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The localisation of acids, sugars, potassium and calcium in developing grape berries

by

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Lokalisierung von Säuren, Zuckern, Kalium und Calcium in sich entwickelnden Traubenbeeren

Zusammenfassung: Traubenbeeren von *Vitis vinifera* cv. Chardonnay wurden in vier konzentrische Zonen aufgeteilt, um die entwicklungsbedingten Änderungen in der Konzentration von Malat, Tartrat, Glucose, Fructose, Kalium und Calcium innerhalb der Haut und des Fruchtfleisches verfolgen zu können. Grüne Beeren zeigten einen von der Haut in Richtung Samen deutlich ansteigenden Konzentrationsgradienten für Malat, während im Fall von Tartrat die höchste Konzentration an der Peripherie und die niedrigste im Beerenzentrum gemessen wurde. Mit fortschreitender Reife nahm der Quotient der Tartratkonzentration in der Haut und des entsprechenden Wertes im Beerenzentrum ab; Malat zeigte das gegenteilige Verhalten. In reifen Beeren konnte auch in axialer Richtung ein Säuregradient gefunden werden, der vom Stielansatz in Richtung Narbe abnahm. Vor dem Einsetzen des Reifeprozesses wurden in der Haut und im Beerenzentrum die höchsten Glucose- und Fructosekonzentrationen beobachtet. Die Akkumulation dieser beiden Zucker im Fruchtfleisch begann ohne deutliche Lag-Phase mit einer Rate von etwa 2 mg/Beere · d. Nach der Véraison wurden die höchsten Konzentrationen im Fruchtzentrum und dem Gewebe unterhalb der peripheren Gefäßbündel gemessen. Sowohl Kalium als auch Calcium waren hauptsächlich in der Nähe der peripheren und zentralen Gefäßbündel lokalisiert. Der Kaliumgehalt stieg während der ganzen Beerenentwicklung mit einer konstanten Rate von 0,04 mg/Beere · d an, während die Akkumulation von Calcium etwa 30 d nach Blühbeginn bei einem Wert von annähernd 0,1 mg/Beere endete.

Key words: berry, growth, translocation, malic acid, tartaric acid, glucose, fructose, potassium, calcium.

Introduction

Studies concerned with the distribution of compounds within the grape berry have usually been limited to differences between the skin, flesh and seeds (Coombe 1973; Hale 1977; Coombe and Matile 1980). However, since the pulp represents as much as 90 % of the total berry weight (Laborde 1907), it is somewhat surprising, that only few reports exist dealing with the location of compounds within this part of the fruit (van der Heide and Schmitthenner 1922; Kriedemann 1969; Possner *et al.* 1983). This fact might be due to the difficulties in separating the relatively soft fruit flesh into several parts, especially with ripe berries, where considerable losses of cellular juice have to be taken into account. The progressive change in consistency of berry pulp tissue makes its exact separation with simple techniques virtually impossible.

In spite of these problems, van der Heide and Schmitthenner (1922) showed that the titratable acidity of ripe berries was lowest in the skin area and highest around the seeds. In 1956, Amerine reported that the opposite was true in very young grapes. According to Steffan and Rapp (1979), peripheral malate can more readily be respired than malate from the berry center, regardless of the stage of ripeness. Correspondingly, Coombe and Matile (1980) found a lower level of malate in the skin of pre-véraison Pinot Noir grapes compared to the flesh, whereas with glucose the reverse was the

case. These findings can partially be explained with the results of SMART and SINCLAIR (1976), who detected a distinct temperature gradient within the fruit, decreasing from the skin towards the center. Obviously, metabolic turnover rates and, therefore, the concentration of certain compounds differ widely throughout the berry, a fact that is often neglected in physiological studies.

The purpose of this investigation was twofold: firstly to describe a simple technique that allows the quantitative determination of compounds in four approximately concentric fruit zones, and secondly to monitor the changes in the concentrations of malate, tartrate, glucose, fructose, potassium and calcium within these different areas during the entire period of fruit development. In addition, the concentrations of these constituents in axial directions of grape berries are reported.

Materials and methods

Grape material: A randomly selected cluster of field-grown Chardonnay grape berries (*Vitis vinifera* L.) was harvested at intervals of 10 d and rinsed in distilled water for about 15 min. Using a razor blade, 60 berries were separated from the pedicel and cleaned with soft paper towels. After weighing and measuring their diameter, the berries were randomly separated into 6 sets of 10 berries each (sets 1 to 6). Set 1 was used for the refractometric estimation of total soluble solids; the other sets were used as described in the next two sections.

Division of berries into four concentric zones: To avoid the obvious difficulties of dividing a single berry into four concentric zones, the following technique was worked out: Using a razor blade, single berries were divided into two zones; the thickness of each zone was varied by systematic alteration of the depth of cutting. Thus, the berries of set 2 served to separate the skin from the pulp by placing two meridional cuts through the outer layers of the fruit and pulling off the skin with a pair of tweezers. Subsequent microscopic examination showed that, using this technique, the peripheral vascular bundles of the fruit represented the border between 'skin' and pulp. The berries of set 3 served to separate a somewhat thicker peripheral zone, i.e. skin with attached pulp representing about one quarter of the fruit radius,

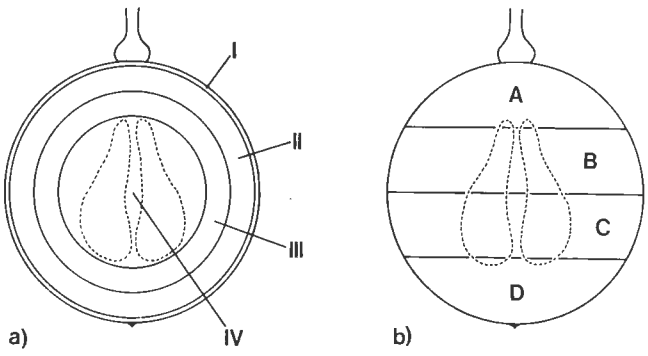

Fig. 1: Division of Chardonnay grape berries in four concentric (a) and four axial areas (b).
Aufteilung von Chardonnay-Traubenbeeren in vier konzentrische (a) und vier axiale Zonen (b).

from the remaining pulp. 10 other berries (set 4) were divided into the skin with about half the radial distance of pulp attached, leaving the fruit flesh of the berry center. A 5th set of undivided berries was used for comparison. After removing the seeds, the fruit parts were put into tared glass beakers, each containing about 10 ml of distilled water, and the gross weight determined. Subsequently, the tissue was boiled for 5 min, pottered, filtrated through Whatman number 4 and the filtrate made up to 30 ml with distilled water. This extract was appropriately diluted and used for quantitatively determining the compounds of interest. After normalizing the values and calculating the differences of the corresponding overlapping areas, the described procedure allowed the determination of the relative shares of the four approximately concentric zones (Fig. 1 a). Thus, the skin (zone I) represented 15.3 ± 3.7 % (n=9) of the berry fresh weight — excluding seeds, which represented 5—14 % of the total berry fresh weight, and losses due to undesired draining of cell juice, which were 5—20 % — the outer part of the pulp (zone II) 36.8 ± 5.5 %, the inner part of the pulp (zone III) 25.3 ± 3.7 %, and the area around the seeds (zone IV) 22.6 ± 5.8 %.

Division of berries into four axial zones: After dividing one set of berries (set 6) into two approximately equal halves by cutting perpendicular to the central vascular bundles, the two resulting portions were cut again parallel to the fruit equator (Fig. 1 b). On a weight basis, the relative portions of the four areas A, B, C, and D were respectively 19.6 ± 1.8 % (n = 9), 27.7 ± 2.4 %, 31.8 ± 1.7 %, and 20.9 ± 1.7 %. All extracts were made as described previously.

Malate and tartrate concentrations were determined by HPLC. The samples were prepared as described by McCord *et al.* (1984). The analysis was conducted using a Bio-Rad HPCL system as follows: Column: fruit quality analysis at 65 °C, 100 × 7.8 mm; guard: cation H^+ at 25 °C; solvent: 1 mM H_2SO_4, 1 ml/min; detector: differential refractometer, model 1770.

Glucose and fructose concentrations were estimated enzymatically using hexokinase, glucose-6-phosphate dehydrogenase and phosphoglucose-isomerase (Boehringer, Mannheim, FRG) as described by Bergmeyer and Bernt (1974). Sucrose could not be detected because of its rapid inversion during extraction (cp. Rapp *et al.* 1977).

Potassium and calcium concentrations were determined by emission and atomic absorption spectroscopy, respectively, using a Perkin Elmer 2380 AA spectrophotometer (Perkin Elmer, Norwalk, CT, USA).

Results

1. Malic and tartaric acid

Fig. 2 shows that both malic and tartaric acid were not evenly distributed throughout the fruit. Green berries were characterized by a distinctive malate gradient, increasing in concentration from the periphery towards the center (Fig. 2 a). After véraison, this gradient was altered only by the relatively high malate concentration of the skin. The areas II, III and IV (cp. Fig. 1) accumulated malic acid during the entire green stage of berry development at an increasing rate from the center towards the periphery, whereas incorporation into area I (mainly skin) started only 10 d before véraison. During this 10 d period, however, the skin accumulated malate at a higher rate than any other fruit part (0.6 mg/g FW · d). The transition to the ripening stage was characterized by a lag phase in malate breakdown. Although the sigmoidal shape of the

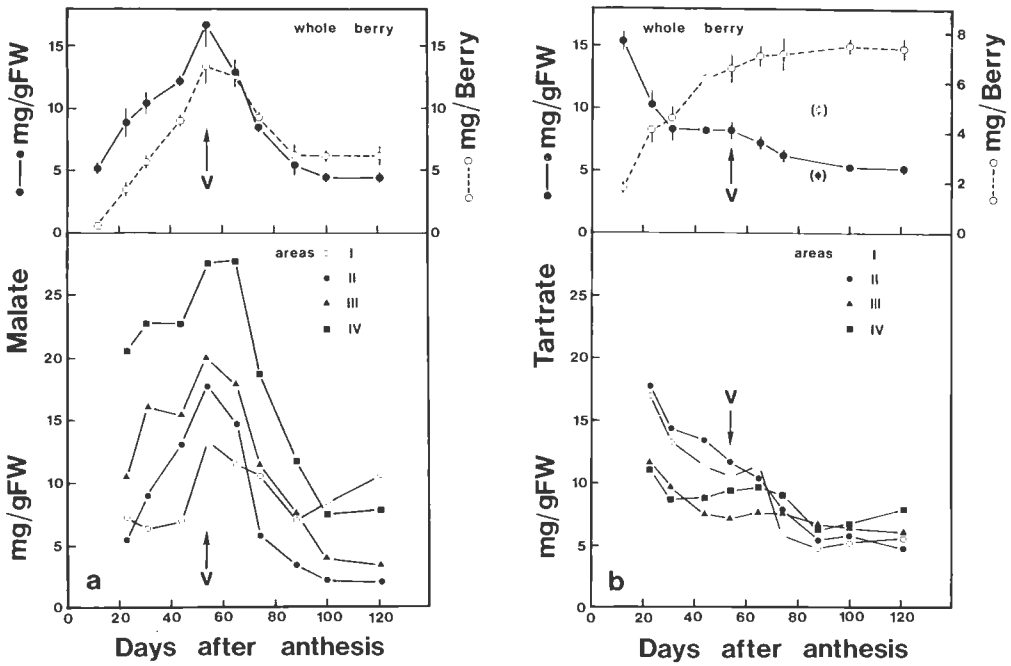

Fig. 2: Developmental changes in the concentration of malate (a) and tartrate (b) in four concentric fruit areas. I = skin, II = outer part of the pulp, III = inner part of the pulp, IV = area around the seeds. V = véraison.

Entwicklungsbedingte Änderungen der Konzentration von Malat (a) und Tartrat (b) in vier konzentrischen Beerenzonen. I = Haut, II = äußerer Teil des Fruchtfleisches, III = innerer Teil des Fruchtfleisches, IV = samenumgebende Zone. V = Véraison.

malate curve was not very conspicious on a fresh weight basis with whole berries (Fig. 2 a, upper graph), it became more obvious in the different fruit areas (Fig. 2 a, lower graph). The rate of malic acid degradation during the first 10 d after véraison again increased from the center towards the periphery. After that period, the areas III and IV aligned their breakdown rates to that of area II reaching a value of about 1 mg/g FW · d. As early as 20 d after the onset of ripening, malate degradation started to slow down in area II, whereas the process continued in zone IV for 20 more days at the maximum rate. With an approximative rate of 0.2 mg/g FW · d, malate breakdown in the skin was considerably slower than in the other berry parts. Although there was no malate decrease detectible on a per berry basis beyond 90 d after anthesis, the malate concentration increased in area I and decreased in the areas II, III and IV (Fig. 2 a and 3).

In contrast to the breakdown of total malate per berry after the onset of ripening, the tartrate c o n t e n t remained essentially constant (Fig. 2 b). However, there was a decrease in tartrate c o n c e n t r a t i o n , due to the diluting effect of the increase in berry volume. As in the case of malate, considerable differences in tartrate concentrations between the peripheral and central zones of the berry were found. Zone III and IV showed more or less the same concentration during the entire period of berry development. In areas I and II, however, the concentration initially was much higher

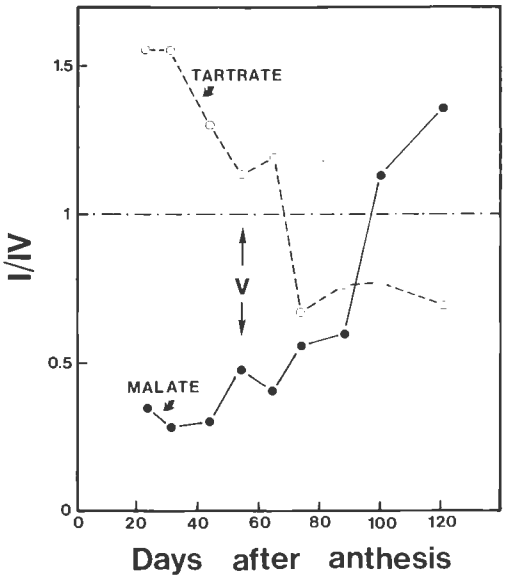

Fig. 3: Changes in the ratio I/IV (concentration in the skin/concentration in the area around the seeds) of malate and tartrate during the berry development. V = véraison.

Änderungen des Quotienten I/IV (Konzentration in der Haut/Konzentration in der samenumgebenden Zone) von Malat und Tartrat während der Beerenentwicklung. V = Véraison.

than in the inner zones and decreased steadily throughout berry development reaching values lower than the fruit center (Fig. 2 b and 3).

Division of berries transversely into four parts (Fig. 1 b) showed that at most sampling dates the tissue proximate to the pedicel had higher malate and tartrate concentrations ($P < 0.05$) than the corresponding distal parts. However, because of the varying distribution of the acids in the radial direction during berry development, the tendency to form a gradient decreasing in concentration from the pedicel towards the stylar scar was obvious only in the ripe fruit (Table).

Axial malic and tartaric acid gradients in ripe grape berries (n = 2) · 'A' is proximate and 'D' is distal to the pedicel with 'B' and 'C' areas in between (see Fig. 1 b for explanation of fuit areas)

Axiale Äpfel- und Weinsäuregradienten in reifen Traubenbeeren (n = 2) · „A" ist proximal und „D" distal zum Stiel, während „B" und „C" die dazwischenliegenden Zonen repräsentieren (für die Erklärung der Beerenzonen s. Fig. 1 b)

Fruit area	Malic acid (mg/g FW)	Tartaric acid (mg/g FW)
A	5.72 ± 0.15	7.63 ± 0.22
B	5.15 ± 0.01	5.86 ± 0.20
C	4.62 ± 0.06	5.66 ± 0.03
D	4.56 ± 0.09	4.79 ± 0.37

2. Glucose and fructose

Although the concentrations of both glucose and fructose were consistently low in all parts of the young grape berry (Fig. 4), the skin (zone I) and fruit core (zone IV) showed significantly higher concentrations than the tissue from the two zones in between ($P < 0.05$). The quotient I/IV (concentration in the skin/concentration in the center) was 2.16 ± 0.39 ($n = 4$) for glucose and 1.60 ± 0.37 ($n = 3$) for fructose during the green berry period of fruit development. After véraison, zone II and IV contained higher sugar concentrations ($P < 0.005$) than zones I and III and zone III tissue was generally higher in glucose and fructose than zone I ($P < 0.005$). Fig. 4 shows that the accumulation of sugars in the fruit flesh started without any apparent lag phase. Sugar accumulation following véraison continued at a rate of about 1.5 mg/g FW · d for approximately 6 weeks and decelerated last in the berry core. No significant differences in concentration of glucose and fructose were observed from the pedicel end to the stylar end of berries, however, there was a trend for higher concentrations at the stylar end.

3. Potassium and calcium

The potassium content increased approximately linearly at a rate of 0.04 mg/berry · d during the entire period of fruit development, reaching a value of nearly 5 mg/berry at the end of the ripening process (Fig. 5 a). In contrast, the calcium content rose for only 30—40 d following anthesis, attaining a level of about 0.1 mg/berry and

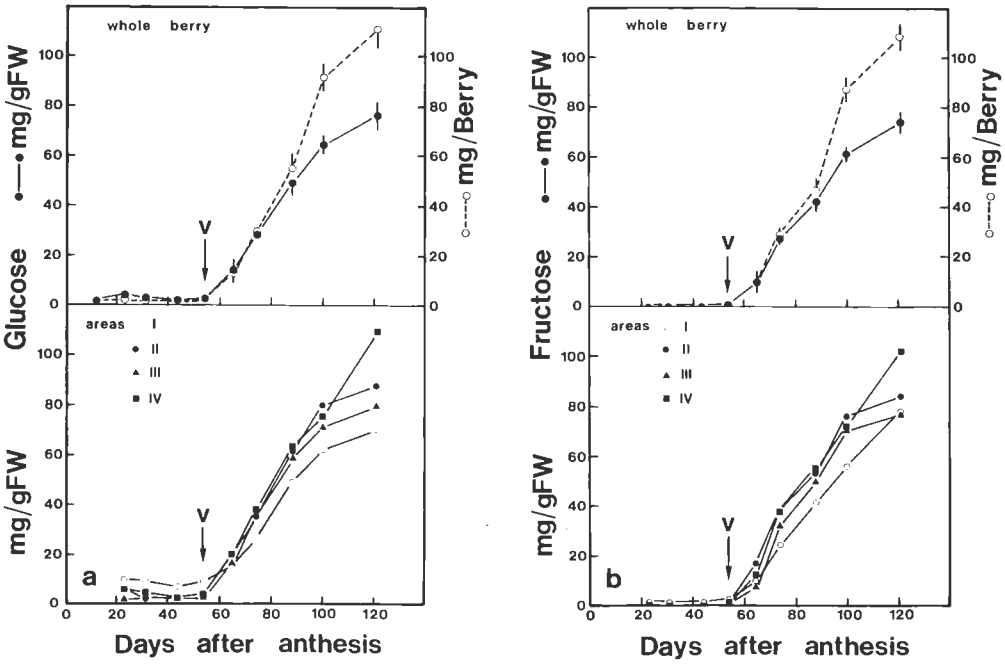

Fig. 4: Changes in the concentration of glucose (a) and fructose (b) (cp. Fig. 2).
Änderungen der Konzentration von Glucose (a) und Fructose (b) (vgl. Fig. 2).

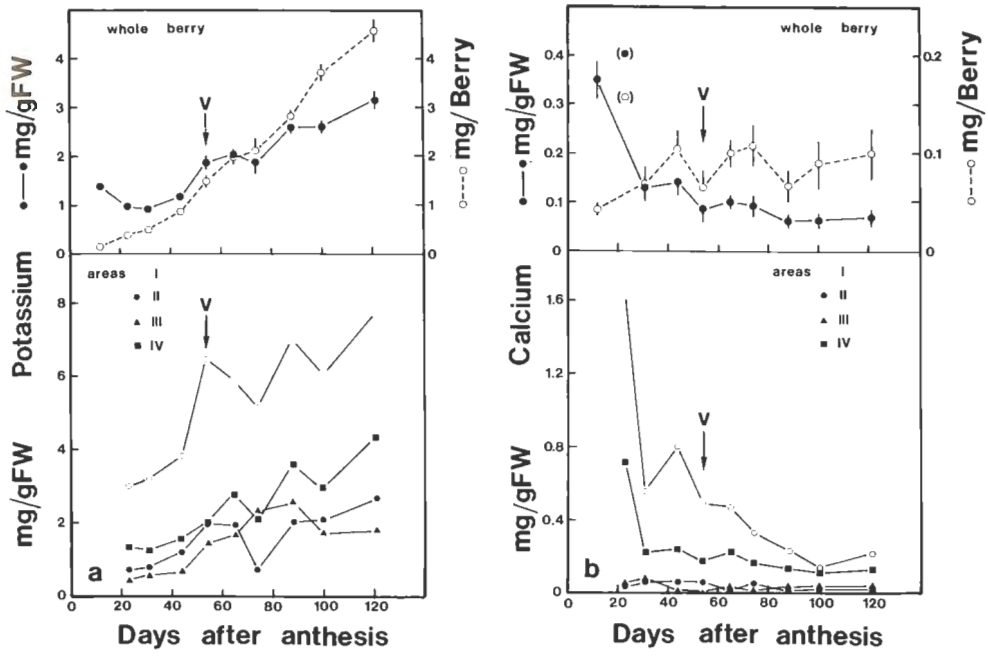

Fig. 5: Changes in the concentration of potassium (a) and calcium (b) (cp. Fig. 2).
Änderungen der Konzentration von Kalium (a) und Calcium (b) (vgl. Fig. 2).

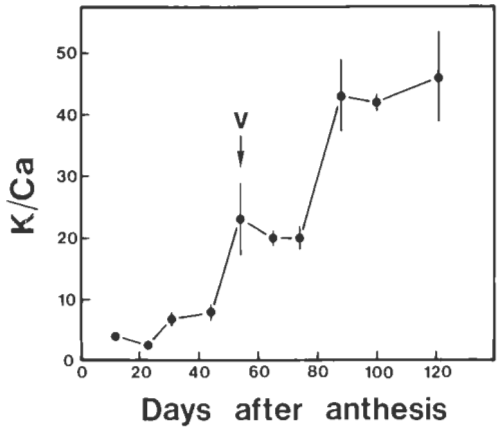

Fig. 6: Developmental changes in the ratio potassium/calcium of whole berries (n = 5). V = véraison.

Entwicklungsbedingte Änderungen des Quotienten Kalium/Calcium ganzer Beeren (n = 5). V = Véraison.

then remained relatively constant for the remainder of the season (Fig. 5 b). The K/Ca ratio, however, did not increase linearly, but was characterized by two distinct plateaus; the first was reached 55 d after anthesis with a K/Ca value of about 20 and the second 90 d after anthesis with a K/Ca value of about 40 (Fig. 6). The highest concentrations of K and Ca were found in the skin and center portion of the fruit (Fig. 5). Despite the quantitative differences between the K and Ca content of grapes, the quotients I/IV for these ions were similar: K = 2.32 ± 0.43, Ca = 2.30 ± 0.58, averaged over 9 sampling dates. No significant difference in concentration of Ca was observed in axial direction of the berry. Potassium, however, showed in green berries higher concentrations ($P < 0.05$) in the area near the stylar scar than near the pedicel, whereas the relationship was reversed after véraison ($P < 0.05$), due to a drastically decelerated incorporation in zone D and a continuing rise in zone A (not shown).

Discussion

VAN DER HEIDE and SCHMITTHENNER (1922) and AMERINE (1956) indicated that there is a gradual increase in titratable acidity from the periphery towards the center of ripe grape berries. The high concentration of malate found in the skin of mature berries (Fig. 2 a), seems to be in contrast to these findings. This discrepancy may be explained by the relatively high levels of potassium (Fig. 5 a) and calcium (Fig. 5 b) in the fruit skin, which are most likely to form salts of malate and tartrate and thus lead to a lower titratable acidity, provided that the released hydrogen ions are either buffered by the cell juice itself or transported to neighbouring cells in an exchange with imported metal cations (BOULTON 1980). STEFFAN and RAPP (1979) showed that peripheral malate can more readily be respired than malate from the berry center. In view of this result, the ability of young grapes to form a gradient, increasing in concentration from the skin towards the seeds, is not surprising. During the ripening process both malic acid (STEFFAN and RAPP 1979) and malic enzyme (POSSNER *et al.* 1983) migrate from the fruit core towards the periphery with the latter even reaching its highest concentration in this part of the fruit (unpublished results). Thus, the metabolic intensity at the fruit periphery presumably determines the malate degradation rate of the whole berry. Owing to the steady supply from the berry interior, the malate concentration in the skin diminishes much slower than in the fruit core (Fig. 2 a). The migrational behaviour of tartaric acid, on the other hand, was characterized by an opposite tendency (Fig. 3). Although it cannot be concluded from the present data whether the decreasing ratio I/IV was due to a direct transport of tartrate from the periphery towards the center or an increasing tartrate import via the central vascular bundles in combination with a decelerated synthesis at the berry periphery, the physiological significance of these shifts to maintain the osmotic concentration is evident.

The tendency of grapes to form an axial organic acid gradient, decreasing in concentration from the pedicelular end to the stylar end, may either reflect differences in the metabolic intensity within the berry, or more likely simply be referred to the natural polarity of the fruit. In contrast to substances such as sugars, which are integrated into the fruit metabolism as soon as they enter the berry, there is no obvious reason for ions, such as potassium or calcium, to stop their migration at the stem end of the fruit. Correspondingly, tartrate and malate, which both can be formed as products of the carbohydrate metabolism (RUFFNER 1982 a, b), reach higher concentrations at the stem end of the berry than at the stylar end during the entire period of berry ontogeny. In the case of tartrate, however, not only higher values but also a steeper gradient were

found (Table), which is probably due to its negligible reintegration into the fruit metabolism (RUFFNER 1982 a). The slightly higher sugar concentration at the stylar end of berries is consistent with the results found in citrus fruit (TING 1969). Accumulation here is probably due to a damming up of surplus carbohydrates at this final point of assimilate transport, and is similar to the behaviour of potassium, which accumulates at the stylar end of green berries. With progressing maturity, however, the pedicelular end of the fruit is filled up, reaching even higher values than the tissue near the stylar scar.

In contrast to K, which is present in relatively high concentrations in all parts of berries (Fig. 5 a), Ca apparently is mainly confined to the tissue with a relatively large number of cells and, correspondingly, also with a high proportion of cell walls, i.e. the inner- and the outermost part of the berry (Fig. 5 b; SWIFT *et al.* 1973). In combination with the fact that the rise in Ca content stops at about the same time as cell division within the pericarp stops, i.e. about 30 d after anthesis (HARRIS *et al.* 1968), this result is in agreement with the well known participation of Ca in cell division (SALISBURY and ROSS 1978) and in the construction of cell walls (KNEE and BARTLEY 1981). On the other hand, the permanent rise in the concentration of K during berry development is most likely to produce high osmotic values (PIERCE and HIGINBOTHAM 1970), especially towards the end of the fruit ontogeny, when the volume increase of the pericarp cells stops (HARRIS *et al.* 1968) — and, consequently, raises the tendency of cells to expand. Based on the almost contrary effects of K and Ca on cell wall stability, the rise in the K/Ca ratio during berry ontogeny (Fig. 6) could contribute to an increase of the cell permeability at an advanced stage of ripening (DVORAK and CERNOHORSKA 1972; SACHER 1973). Interestingly, the breakdown of malic acid started at about the same time as the K/Ca ratio reached its first plateau and stopped as soon as the quotient attained the second plateau. One possible explanation for this is that, when the first threshold value is exceeded, it stimulates the efflux of vacuolar malate into the cytoplasm (VICKERY and BRUINSMA 1973), where malate causes both its decelerated synthesis (LAKSO and KLIEWER 1975) and its accelerated breakdown (POSSNER *et al.* 1981), but still in a well organized environment (RUFFNER 1982 b). When the second K/Ca threshold value is reached, this could trigger the dissolution of the middle lamella and the disintegration of fibrillar material throughout the cell wall, as observed in apples and pears (BEN-ARIE and KISLEV 1979), thus leading to a beginning of senescence. The sudden decrease in malic enzyme activity at the end of the ripening process (RUFFNER *et al.* 1984) favors the latter assumption.

Compared to the previously discussed compounds, glucose and fructose were approximately evenly distributed throughout the berry (Fig. 4). However, before the onset of ripening, the skin and fruit core zones are characterized by significantly higher concentrations than the tissue in between. These results correlate well with the finding, that ^{14}C-labelled photosynthate enters the berry predominantly via the peripheral and central vascular systems (KRIEDEMANN 1969). After a comparison between the quotients I/IV for glucose and fructose on one hand and potassium and calcium on the other, it can be assumed that the sugar contents of the different areas in green berries mainly reflect the distribution pattern of imported sugar and not *in situ* photosynthetically produced carbohydrates, which are most likely to be completely integrated into the fruit metabolism (KOCH and ALLEWELDT 1978). The high tartrate values at the berry periphery (Fig. 2 b; HALE 1977) may indicate the predominance of photosynthetic activity in the outer parts of the fruit. At véraison, the sugar content in the pulp increased without any apparent lag phase, in agreement with findings of COOMBE and PHILLIPS (1982). At least in the case of glucose, however, the rise in concentration in the skin starts much slower and, consequently, the values are lower during the entire

post-véraisonal phase. In this context it must be born in mind that the ability of grapes to metabolize exogenous sucrose is not confined to the time before véraison (POSSNER *et al.* 1983). Thus, the relatively low sugar concentrations in the skin of ripening berries are probably due to a higher respiration in this part of the fruit, similar to the behaviour of malic acid. The lack of a slow beginning in the accumulation of sugars following véraison suggests that the incorporation of carbohydrates into the ripening fruit displays the end of the process, that had already started before véraison, with the basic difference, however, that green berries need virtually all the imported sugar for the production of energy and for anabolic reactions, such as cell wall synthesis, whereas in ripening berries the hexose surplus is transported into the vacuoles and the respiration of stored malate can help to meet the energy demands (RUFFNER 1982 b). To estimate the maximal carbohydrate incorporation until véraison, the glucose and fructose accumulation curves were extended back to the date of anthesis. In this way, for each hexose a value of 0.5 mmol/berry was obtained, which theoretically could lead to the production of up to 1.5 mmol malic acid. However, the amount of malate stored at véraison was only 0.1 mmol/berry or 7 % of the maximal possible yield. Assuming that about 50 % of exogenous sucrose is respired (POSSNER *et al.* 1983), only 3.5 % of the imported C-atoms are stored as malic acid. Similar results can be obtained by balancing the sugar incorporation r a t e s (10 µmol/berry · d for both glucose and fructose) with the malate accumulation rate (2 µmol/berry · d).

Summary

Grape berries (*Vitis vinifera* cv. Chardonnay) were divided into four concentric zones in order to follow the developmental changes in the concentrations of malate, tartrate, glucose, fructose, potassium and calcium within the skin and the fruit flesh. Green berries showed a definite malate gradient, increasing in concentration from the skin towards the seeds; tartaric acid, on the other hand, was highest in concentration at the periphery and lowest in the berry center. With progressing maturity, the ratio between the tartrate concentration in the skin and the corresponding values in the berry core decreased, whereas the reverse was true for malate. In the ripe berry an acid gradient could also be found in the axial direction, decreasing from the pedicel towards the stylar scar. Before the onset of ripening, the highest glucose and fructose concentrations were observed in the skin and the berry center. The accumulation of these sugars in the pulp began without any apparent lag phase at a rate of about 2 mg/berry · d. After véraison, the highest concentrations were found in the fruit core and the tissue below the peripheral vascular bundles. Both potassium and calcium were mainly localized near the peripheral and central vascular bundles. The potassium content increased during the entire period of berry development at a constant rate of 0.04 mg/berry · d, whereas calcium accumulation stopped about 30 d after anthesis. At this time, the calcium content was approximately 0.1 mg/berry.

Acknowledgements

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