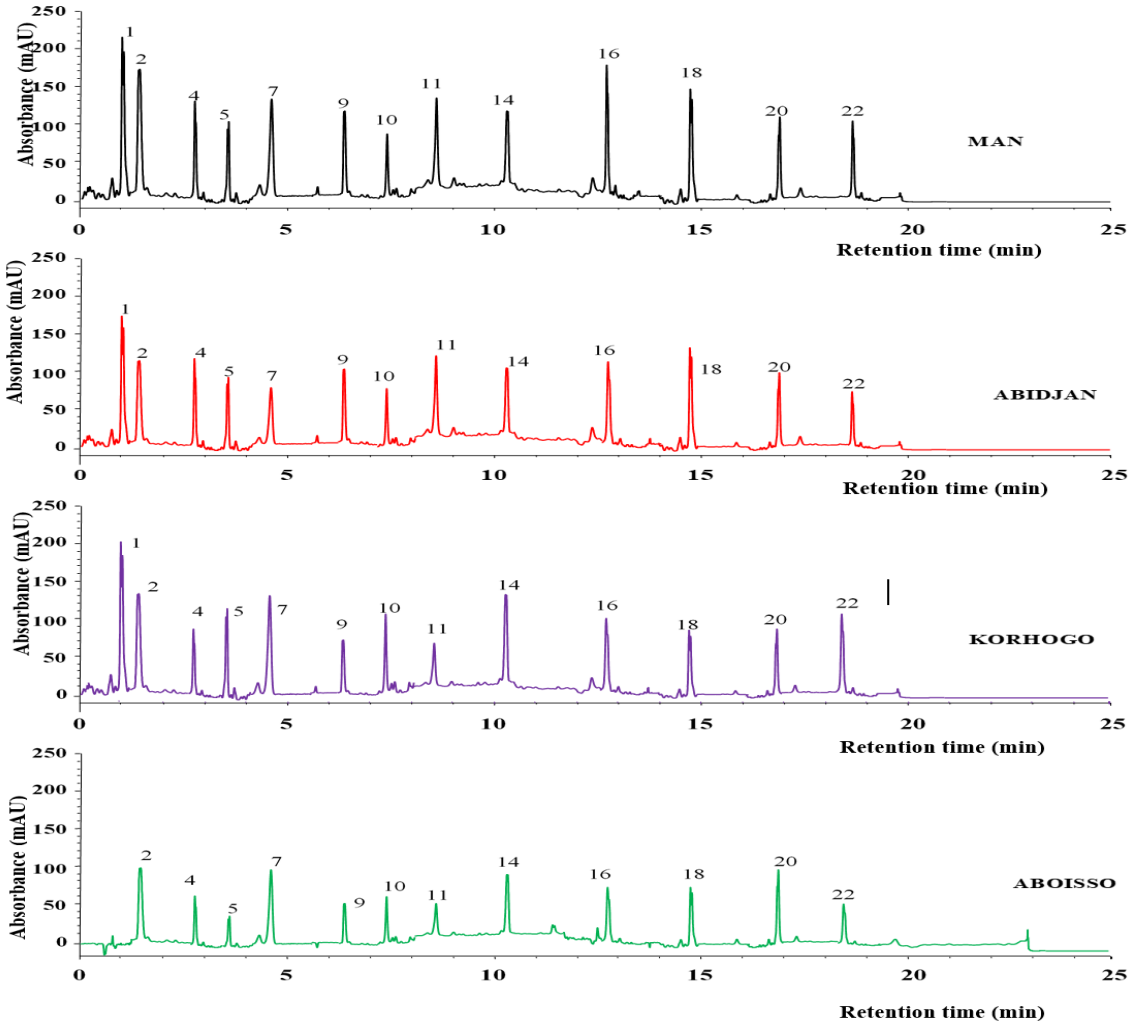


Figure 5: Chromatographic profile of the phenolic content of grapes from the vine variety Béquigno

DISCUSSION

The total sugar content of grapes from the four experimental sites was analysed. The results showed that whatever the experimental plot, the total sugar content of the Béquignol grape was the highest. From an agronomic point of view, the Béquignol variety was the least productive, with bunches and berries of average size (INRA, 2021). This low fruit load would have raised the sugar content of the grapes from this variety. Similar observations were made by Yara (2022). This author believes that the level of sugar accumulation depends in part on the number of bunches and berries on the vine. When the vine is less crowded, the sugar content is higher. On the other hand, it is diluted. From a nutritional point of view, the high sugar content of

Béquignol grapes could be explained by the fact that this variety assimilates a large amount of nitrogen. According to Yara (2022), the more nitrogen available to the vine, the higher the sugar content of the grapes and the higher the amount of residual sugar in the wine. In contrast, total sugar levels in Aleatico grapes were lower in all experimental plots. The low total sugar content observed in Aleatico grapes could be explained by the fact that they are sufficiently rich in acid to maintain vinification (Groeninger, 2016). The high acidity of the grapes from this variety would make it possible to reduce their total sugar content. Research by Neethling et al (2011) has shown that sugar content is negatively correlated with total grape acidity. In fact, an increase in acidity reduces the total sugar content of the grapes. In addition, the sugar content of grapes from these varieties varies from one growing site to another. This could be explained by the fact that these sites are very different in terms of climate and soil characteristics. For example, the total sugar content of the berries of the Béquignol and Aleatico grape varieties was higher at the Man site than at the other three sites. This could be explained by the fact that the climate and characteristic soil conditions of the Man agro-ecological zone are favourable to sugar synthesis in the grapes of the different grape varieties. According to Neethling et al (2011), the local climate and soil characteristics are one of the most important factors influencing grape composition. Evaluation of the polyphenol content of the grapes showed a dissimilar trend from one growing site to another. This difference in polyphenol content could be due to the influence of the terroir in each agro-ecological zone. Moreover, numerous studies have shown that the mineral conditions of the soil and the amount of sunlight in the berry can explain this variation in polyphenol content (Zouid, 2011). Excessive soil fertility not only affects berry composition, but also leads to a reduction in berry colour (anthocyanins). However, berries exposed to sunlight have higher concentrations of anthocyanins than those exposed to shade (Downey et al., 2004). In a general way, the total phenols issue of Aleatico have been more than those of Béquignol for the four study sites. This could be explained by the fact that Aleatico is a red wine grape variety. According to Duco-du-vin (2022), phenolic compounds are more abundant in red wine grapes than in whites. This high phenolic content could also be linked to the grape variety. According to Mylène (2018), certain grape varieties such as Merlot, Pinot Noir and Saint-Laurent are known to produce wines with high phenolic concentrations. This could be the case for the Aleatico variety. As a result, the phenolic content of the different grape varieties (Aleatico and Béquignol) rose more at the Man site than at the other sites. This could be explained by the fact that the climate and characteristic soil conditions of the Man agro-ecological zone are favourable to the phenolic synthesis of the different grape varieties. In fact, from a climatic point of view, the Man site is under the influence of a tropical climate, with high mountains and plains in places, combined with a cooler, wetter climate. However, it was very sunny, but the average monthly temperature was moderate, although not excessively hot. Soil-wise, the Man site is rich in nitrogen and many other minerals. In addition to this, the soil (Man) has a good soil temperature, as well as good water availability thanks to the river (Mian). These different soil and climate parameters make this site an ideal location for growing vines. As well as being favourable to the productivity of the grape varieties, this site also influences the phenolic composition of the grapes through these pedoclimatic factors. According to Neethling et al (2011), the climate and characteristics of the local soil are one of the factors that most influence the phenolic composition of the grapes. Similarly, it has been reported that soil factors, soil temperature, water availability and nitrogen availability can have a major impact on the phenolic composition of grapes (Oeno One, 2020). Wine quality depends

on many factors relating to grape quality, which is largely determined by its phenolic content (Zouid, 2011). In order to assess the phenolic richness of the grapes from the different experimental plots, a biochemical study was carried out to identify the compounds using Ultra High Performance Liquid Chromatography. This method is ideally suited to determining and quantifying small quantities of polyphenols. During this chromatographic study, the nature and amplitude of the polyphenols varied according to the grape variety. U-HPLC analysis was used to isolate 23 compounds from Aleatico grapes only at the Man, Abidjan and Korhogo experimental sites, and 22 compounds at the Aboisso site. As for the Béquignol grapes, U-HPLC analysis identified 13 phenolic compounds at the Man, Abidjan and Korhogo sites, followed by 12 compounds at the Aboisso site. These compounds are common to Aleatico grapes and are divided into five main chemical groups: phenolic acids (gallic acid, protocatechic acid, cinnamic acid, hydroxybenzoic acid, ferulic acid, caftaric acid, p-coumaric acid and gentisic acid), flavanols (catechin and epicatechin), flavonols (kaempferol, myricetin, isorhamnetin, quercetin and delphinidin 3-O-glucoside), anthocyanins (malvidin 3-O-glucoside, petunidin 3-O-glucoside, peonidin 3-O-glucoside and cyanidin 3-O-glucoside) and stilbenes (resveratrol, pikeid, astringin and pallidol). In the experimental plots in Man, Abidjan, Korhogo and Aboisso, grapes from the Aleatico variety produced high levels of phenolic compounds. This could be explained by the fact that this is a red wine grape variety. Duco-du-vin (2022), confirms the hypothesis that phenolic compounds are more widely present in red wine grapes than whites. This makes Aleatico grapes grapes with a good phenological profile. This high phenolic content in the grapes from the different experimental sites could also depend on the grape variety (Aleatico). According to Mylène (2018), certain grape varieties such as Merlot, Pinot Noir and Saint-Laurent are known to produce wines with a higher concentration of polyphenols. Aleatico grapes from the experimental plot in Aboisso induced the synthesis of (1) less compound. This was compound 22 (astringin), which did not appear. The Aboisso site also recorded the lowest amplitudes of phenological peaks, unlike the Man, Abidjan and Korhogo sites. This could be due to the influence of the terroir of the study areas. The work of Mylène (2018) has shown that terroir, especially climate, affects the metabolism of polyphenols, mainly stilbenes. This could affect the synthesis of compound 22, especially as it also belongs to the large group of stilbenes. Furthermore, the chromatographic profiles of the phenolic content of Béquignol grapes from the Man, Abidjan and Aboisso experimental sites were all similar. In fact, the grapes from this vine variety synthesised the same number of compounds (13), as well as the same amplitudes of phenolic peaks on each experimental plot. Unlike the Aleatico grapes, the Béquignol grapes induced the synthesis of a small number of polyphenols within the five main groups: phenolic acids (gallic acid, protocatechic acid, hydroxybenzoic acid, gentisic acid, p-coumaric acid), flavanols (catechin, epicatechin), flavonols (kaempferol, quercetin, delphinidin 3-O-glucoside), anthocyanins (malvidin 3-O-glucoside, peonidin 3-O-glucoside) and stilbenes (pikeid). This low number of polyphenols in the polyphenolic groups could be explained by the fact that Béquignol is a grape variety that produces wines with low polyphenolic concentrations. These results are similar to those reported by INRA (2021), which states that this grape variety can be used to make light, low-tannin wines that can be consumed quickly. Analysis of the results after quantification of polyphenols reveals an absence of certain compounds in Béquignol grapes. These include phenolic acids (cinnamic acid, ferulic acid, caftaric acid), flavonols (myricetin, isorhamnetin), stilbenes (resveratrol, astringin, pallidol) and antocyanins (peonidin 3-O-glucoside, cyanidin 3-O-glucoside). It is important to note that these polyphenolic groups are

defined as polyphenols with antioxidant, cardioprotective, anti-cancer, anti-inflammatory, cardiovascular and anti-diabetic activities (Mylène, 2018). The absence of all these compounds makes Béquignol a less rich variety than Aleatico.

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