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Physicochemical behavior of Riesling x Silvaner grapevine fruit under the high altitude conditions of Colombia (South America)

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Summary

The Valle del Sol (Sun Valley) of the Boyacá department is a zone with temperate tropical climate conditions (2,500 m above sea level) that is suitable for the production of grapes for quality wine. The objective of this investigation was to study the physical and chemical behavior during growth and development of the grapevine fruit var. Riesling x Silvaner, produced for winemaking, in the municipality of Corrales (Boyacá, Colombia). To determine the physical and chemical characteristics of the fruit starting at 28 days after anthesis (daa), 14 weekly samplings were carried out, in each of which three clusters were taken from randomly selected plants. The development of the berry lasted 119 daa in which three stages were defined: herbaceous, veraison and maturation[1]. The herbaceous stage ended at 63 daa, the veraison period lasted 14 days and ended at 77 daa, whereas the maturation and ripening stage lasted 42 days; no period of overmaturity was observed. The behavior of the fresh mass, dry mass and diameter of the fruit followed a double sigmoid curve. During berry development, total soluble solids (from 5.03 to 23.73 °Brix at the harvest point), pH (from 2.88 to 3.71) and technological maturity index (from 2.27 to 21.84) all increased, whereas total titratable acidity decreased from 3.96 to 1.11%.

Introduction

Vinifera grapevines were introduced during the last 450 years to North and South America, South Africa, South East Asia and Australia; and as a result, about 50 years ago, a grapevine industry developed in the tropics with entirely different climatic and growing conditions than those in the "traditional" subtropical and temperate regions (Lavee, 2000). It is well known that the tropics are characterized by a uniform annual photoperiod and uniform temperature conditions without marked temperature seasons in all altitudes (Lauer, 1986).

Phenology is the study of phases, physiological events or periodic and repetitive activities of the life cycle of plants that occur seasonally throughout the year in response to the climate (Mantovani et al., 2003; Gris et al., 2010). Lambers et al. (1998) defined growth as an increment in dry mass, volume, length or area, which in many cases involves the division, expansion, and differentiation of cells. According to the above, knowing the phenology of a species, we can determine the potential of a region to produce a crop within the limits of its climate pattern (Morlat and Bodin, 2006; Webb et al., 2007). And with studies of growth and development, we can establish how a plant or organ, in this case the fruit, behaves under certain conditions, which may be useful, among other factors, for scheduling cultural practices such as fertilization, irrigation, plant protection and time of harvest (Mullins et al., 1992).

The growth of the grapevine fruit (*Vitis vinifera* L.) consists of two successive sigmoid cycles, each with distinct characteristics (Coombe, 1992). The first cycle of the berry formation starts with increased cell division in the pericarp tissues, this process gradually changes to cell elongation, which slows at the end of the first sigmoid cycle. In this state, the berry is green and grows slowly, but malic acid accumulation in the pericarp occurs. The second cycle begins with the accumulation of sugar, softening and coloring of the berry and increase in size (Coombe, 1992; Hidalgo, 2002; Salazar and Melgarejo, 2005).

According to Conde et al. (2007), in many grape cultivars, the first growth phase is followed by a phase where growth is temporarily stopped. The duration of this phase is specific for each cultivar and ends with the completion of the herbaceous berry stage. After this period of non-growth, a second stage takes place, veraison, in which the skin of the berry starts changing color, indicating the onset of maturation. The most representative changes of the composition of the grape fruit occur during this maturation stage. Conde et al. (2007) mention that the berry passes from a state where it is small, hard and acidic, with low sugar content to a state where the fruit becomes larger, softer and sweeter, with a lower acid content, and with a characteristic flavor and color particular to the variety.

During fruit development of the grape, major changes occur at the biochemical level that lead to fruit ripening and determine its quality, of which, the most representative are the accumulation of total soluble solids (mainly sugars), increased pH and technical maturity index, decreased acidity (Gris et al. 2010; Almanza-Merchán and Balaguera-Lopez, 2009; Almanza et al., 2010), degradation of chlorophyll, accumulation of pigments in the epidermis, the synthesis of aromatic substances and flavor changes (Conde et al., 2007; Ali et al., 2011). These features are important to the monitoring of the development and ripening of the berry, mainly in areas where new varieties of grapes are to be introduced (Gris et al., 2010) in order to determine its productive potential.

The Valle del Sol (Sun Valley) of the Boyacá Department (Colombia) is characterized by a cold tropical climate zone suitable for the cultivation of grapevine for the production of quality wines (Quijano, 2004). Early studies in these agro-ecological conditions were performed with the Pinot Noir variety, which identified four phenological stages, herbaceous, ripening, maturation and over-ripening (Almanza-Merchán and Balaguera-Lopez, 2009), and showed that the development of the fruit until harvest lasted 126 days after anthesis (daa), during this lapse 826.2 growing degree days accumulated (Almanza et al., 2010).

Because the physicochemical behavior of some important varieties for winemaking under conditions of high tropics is not known, the objective of this research was to study the behavior during growth and fruit development of grapevine 'Riesling x Silvaner'.

Materials and methods

This research was conducted between July and November of 2009, in a commercial vineyard in the municipality of Corrales (Boyacá-Colombia), situated at 5°48'30 N and 72°58'35" W at an altitude of

[1] In this article the maturation stage also includes the ripening of the berry on the plant

2,450 m. The climate conditions during the experiment are shown in Tab. 1, where the average temperature value was 16.7 °C (maximum and minimum temperatures 22.3 °C and 10.4 °C, respectively), the relative humidity was 86.3% and the total rain fall was 243.11 mm. An average of 4.2 h d^{-1} of direct sunshine (Tab. 1) and a high solar insolation, with a mean value of 476 cal cm^{-2} d^{-1}, were recorded. The soils are stony and a light-textured, sandy loam type with a low natural fertility.

The evaluated variety corresponds to the clonal selection of *Vitis vinifera* L., 'Riesling x Silvaner', also known as Riesling Becker, provided by the vineyard "Loma de Puntalarga" in Nobsa (Boyacá). The crop was established 8 years ago and set at a distance of 1.2 x 0.8 m between rows and plants, respectively, using the Guyot simple pruning type at a three-wire trellis. For the experiment, 42 clusters were randomly selected from an equal number of plants. Each week three clusters were harvested randomly. From each cluster, 10 fruits were taken to determine the physical characteristics and 10 fruits for the chemical characteristics. Fourteen samples were taken from the time the fruit size allowed for the taking of these measurements (28 daa).

The fruit variables measured were: fresh and dry mass (balance, 001 g precision; dry mass after subjecting the fruit to 75 °C for 48 h), equatorial diameter and length, absolute growth rate (according to Hunt, 1990), total titratable acidity (TTA; AOAC, 1990; expressed as percentage of tartaric acid), total soluble solids (TSS; digital refractometer Hanna Instruments HI 96801, 0-85 °Brix; Clarkson Laboratory & Supply Inc., Chula Vista, CA), and pH (potentiometer previously calibrated with buffer solutions of pH 7.0 and 4.0). The technological maturity index (TMI) was obtained through the relationship between TSS and TTA.

Each cluster corresponded to one repetition in each sample. The data were analyzed using descriptive statistics and the average and standard error were calculated. In addition, statistical models with the greatest adjustment were determined. For the data analysis, SAS v. 8.1e (Cary, NC) program was used.

Results and discussion

Phenological stages of the grapevine fruit

The results indicate that the 'Riesling x Silvaner' fruit presented three well-defined stages of development: herbaceous, veraison and maturation and ripening (Fig. 1). The herbaceous stage lasted from fruit set to 63 daa, representing the longest stage. Agustí (2008) and Reynier (1995) indicate that in this period cell division is predominant which favors rapid growth of the fruit; and the berry begins its evolution and has a green color with a high photosynthetic capacity and consistency.

The veraison stage ended at 77 daa with a period of 14 days. Some authors state that this stage marks the onset of maturation; the berries lose their firmness and change color (Ali et al., 2011; Conde et al., 2007). However, with Riesling x Silvaner, a cultivar with "white" fruits in this stage, the berries take on a translucent appearance. According to Mullins et al. (1992), at this stage the seed reaches physiological maturity, while respiration, photosynthesis and chlorophyll content decrease; the fruit is covered with pruina (waxy epicuticular coating of the grape) and herbaceous aromas decline (Hidalgo, 2002; Reynier, 1995). The maturation stage culminated at 119 dda, registering a span of 42 days. During this period, the berry has a quick gain of biomass (Fig. 1) and an accelerated evolution of biochemical components (Hidalgo, 2002; Salazar and Melgarejo, 2005).

Contrary to that reported by other authors for 'Pinot Noir' (Almanza-Merchán and Balaguera-López, 2009; Almanza et al., 2010), with 'Riesling x Silvaner' there wasn't an over ripeness stage, but the onset and duration of the other periods coincide with those found by Almanza-Merchán and Balaguera-López (2009) and differs only in duration of maturation which was observed by Almanza et al. (2010) for 'Pinot Noir' as lasting 35 days. These results confirm that the duration of the phenological stages can vary representatively between varieties, climate and geographical location (Bodin and Morlat, 2006; Webb et al., 2007; Gris et al., 2010). Knowing the duration of the phenological phases in specific

Tab. 1: Environmental conditions of the Corrales municipality (Boyacá, Colombia) during the study period (July-November, 2009).

Days after antesis (daa)	Precipitation (mm)	Mean temp. (°C)	Mean max. temp. (°C)	Mean min. temp. (°C)	Sun shine (h d^{-1})	Relative humidity (%)
7	2.8	16.7	21.6	10.5	3.8	87.4
14	14.3	16.8	21.2	10.7	4.4	89.5
21	2.31	16.6	21.6	10.0	5.5	90.2
28	5.93	17.1	23.5	10.8	5.5	89.8
35	28.11	16.3	21.1	10.4	4.3	90.4
42	0.61	16.6	21.6	10,4	5.3	86.6
49	2.2	16.7	22.4	9.8	5.2	87.0
56	40.9	16.1	21.6	10.0	2.9	87.6
63	0.7	16.9	21.8	9.7	4.8	85.6
70	2.2	16.8	21.5	9.5	4.1	86.5
77	0.0	16.9	23.2	10.2	4.4	85.1
84	22.0	16.3	22.3	10.1	3.1	79.1
91	48.71	16.8	23.4	11.0	2.1	88.4
98	11.6	17.0	22.3	11.1	2.5	87.0
105	28.72	17.1	23.4	11.8	3.0	87.3
112	0.0	17.2	22.8	10.7	5.2	85.5
119	32.02	17.5	23.8	11.0	6.8	74.5
Mean value	**243.11***	**16.7**	**22.3**	**10.4**	**4.2**	**86.3**

*Accumulated precipitation during the study period agro-ecological conditions is essential in viticulture, as it facilitates the selection of crop management practices.

Physical fruit characteristics

The accumulation of dry and fresh weight of fruits conformed to a logistic growth model and had a typical behavior of a double sigmoid curve (Fig. 1), characteristic for this species (ALMANZA et al., 2010; OPARA, 2000; COOMBE, 1976). The dry mass of the fruit presented lower values until 63 daa, when the first sigmoid phase was completed, and coincided with the herbaceous stage, characterized by active cell division (HERNANDEZ, 2000). In this period, the absolute growth rate (AGR) was also low, but then the dry mass showed a continuous rise until the harvest at which point 0.33 ± 0.019 g was accumulated by the berry. The mass gain was more pronounced between 77 and 84 daa, and from day 105 on (Fig. 1A).

The absolute growth rate for dry mass had maxima at 49 and 84 daa and showed a dramatic increase from 112 daa until harvest, when it reached its maximum value (0.0129 g d^{-1}). This behavior also indicated that the dry mass did not follow an asymptotic curve (Fig. 1A and C), possibly due to the continuous accumulation of photoassimilates in the last stage of fruit growth (GRANGE, 1996).

A low gain of fresh mass up to 42 daa was observed, after that, a rapid increase ending at 49 daa followed by a slowing until 63 daa was observed. Therefore, AGR peaked at 49 daa (0.04 g d^{-1}) and then declined. Subsequently, increases were observed for fresh mass, mostly between 70 and 77 daa and after 112 daa, meaning AGR showed high values in the same periods. At harvest, the fresh mass and AGR were 1.94 ± 0.05 g and 0.03 g d^{-1}, respectively (Fig. 1B y C). CONDE et al. (2007) mentioned that berry size from veraison to harvest almost doubles. At this stage, the cell elongation is the predominant process, influenced by the plasticity of the cell walls and the turgor pressure of the cells responsible for the sharp increase in volume and mass (COOMBE, 1960). It is possible that the increase in the concentration of sugars during ripening causes a decrease in osmotic potential and therefore an increase in fruit fresh mass produced by an increase in water holding capacity (COOMBE, 1960).

Longitudinal and equatorial diameters were adjusted to a cubic model and had a double sigmoid trend. The completion of the first sigmoid stage (herbaceous stage) was at 63 daa similar to the behavior of fresh and dry weight of the fruit, but with the difference that diameter had a asymptotic growth at the end of the second sigmoid stage. A similar behavior was reported in the grape by DOKOOZLIAN (2000). The longitudinal diameter was greater than the equatorial one during the whole development of the fruit and at harvest the obtained values were 1,49±0,03 cm and 1,42±0,04 cm, respectively (Fig. 2), which indicates that these fruits are bigger than those of the variety ‘Cabernet Sauvignon‘ which presents an average of 12 mm.

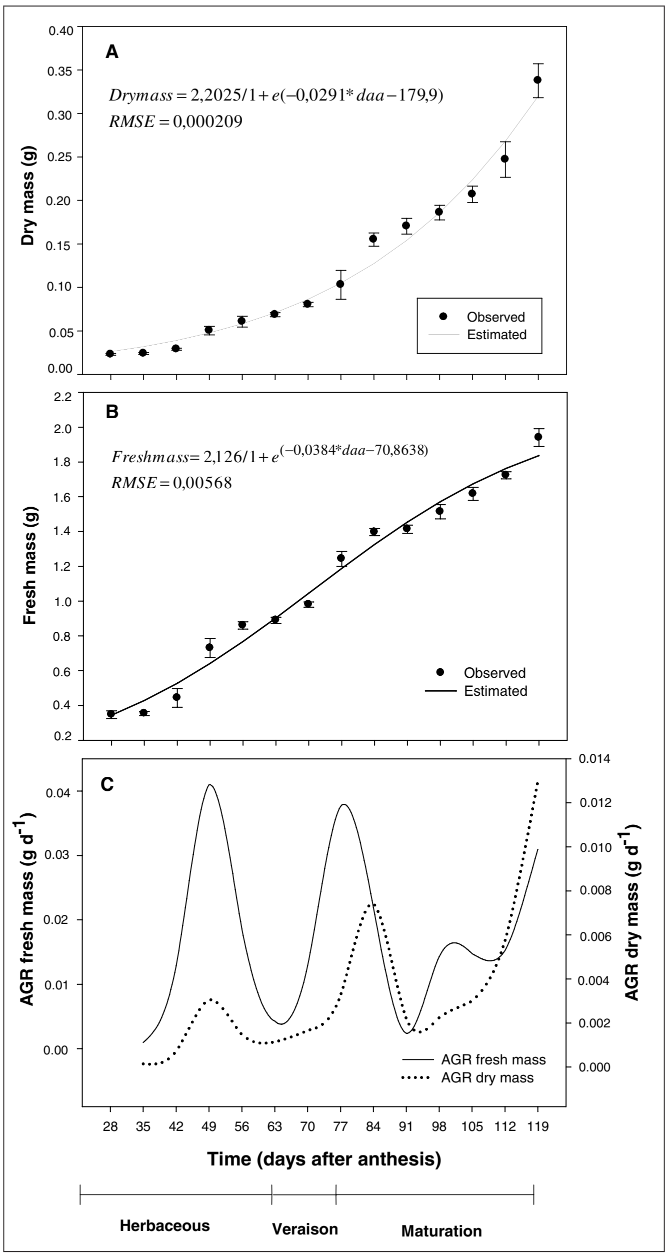

Fig. 1: Behavior of dry mass (A), fresh mass (B) and absolute growth rate (AGR) (C) of the grapevine ‘Riesling x Silvaner’ fruit during growth and development under high altitude tropical conditions. Vertical bars indicate the standard error of each mean (n = 3). RSME, root square mean error.

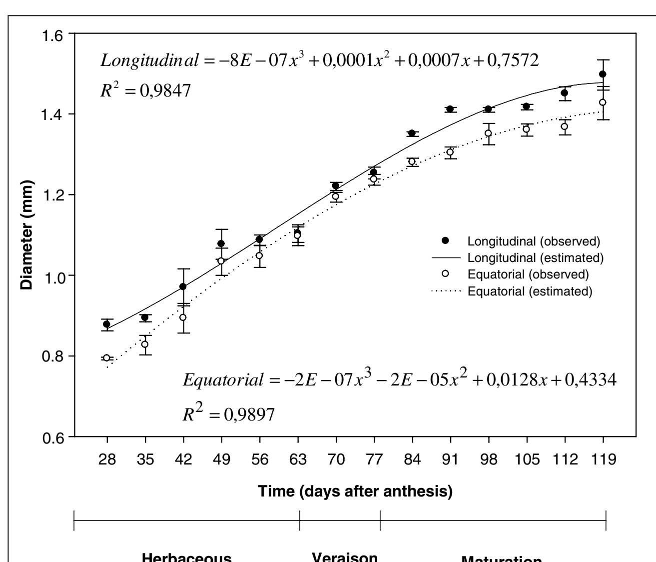

Fig. 2: Behavior of the diameter of the grapevine ‘Riesling x Silvaner’ fruit during growth and development under high altitude tropical conditions. Vertical bars indicate the standard error of each mean (n = 3).

Chemical characteristics of the fruit

The concentration of chemical substances is important to the quality and quantity in the berries of the grape for growth and yield regulation (GIL, 2004). The increase of the TSS during the grape fruit development was described by a third degree polynomial. Between 28 and 42 daa, 63 and 77 daa (veraison stage) and 98 and 112 daa, the TSS remained almost constant, at other times there was a representative increase. At the end of the herbaceous stage, veraison and harvest, the fruit of 'Riesling x Silvaner' presented 14.53±0.35, 15.4±0.3 and 23.73±0.49 °Brix, respectively (Fig. 3A). Accordingly, DOKOOZLIAN (2000) affirms that the grapes accumulate more sugars during maturation than many other fruits. The TSS content found in our study at harvest is higher than those of the Cabernet-Sauvignon grape (GIRIBALDI et al., 2010) and the Superior cultivar (GRANGEIRO et al., 2002), but lower than those in 'Pinot Noir' berries cultivated in a cold tropical climate (ALMANZA-MERCHÁN and BALAGUERA-LÓPEZ, 2009; ALMANZA et al., 2010).

80 to 95 % of the total soluble solids are composed of sugars and the content is associated with the sugars dissolved in the cellular juice (OSTERLOH et al., 1996), to a lesser extent they also contain organic acids, proteins, fats and several minerals. The sugar necessary for the growth and maturation of the berries must be imported principally from the leaves and to a lesser extent from the trunk and roots (DOKOOZLIAN, 2000), because sucrose is the principal sugar transported to the fruits, although it seems that a high activity of the acid invertase enzyme exists in the grape fruit (FILLION et al., 1999) because the sugars with the highest concentrations are fructose and glucose (ALI et al., 2011; HERRMANN, 2001).

GIL (2004) also reports that the principal cause of the increase of TSS is the transport of sucrose from the leaves and reserve sites of the vascular and peripheral system, with a rate of 27 to 30 cm h^{-1}. CONDE et al. (2007) mention that after the veraison period a continuous amount of glucose and fructose is accumulated in the vacuoles of the mesocarp cells, which possibly explains the increase of the TSS of the 'Riesling x Silvaner' fruits. Also, DOKOOZLIAN (2000) affirms that the sugars also come from skeletons of carbon generated by organic acids and amino acids.

There was a quadratic decrease of the TTA as the fruit development increased; in the beginning of the herbaceous period, the acidic contents were high with values of about 4 %, nevertheless, in the herbaceous stage the TTA remained almost constant with values about 2 %, whereas at harvest it presented the lowest concentrations with 1,09±0,06 % (Fig. 3B), a value that can be considered to be a high as compared to the contents found in Cabernet Franc, Merlot, Sangiovese and Syrah varieties (GRIS et al., 2010), but it is similar to the one found in 'Pinot Noir' (ALMANZA-MERCHÁN and BALAGUERA-LÓPEZ, 2009) under high altitude tropical conditions, which confirms those mentioned by BLUSKE (2008), that is, in high altitudes the values of acidity are high because there is minor respiration of malic acid compared with that of tartaric acid, and consequently produce wines that are too smooth.

Nevertheless, HERRMANN (2001) states that during grape maturation there is a greater decrease in malic acid than in tartaric acid. Also, the continuous precipitations that appeared during the development of the berries could increase the TAA, as was found by JUBILEE et al. (2010) in fruits of 'Cabernet Sauvignon', produced in the off-season. The berries synthesize only a small part of the acids (HARDY, 1968), most of them are translocated from the leaves, as a consequence of the photosynthetic activity which is closely involved to its synthesis (GIL, 2004).

Tartaric acid is accumulated during the initial stage of the berry development and its concentration is higher in the periphery of the developing berry. On the contrary, malic acid is stored in the cells of the pulp at the final stage of the first phase of growth (CONDE et al., 2007; DE BOLT et al., 2006). During maturation, this acid is metabolized, transformed into sugars and used as a source of energy during the maturation phase (CONDE et al., 2007), also it can be diluted by the water that accumulates in the fruit (ALMANZA-MERCHÁN and BALAGUERA-LÓPEZ, 2009) (processes that would explain its decrease [Fig. 3B]). These acids give the acidity to the wine and in part determine its quality (CONDE et al., 2007). DOKOOZLIAN (2000) mentions that malic and tartaric acids make up approximately 90 % of the whole acidity. The remaining percentage in the berries depends on other acids such as citric (up to 5 % [HERRMANN, 2001]), succinic, lactic and acetic acids that are present principally in the maturation stage (CONDE et al., 2007).

The flavor of the grape fruit is principally the result of the acid/sugar ratio and the synthesis of aromatic compounds, or of precursors that take place at the same moment. The development of these characteristics will determine to a great extent the quality of the final product (BOSS and DAVIES, 2001). In congruity, the technological maturity index (TMI) of 'Riesling x Silvaner' increased rapidly up to harvest; conforming to a quadratic function, with the exception of the herbaceous stage, where the TMI remained almost constant. At harvest, the TMI was 21,84±1,27 (Fig. 4A), this value is considered to be low as compared to indices found by GRANGEIRO et al. (2002); GRIS et al (2010) and ALMANZA et al. (2010). This might be explained by the high acid content (Fig. 3B), since the measured TSS

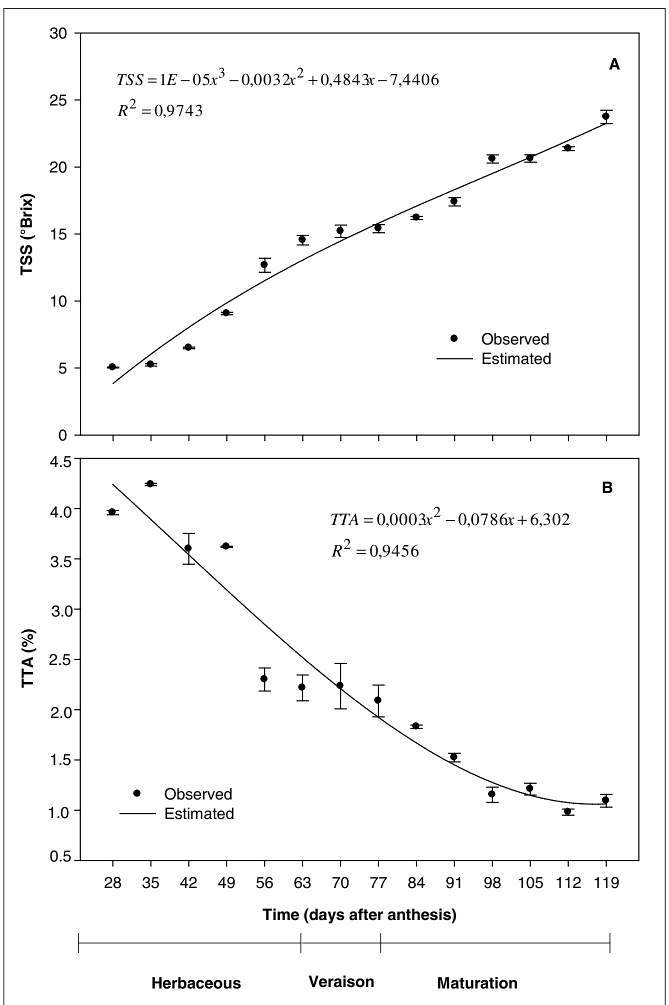

Fig. 3: Behavior of the total soluble solids (TSS) (A) and the total titratable acids (TTA) (B) of the grapevine 'Riesling x Silvaner' fruit during growth and development under high altitude tropical conditions. Vertical bars indicate the standard error of each mean (n = 3).

was considered high (Fig. 3A).

Jubileu et al. (2010) mention that the use of the maturation index must be done carefully, since an increase of sugar does not always represent a decrease of acidity and one cannot compare the different varieties of grape (Rizzon and Miéle, 2002), but it might be useful to compare the behavior of the same variety under different growing conditions. Meanwhile, Blouin and Guimberteau (2004) affirm that this index serves as a reference for an ideal harvest time from the wine point of view. Nevertheless, Jubileu et al. (2010) and Gonçalves et al. (2002) coincide in affirming that the TSS and the TTA contents are of fundamental importance in monitoring the harvest point of grape fruits, making a better control of the quality of the raw material for wine making possible.

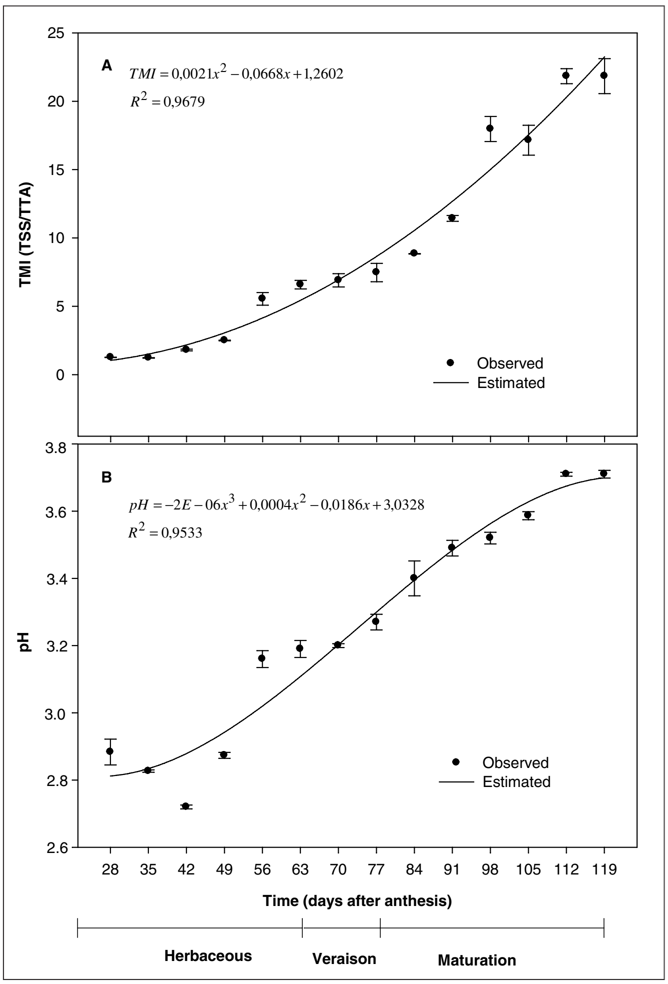

Fig. 4: Behavior of the technological maturity index (TMI) (A) and the pH value (B) of the grapevine 'Riesling x Silvaner' fruit during growth and development under high altitude tropical conditions. Vertical bars indicate the standard error of each mean (n = 3).

The pH conformed to a third degree polynomial, which was characterized by a slight decrease at the beginning of the herbaceous stage and a rapid increase towards the end of the same period, whereas in the veraison phase it remained stable and again it increased up to 112 daa to remain stable up to the harvest (Fig. 4B). In congruity, Dokoozlian (2000) affirms that the pH is a measurement of the concentration of ions hydrogen in the berry and is generally related to the acidity of the juice. In accordance with the latter author, the pH is relatively constant during the first stage of the fruit development with values near to 2.5, and then it increases gradually during maturation due to the decrease of malic acid. This behavior aligns with Dokoozlian (2000) who affirms that the pH is a measurement of the concentration of hydrogen ions in the berry and is generally related to the acidity of the juice. The measured pH at the crop harvest was 3.71±0.01 (Fig. 4B), an advisable value for securing quality wines, since, in accordance with Rizzon and Miéle (2002), and values of pH lower than 3.3 can negatively affect wine quality.

Conclusions

Under the agroecological conditions of the Corrales municipality in Boyacá (Colombia), the fruit development of the 'Riesling x Silvaner' grape was 119 days from anthesis to harvest and its development could be explained efficiently by a double sigmoid curve, with three definite stages: herbaceous, veraison and maturation. The herbaceous stage finished at 63 daa and was the longest period. The veraison period was the shortest (14 days) and finished at 77 daa, whereas the maturation stage lasted 42 days. During the development of the berries, TSS increased, as did pH and TMI, but TAA decreased.

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