STUDIES ON THE COMPOSITION OF PULP AND SKIN OF RIPENING GRAPE BERRIES

by

Patrick G. Iland. B. App. Sc. (Q.I.T.)

Roseworthy Agricultural College
Roseworthy
South Australia

Thesis submitted for the Degree of
Master of Agricultural Science

Department of Plant Physiology
Waite Agricultural Research Institute
The University of Adelaide
South Australia

March 1984

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to JUDITH

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SUMMARY

Changes occurring in the content of some organic acids, monovalent cations, total anthocyanins and total phenols in the pulp and skin of ripening 'Shiraz' grapes were studied. During berry ripening, amounts of malate in the pulp declined, sometimes to 1/5 of the value at veraison, while in the skin malate levels doubled during the same period. Levels of potassium increased significantly in both pulp and skin and during the period from veraison to ripeness the potassium content of whole berries increased 3 to 4 fold. The total amount of tartrate per berry remained relatively constant during berry ripening; however differential extraction of berry pulp with 80% ethanol showed that the free acid form (H_2T) of tartrate was progressively converted to salt forms (HT^-, $T^=$). Malate existed entirely as the free acid (H_2M) at all stages of berry ripening.

Effects of canopy structure and vine water status on the amounts of the above components in the pulp and skin of ripening 'Shiraz' grapes were investigated. Ripe fruit from shaded canopy environments had higher potassium, malate and pH values in the berry pulp and decreased levels of total anthocyanins in the berry skin. Shaded conditions induced high potassium in berries at veraison and this correlated positively with potassium levels in ripe berries. The interrelationship between improved vine water status and berry composition appeared dependent on the extent to which the applied treatment modified the canopy structure.

Compartmentation and properties of cell membranes in relation to the above compounds in berry cells during ripening were investigated using efflux techniques. As ripening progressed, the speed with which tartrate, malate and potassium leached out of pulp tissue increased, indicating an increased membrane permeability with berry ripening. Malate leached out faster than the other compounds. It is postulated that malate was contained in cells separate to those storing tartrate and potassium.

Potassium, total anthocyanins and total phenols were the major components extracted from the skins of 'Shiraz' and 'Cabernet Sauvignon' grapes during vinification. Higher amounts of extractable potassium were associated with initially natural low pH and low potassium levels in the juice. The rise in pH associated with the fermentation of black grapes was greatest when natural juice pH was low.

When grape berries are crushed varying percentages of compounds originally in the pulp are found dissolved in the resultant juice - 100% of malate, 66% of potassium and 55% of tartrate. Thus grape juice or must samples represent solutions in which varying amounts of H_2T, HT^-, $T^=$, H_2M, K^+ and Na^+ have been dissolved. Higher juice pH values would be associated with higher amounts of dissolved HT^- and $T^=$ which corresponds also to higher potassium concentration in the juice. Increased potassium uptake in the berry pulp during berry ripening indirectly lowered the titratable acidity and raised the pH of the juice extracted from the ripe berry, suggesting that viticultural practices should aim at limiting the uptake of this cation during berry ripening.

STATEMENT

I hereby declare that the thesis here presented is my own work, that it contains no material previously published, except where due reference is made in the text, and that no part of it has been submitted for any other degree.

I consent to this thesis being made available for photocopying and loan if accepted for the award of M. Ag. Sc.

Patrick G. Iland

PREFACE

This thesis explores aspects of red wine grape composition and aims at improving knowledge at the interface of viticulture and oenology.

I am very grateful to my supervisor, Dr. Bryan Coombe, who through his scientific thought and persistence to detail has taught me the reality and rewards of scientific research.

I am also indebted to my other supervisor, Dr. Chris Somers, whose expertise in the area of grape and wine phenolics has helped immensely in this part of my thesis.

I am grateful to Roseworthy Agricultural College, The Waite Agricultural Research Institute and The Australian Wine Research Institute, Penfolds/Kaiser Stuhl Wines Pty. Ltd., Primo Estate Winery and Rothbury Estate Winery for use of either facilities or vineyards during the various experiments.

Many people have helped me during the course of this thesis. A special thanks to Dick Smart, Dave Bruer and Noel Richardson, who urged and supported the commencement and continuity of this work. Chris Brien helped with statistical advice and Eleanor Berridge with typing of the graphs. Their assistance is appreciated. The help of Paul Monk in loaning his equipment for the analysis of the organic acids, and for his advice is also appreciated.

My thanks to Mary and Graham Collins for their care and patience in typing this thesis.

My family have supported me throughout this period and this thesis indicates their patience and understanding in contributing to my final goal.

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CHAPTER 1

LITERATURE REVIEW

LITERATURE REVIEW

1.1. THE RIPENING STAGE OF BERRY GROWTH

The growth of the grape berry may be considered to comprise two distinct periods: development and ripening.

From flowering until veraison grape berries remain hard and green and undergo cell division phasing to cell enlargement. At veraison, there is a sudden and dramatic change in the course of development of the berry. The berry softens and increases in volume, metabolic pathways switch from acid synthesis to sugar accumulation, and for black berries the skin becomes coloured (Coombe 1975).

The period from veraison to ripeness is defined as the RIPENING STAGE. Other terms used throughout this text to describe this growth stage will be the ripening period, the sugar accumulation stage, berry or fruit ripeness or ripe berries.

Criteria for assessing ripeness include sugar content, acidity level and flavour intensity; suitable levels of these criteria are dictated by the wine style that will be produced from those berries. Normally, harvest occurs when juice sugar content is greater than 18 °Brix, often 22 - 24.

1.2. PHYSICAL CHARACTERISTICS OF THE PULP AND SKIN OF THE GRAPE

Each grape berry may be divided into skin, enclosed tissue (here termed the pulp) and a variable number of seeds (Fig 1.1). The pulp consists of cells with large vacuoles containing the cell sap which, it is thought, provide the major constituents of the juice when the berry is crushed. Small thick-walled cells characterise skin tissue (Peynaud and Ribéreau-Gayon 1971). The pulp generally constitutes 65-91%, the skin 6-20% and the seeds 2-6% of the total weight of the berry, depending on variety and stage of maturity.

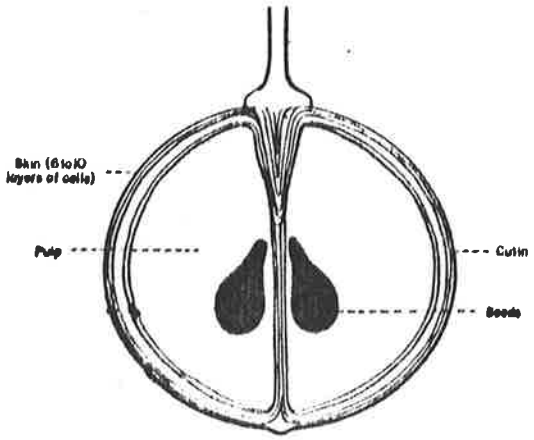

Fig 1.1. Cross-section through a mature berry
(adapted from Peynaud and Ribéreau-Gayon 1971)

During ripening the pulp increases in volume and weight considerably, while the skin tends to stretch without major weight gain (Bourzeix and Coll, 1976). Since the skin contains pigment, aroma and flavour components that may be extracted during vinification, high skin/pulp ratio, a feature of small berries, is considered an important physical characteristic of ripe berries.

1.3. SOME COMPOSITIONAL ASPECTS OF BERRY RIPENING

1.3. a. ORGANIC ACIDS

1.3.a.i. Organic acids - nature and chemistry

The principal organic acids of the grape berry are L(+) tartaric acid and L(-) malic acid (Fig 1.2), constituting 90 percent or more of the total acidity, plus variable but smaller amounts of other acids, notably phosphoric, citric and various acids present as intermediates in metabolic pathways (Winkler et al 1974).

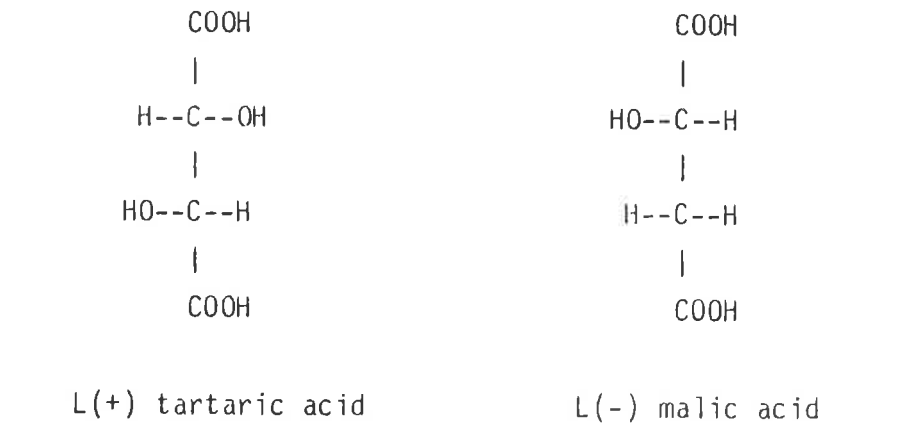

Fig 1.2. Structure of the two major organic acids of the grape berry.

Aqueous solutions of these acids exhibit pH-dependent equilibria (Fig 1.3 and Table 1.1).

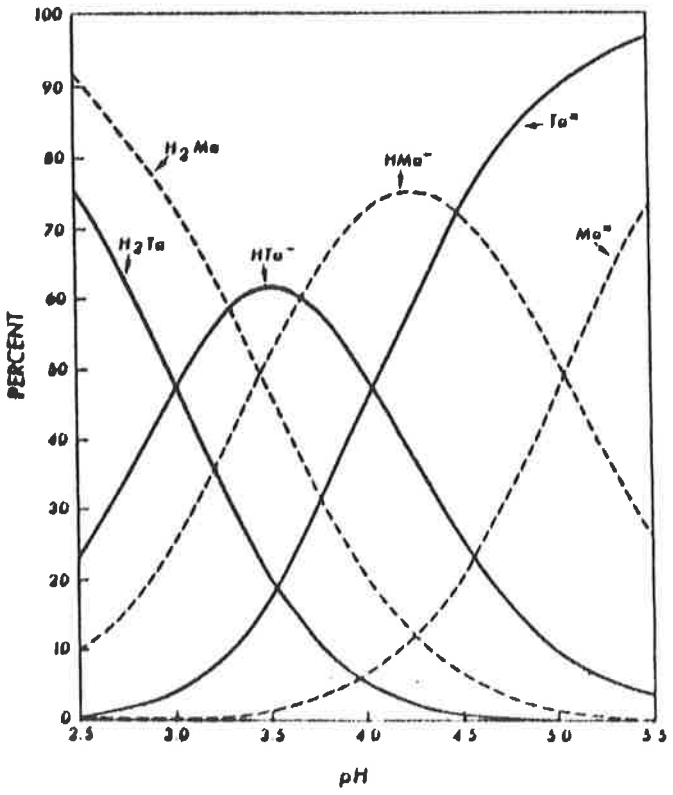

Fig 1.3. Relative concentrations of tartrate and malate ionic species in water at various pH values (from Mattick et al. 1980).

Table 1.1: *Dissociation constants (K) and pK values for tartaric acid and malic acid.

	Step 1		Step 2	
	$H_2T \rightleftharpoons H^+ + HT^-$		$HT^- \rightleftharpoons H^+ + T^=$	
Tartaric acid (H_2T)				
	K_1	1.04×10^{-3}	K_2	4.55×10^{-5}
	pK_1	2.98	pK_2	4.34
	$H_2M \rightleftharpoons H^+ + HM^-$		$HM^- \rightleftharpoons H^+ + M^=$	
Malic acid (H_2M)				
	K_1	3.90×10^{-4}	K_2	7.80×10^{-6}
	pK_1	3.40	pK_2	5.11

* figures from Weast, R. C., 1971-72. (ed). C.R.C. Handbook of Chemistry and Physics

Some physical constants for various forms of these acids are given in Table 1.2. The preferential solubility of the free acids into ethanol provides a method for differential extraction of the free acids and their salts from plant tissue. This partitioning technique has been applied to berries of Vitis labruscana.B. 'Delaware' (Saito and Kasai 1968). Results of this study are discussed later in this review.

The interchangeable use of the terms tartaric acid, tartrate, malic acid and malate has often caused confusion in the literature. For this thesis the terms tartrate and malate refer to the total amount of each compound irrespective of form type, ie, all undissociated (free) tartaric acid plus any of its dissociated or salt forms and similarly for the term malate. Where an individual form is of importance then the species will be clearly identified.

The importance of acidity expressions involving these acids is discussed in Chapter 6.

TABLE 1.2. Some physical constants for various forms of the major organic acids of the grape

	Molecular weight (g)	*Equivalent weight (g)	Solubility		
			cold water	hot water	alcohol
Acids and anions					
[1]Tartaric acid, $C_4H_6O_6$,	150	75	very	very	very
[2]Tartaric acid	-	-	[b]139	-	25
Bitartrate anion, $C_4H_5O_6^-$, (HT^-)	149	149	-	-	-
Tartrate anion, $C_4H_4O_6^=$, ($T^=$)	148	-	-	-	-
[2]Malic acid, $C_4H_6O_5$ (H_2M)	134	68	very	very	very
Bimalate anion, $C_4H_5O_5^-$, (HM^-)	133	133	-	-	-
Malate anion, $C_4H_4O_5^=$ ($M^=$)	132	-			
Potassium salts					
[1]potassium bi tartrate $KHC_4H_4O_6$ (KHT)	188	188	[a]0.37	[a]6.1	insoluble
[2]potassium tartrate $K_2C_4H_4O_5.\frac{1}{2}H_2O$	235	-	[a]150	278	slightly soluble
2 " " "	-	-		[b]278	slightly soluble
[1]potassium malate, $K_2C_4H_4O_5$ (K_2M)	210		soluble	soluble	-
Sodium salts					
[1]sodium bi tartrate, $NaHC_4H_4O_6.H_2O$	190	-	[a]6.7	[a]9.2	-
2 " " "		-	[b]11.0	[b]50	slightly soluble
sodium tartrate, $Na_2C_4H_4O_5.2H_2O$	230		29	66	insoluble
Calcium salts					
[1]calcium tartrate, $CaC_4H_4O_6.4H_2O$	260	-	[a]0.027	[a]0.069	slightly soluble
2 " " "			[b]0.037	[b]0.22	slightly soluble
[1]calcium malate, $CaC_4H_4O_5.2H_2O$	208		[a]0.812	1.22	

[1] Weast, R.C., (1971-72).(ed). C.R.C. Handbook of Chemistry and Physics.

[2] Lange, N.A., (1956). Handbook of Chemistry. 9th edition.

a. solubility in g. per 100 mls. solvent

b. solubility in g. per 100 gm. solvent

* equivalent weight is based on the neutralisation reaction with NaOH.

1.3.a.ii. Organic acids - biochemical pathways

The major pathways for the metabolism of organic acids in plants involve respiratory oxidations and carboxylations or decarboxylations, but specific reactions may be involved for individual acids of certain fruits (Hulme 1970).

All the acids of the Krebs (TCA) cycle are present in grape berries (Kliewer 1964). However for the grape it has been demonstrated that, although the reactions from pyruvate to citric acid are particularly active, the remaining reactions to complete the cycle show little activity (Ribéreau-Gayon 1966). Consequently this pathway does not contribute largely to malate metabolism in the grape berry.

In immature grapes Ribéreau-Gayon (1968) found the main biosynthetic pathway leading to malate formation was carboxylation of pyruvic acid by atmospheric CO_2 and PEP carboxylase, Fig 1.4.

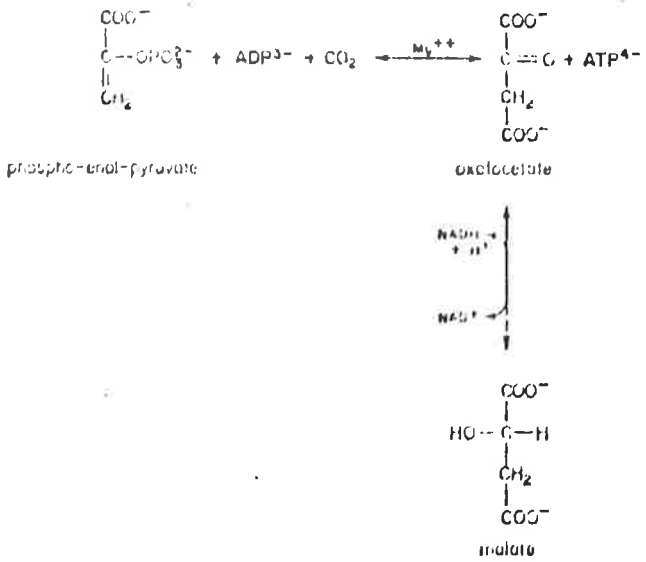

Fig 1.4. Malic acid synthesis through carboxylation of phospho-enol pyruvate (from Peynaud and Ribéreau-Gayon 1971)

Changes in activity of various enzymes associated with berry development have been reported by Hawker (1969) and Ruffner and Kliewer

(1975). A peak in activity at about veraison was observed for both malic enzyme and malic dehydrogenase enzyme (Hawker 1969). The decline in malate at the onset of ripening is thought to be due to the decarboxylation of malate by malic enzyme to pyruvate and subsequent respiration of this pyruvate (Hawker 1969), as shown below.

$$\text{malate} + NAD^+ \xrightleftharpoons{\text{malic enzyme}} \text{pyruvate} + NADH + CO_2$$

In air the equilibrium favours the decarboxylation but possibly in vitro removal of malate to inert pools allows the reverse reaction to occur and malic enzyme may have a dual role involved in both malate accumulation and degradation dependent on stage of berry development (Hawker 1969). The same studies indicated increasing activity of the Kreb's cycle enzyme, malic dehydrogenase, near the end of the ripening period, which is of interest since this, when coupled with carboxylation catalysed by phosphopyruvate carboxylase, provides a mechanism for synthesis of malate. Hawker (1969) ascribed this increase to synthesis of cytoplasmic-located enzyme rather than mitochondrial activity. The PEP carboxylase activity in Sultana fruit (berries had been stored frozen) reached a peak of activity 3 weeks after anthesis and then steadily declined until veraison, but overall remained low throughout berry ripening (Hawker 1969). On the other hand, fresh Pinot Noir fruit demonstrated high PEP carboxylase activity throughout fruit growth and ripening, only exhibiting a temporary sharp decline at veraison (Ruffner and Kliewer 1975). Discrepancies in activity pattern were attributed to: variable methods of enzyme extraction, fresh versus frozen samples and varietal differences. Further experiments are required to clarify this aspect of malate metabolism in grape berries.

The simultaneous degradation of malic acid and the accumulation of sugar during the period from veraison to ripeness have led to suggestions of gluconeogenesis activity within grape berries (Ruffner et al. 1975). Ribéreau-Gayon (1966) has shown that immature grapes readily synthesise carbohydrates from malic acid and proposed the mechanism shown in Fig 1.5.

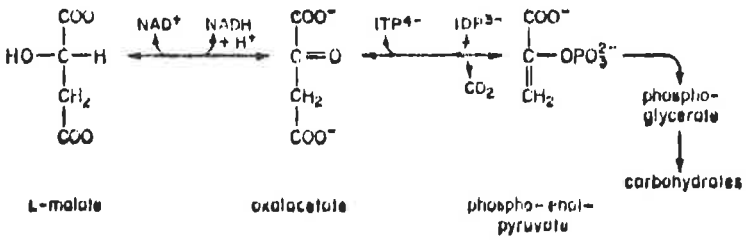

Fig 1.5. Carbohydrate synthesis from L-malic acid (Karlson 1964) (in Peynaud and Ribéreau-Gayon 1971)

P.E.P. carboxykinase is the only enzyme known to be capable of catalysing a freely reversible carboxylation between phospho-enol-pyruvate and oxalacetate and is considered a key enzyme in gluconeogenetic transformation (Ruffner and Kliewer 1975). These authors suggested that during the post-veraison period, the PEP carboxykinase enzyme primarily acts towards sugar synthesis from accumulated acids. However the activity pattern for this enzyme on a weekly basis during berry growth did not show any dramatic change around veraison (Ruffner and Kliewer 1975).

When radioactive malic acid was fed to post-veraison berries 25% of the label was found in sugars, conversion being maximised around veraison (Drawert and Steffan 1966). On the other hand, Hardy (1968) found little evidence for gluconeogenesis in pre-veraison fruit. The extent to which gluconeogenesis contributes to hexose accumulation is difficult to quantify, since both this mechanism and continuous influx of leaf synthesised sugar occur concurrently. Based on the extent of malic acid decrease Ruffner and Hawker (1977) suggested that at best only 5% of the total sugar accumulation could be explained by gluconeogenesis. Thus the principal reactions of malic acid in post-veraison berries are likely to be of a respiratory nature. Rapp et al. (1971) showed that about 60% of radioactively labelled malic acid was consumed in respiration within 15 hours during the preveraison growth period. After veraison more than 90% was respired within this same time period. This high incorporation into respiratory CO_2 indicates the large extent to which malic acid is used as a respiratory substrate in the ripening process. Tartrate on the other hand

contributes only to a small extent in respiratory reactions (Rapp et al. 1971).

It appears that respiration reactions are confined to specific zones of the berry. Steffan and Rapp (1979) found by administering ^{14}C-malic acid separately by either pedicel application or injection into the berry that malate existed in either of two pools, inactive in the interior of the berry or active near the periphery. Ruffner (1982b) suggests that two distinct types of cells are associated with these malic acid pools, those peripherally located, termed "non-storage-type" cells, which in addition to metabolically active compartments contain a reserve of the acid either in a transitory pool or a true storage compartment (Fig 1.6). Specialized cells, more centrally positioned, act as storage sites for sequestration of imported malic acid from the peripheral areas.

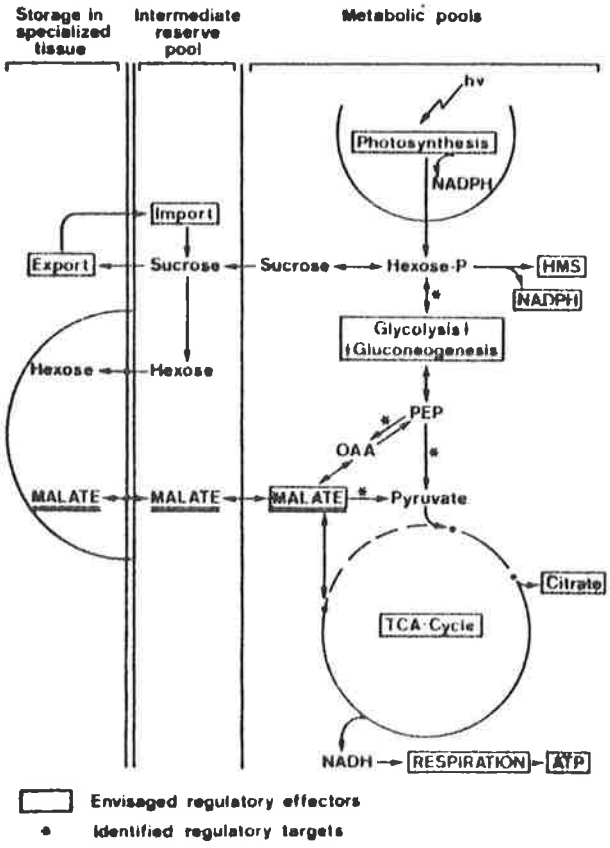

Fig 1.6 Biochemistry and compartmentation of malate/sugar metabolism in the grapevine (from Ruffner 1982b).

At veraison, there is a dramatic switch in metabolism. Demands for cellular energy may now be met by malic acid respiration (Boulton 1980). Owing to an increase in membrane permeability, previously stored acid is radially transported to the berry periphery and dissimilated with berry ripening (Ruffner 1982b). The timing of the process whereby malate respiration is initiated may be crucial in determining final acid level of the ripe fruit (Ruffner et al. 1976).

Various authors have discussed changes in membrane permeability during fruit ripening (Sacher 1973, Vickery and Bruinsma 1973). Reports specific to grapes include those by Hale (1977), Ruffner et al. (1976), Ruffner (1982b) and Steffan and Rapp (1979). Most reports suggest that membrane permeability increases during the ripening phase. Temperature is thought to influence membrane permeability during fruit ripening, but this effect may be modified by potassium levels in grape tissue (Hale 1977). In these studies higher berry potassium levels were associated with higher amounts of malate in the berry at the final stage of ripening; it was suggested that potassium somehow regulated the permeability of membranes associated with malate movement to active metabolic zones.

The grape is one of only a few plant species that accumulates tartaric acid as a major acid component. There is no apparent link between the metabolic pathways of tartrate or malate in the grape and in fact they differ considerably.

Synthesis of tartrate is more active in pre-veraison berries and a link between tartrate synthesis and cell division has been suggested (Hale 1962). Experiments have shown that tartaric acid can be formed in the grape from a number of precursors e.g. glucose (Ribéreau-Gayon 1968), ascorbic acid (Saito and Kasai 1969, Williams et al. 1979), sucrose, gluconate and less so glucuronate and glucurono-lactone (Saito and Kasai 1978). These studies indicate that tartaric acid is formed in a secondary process from the primary carbohydrate products of photosynthesis (Ruffner 1982a). When berries were fed ^{14}C labelled tartaric acid a diurnal pattern for tartrate dissimilation was apparent

during the acid accumulation period, but not during the ripening stage (Takimoto et al. 1976). Nevertheless 31% of tartaric acid was converted to $^{14}CO_2$ during the ripening stage, indicating that tartrate dissmilation occurs during this phase of berry growth. Previous studies based on high RQ values in the ripening grape berry also suggested remetabolism of tartrate, particularly at high temperatures (Peynaud 1958).

Despite the above, it is a fact that tartrate concentrations remain fairly constant during berry ripening (see Section 1.3.a.iv). Ruffner (1982a) questions whether remetabolism of tartrate does occur during this growth period; he argues that high RQ values could also be attributed to fermentation processes occurring within the berry. Conclusive evidence relating to tartrate dissimilation is wanting and the physiological significance of tartrate in grapes remains puzzling (Ruffner 1982a).

1.3.a.iii. Organic acids - translocation patterns

Leaves are capable of synthesizing malic and tartaric acid (Stafford and Loewus 1958) and there is some evidence that both compounds are translocated to the berries (Peynaud and Maurie 1958). Kliewer and Nasser (unpublished data) identified tartaric and malic acids in the phloem exudate of grapevines during the green berry stage of berry development but not during the ripening stage (Winkler et al. 1974). Radioactive malic and tartaric acids have been isolated from Sultanina grape berries after the shoots had been treated with $^{14}CO_2$. Malic acid was formed more rapidly than tartaric acid. The labelling of organic acids in the berry was greatest when $^{14}CO_2$ was supplied to vines bearing immature fruit compared with nearly ripe fruit (Kliewer and Schultz 1964). However in this type of experiments it is unclear whether the compounds becoming labelled in the berry are formed in the leaves and then translocated to the fruit, or if they are formed in the berry from translocated radioactive sucrose (Hardy 1968).

Experiments by Hardy (1968), who administered radioactive compounds to individual excised berries, suggest that most of the malate and tartrate in immature berries is formed from glucose and fructose. It was proposed that the changes in levels of organic acids during ripening were related to changes in the ability of the berry to synthesise them. Hale (1962) exposed leaves to $^{14}CO_2$ and followed the incorporation of radioactivity into the organic acid component of berries. He found that incorporation increased with time, even when the translocation pathway was blocked by girdling. Malate had greater activity than tartrate. The high level of incorporation of tartaric acid in immature berries in the experiments by Hale (1962) relative to that found in mature leaves by Stafford and Loewus (1958) led Hale to postulate that tartaric acid synthesis in the grape may be related to some aspect of growth metabolism. More recent studies showed that no transfer of ^{14}C label occured in berries after radioactive tartrate had been injected into the major veins of a leaf (Ruffner 1982a). The above studies indicate that the berry itself is the important site of synthesis for the major organic acids of the grape.

1.3.a.iv. Organic acids-changes during berry ripening

In the early stages of berry growth tartrate and malate present mainly as the free acids, are accumulated in the berry, reaching a maximum at veraison. During ripening the concentration of each acid decreases resulting from dilution due to berry growth and/or catabolism (Winkler et al. 1974). Only malate is reduced on an absolute basis (Hale 1977, Hardie 1981). For 'Muscat Gordo Blanco' berries, Coombe and Phillips (1982) showed that when ripening events were synchronized with berry deformability, the decrease in malate concentration began one day later than the sudden and rapid increase in hexose levels. In berries of Muscadine (Vitis rotundifolia) grapes, cv 'Carlos' and 'Noble', malic acid per berry showed a threefold decrease from the immature stage (933 mg/berry) to the mature stage (311 mg/berry), while tartaric acid per berry remained nearly constant at 43 mg/berry (Carroll and Marcy 1982).

During the ripening stage the increase in ratio of acid salts to free acids, malate degradation and dilution by water due to berry growth, are all reflected by increasing juice pH. Data from Rankine et al. (1971) and Smart (1982), indicate that for juice from 'Shiraz' grapes at maturity, pH values may range from pH 3.4 to 3.8; and under certain conditions, pH values as high as pH 4.2 have been observed (the author, unpublished data).

Although the effects of environmental and cultural practices on pH, acids and cations in the grape berry have been frequently investigated, most of these studies are based on analysis of juice extracted from crushed grape samples. Reports in which whole berries (pulp and skin) have been studied include Peynaud and Maurie (1953), Saito and Kasai (1968) and Hale (1977, 1978). Peynaud and Maurie (1953) showed that one characteristic feature of ripening, namely the decline in malate concentration in the pulp is not observed in the skin of the grape. In fact malate levels in the skin may even increase during the period of ripening (Fig 1.7). Skin tartrate levels also showed some increase during berry ripening (Fig 1.7).

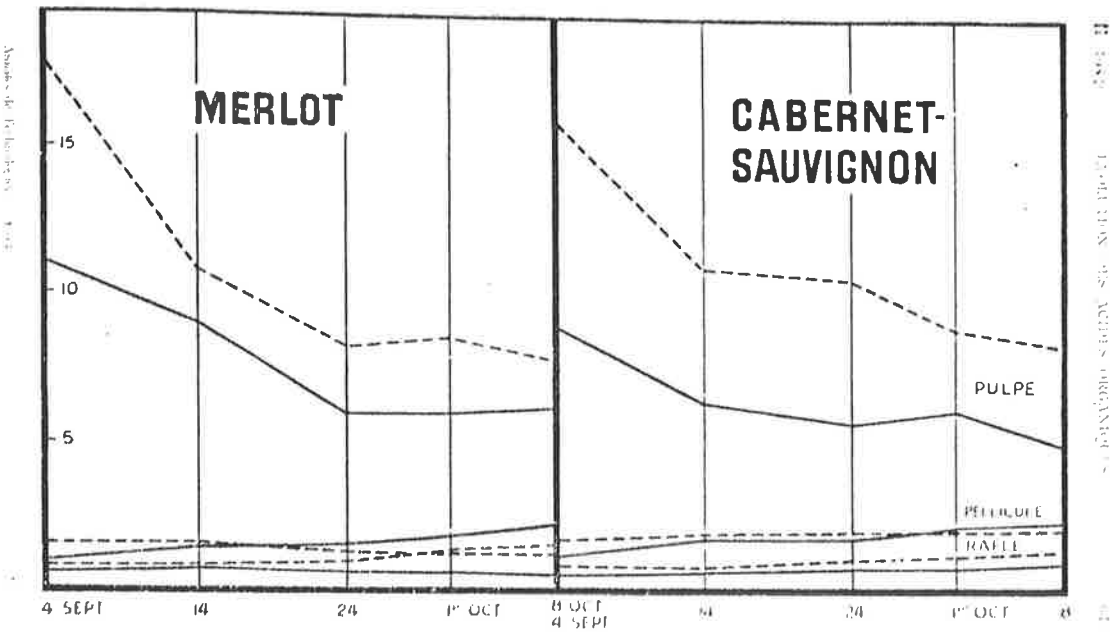

Fig 1.7. Change in concentration of tartrate --- and malate —— in the pulp and skin of berries during ripening. (from Peynaud and Maurie 1953).

1.3.a.v. Organic acids - distribution within the berry

A number of reports indicate that the organic acids are present in both pulp and skin tissue (Table 1.3). The variations in distribution shown in Table 1.3 could be attributed to differences in variety, stage of maturity, climate or cultural practices. It is evident, however, that the contribution by the skin to the acid composition of the berry is not insignificant.

Table 1.3. Distribution of tartrate and malate, between the pulp and skin of ripe grape berries, as reported by various authors.

Reference	Grape Variety	Compound	% of total* Pulp	Skin
Peynaud and Maurie (1953)	Merlot	tartrate	74	26
		malate	88	12
	Cabernet Sauvignon	tartrate	70	30
		malate	81	19
Hale (1977)	Sultana	tartrate	67	23
		malate	80	20

* the total is the sum of the amount of compound present in the pulp plus the amount present in the skin.

Alwood (1914) appears to have pioneered investigation of crystalline deposits in the ripening grape berry. Microscopic examination showed an abundance of minute crystals, varying in shape and size, in both skin tissue and the soft cells just beneath the skin. An observation that the crystals found in the berry tissue did not conform in type to crystals of bitartrate prepared from pure cream of tartar did raise doubts about their origin; but, segmentation of the berry into three zones and subsequent analysis of tartaric acid content of these zones suggested that the crystalline deposits were potassium bitartrate. Scant experimental detail and lack of reasonable chemical rationale for the distinction between free acid and cream of tartar forms does however make these early observations questionable.

The interpretation of intracellular crystals in grapes as potassium bitartrate has been widely accepted (Von der Heide and Schmitthenner 1922, Winkler et al. 1974). Recently Ruffner (1982a), has argued that

although abundant berry potassium content suggests potassium to be the natural counter-cation in the crystalline salt formation, it is not necessarily a true assumption. Ruffner (1982a) has clearly defined the structure of crystalline deposits often seen in the ripe grapes to be exclusively the calcium salts. These calcium salts were sequestered in huge specialized cells, the idioblasts. The relative inertness of tartrate in the ripening grape berry may be due to its conversion to insoluble salts which are scarcely attacked by metabolizing enzymes during fruit development (Saito and Kasai 1968).

Amerine (1956) analysed separate zones of the grape berry and found titratable acidity was least near the skin and greatest near the zone around the seeds. Since salt forms (whether they be KHT, K_2T or CaT) have less available titratable protons than the equivalent amount of undissociated acid, the observed distribution pattern of titratable acidity more likely reflects an increasing gradient of the cations, potassium and calcium across the berry rather than decreasing tartrate content. However malate may also be unevenly distributed within the berry (Steffan and Rapp 1979) and since expression of acids in terms of titratable acidity is influenced oppositely by increasing acid and cation content it is difficult to interpret the trend in an expression that has contributions from a number of components. A more specific study of individual acid and cation content is needed to describe their distribution pattern within the grape berry.

On a cellular level, the acids are likely to be compartmented into either metabolic sites (the cytoplasm) or storage areas (the vacuole). It is possible that further compartmentation occurs within these broad areas. Compartmentation is dependent on transport across the membranes of the cell. Movement across the plasmalemma results in nett gain or loss of component, while transport across the tonoplast represents only a redistribution within the cell. Compartmentalization (availability of substrate) may be more important in regulating malic acid levels during ripening than differences in total enzyme activity (Ruffner 1982b).

The processes occurring in each compartment are not independent, and the metabolism of malate (previously described) demonstrates this. With

tartrate, Ruffner (1982a) emphasises that even if the molecule is stored as a sparingly soluble salt, these salts must still be in equilibrium with undissociated acid in the storage compartment. Remetabolization of one species would lead to replenishment by the other. Mechanisms for maintaining tartrate in its fully protonized stage, i.e. tartaric acid, within the vacuole remain unsolved.

Investigations of the anion content of skin extracts and vacuole lysates from ripe berries (cv 'DeChaunac') indicated that tartrate and malate were major constituents in both types of extracts (Moskowitz and Hrazdina 1981). These authors also reported a method, based on anthocyanin equilibrium, for pH determination in intact skin vacuoles and found an average figure of pH 2.7. The concentrations of vacuolar constituents (tartrate 1.09M, malate, 0.21M and potassium 2.73M), led these authors to suggest that this low vacuolar sap pH, may be attributable to the high concentrations of tartrate present partially as the free acid. Thus, as suggested by Ruffner (1982a), in certain cases free tartaric acid can effectively be stored in the vacuole.

1.3.b. MONOVALENT CATIONS

1.3.b.i. Monovalent cations - nature and chemistry

Potassium ranks in importance with nitrogen and phosphorus as a necessary mineral nutrient for plant growth. In plants potassium has four physiological-biochemical roles: enzyme activation, membrane transport processes, anion neutralization and osmotic potential regulation (Clarkson and Hanson 1980). Sodium may substitute for potassium for some functions e.g. stomatal opening and turgor control but although a physiological role of sodium is apparent it does not appear to be an obligatory one (Clarkson and Hanson 1980).

Grape berries, like most plant tissues, contain much more potassium than sodium: 'Shiraz' grapes grown in various Australian viticultural areas, had juice potassium concentrations that were about 10 times those of sodium when both cations were expressed as meq L^{-1} (Rankine *et al.* 1971). Mature 'Zinfandel' grapes had concentrations of potassium about 30 times larger than those of sodium (Hardie 1981).

1.3.b.ii. Monovalent cations - translocation patterns

Absorption of cations occurs through the root system and initial movement in the xylem is controlled by both vegetative transpiration rate, and the availability of moisture to the root zone. However, once absorbed potassium and sodium are readily mobile in both the phloem and xylem streams (Freeman 1982). He also suggests that xylem flow would most likely be a minor source of potassium for grape berries, since they lack functional stomata and transpiration is negligible.

Redistribution of potassium from the leaves and shoots is directed towards the berry during ripening (Downton 1977, Freeman 1982, Smart *et al.* 1981 and Smart *et al.* 1984), but although potassium may be distributed from older plant parts to new growth sites, any major nett increase in plant size must be associated also with further acquisition of the mineral from the root zone (Clarkson and Harrison 1980). From studies of the effects of irrigation on mineral uptake of 'Zinfandel' grapes

Hardie (1981) suggested a dual contribution from foliage-derived potassium and also a direct effect of mineral transport from the roots; in any one situation, the level to which potassium is accumulated in the fruit will be dependent on the extent of these two concurrent translocatory pathways.

An involvement of the enzyme system potassium/hydrogen adenosine triphosphatase (E.C. 3.6.1.3) in grape vines has been proposed by Boulton (1980c). He suggested that uptake of monovalent metal cations from the soil is achieved by ATPase activity in the roots of grapevines and that the presence of this ATPase in berries enables cation transport across the plasmalemma in exchange for internal protons derived from the organic acids.

1.3.b.iii Monovalent cations - changes during berry ripening

Potassium and sodium are accumulated in the berry during its development (Downton 1977). Sodium levels increased in all tissues examined (petioles, laminae and berries), while potassium concentration decreased in petioles and laminae but increased in the developing fruit. Similar studies (Freeman 1982) with potted 'Cabernet Sauvignon' vines showed a nett flux of K^+ into the berries during ripening; laminae, petioles and shoots contributed about 44% of the total K^+ present in the clusters. Freeman (1982) and Freeman et al.(1983) also showed that the decrease in lamina K^+ concentration (expressed as percent dry weight) with increase in leaf age was partially due to an increase in lamina dry weight density. He recommends that expression of K^+ concentration on a unit area basis gives a better reflection of K^+ fluxes within the vine.

There is evidence that final potassium levels reached at harvest appear related to conditions set earlier in berry development since in Downton's (1977) studies vines on rootstock contained both higher petiole, laminae and berry potassium both at veraison and at maturity. It was suggested that potassium uptake is somehow controlled by potassium status of the vine. In another study (Morris et al. 1982), where potassium status of the vine was manipulated by varying soil

potassium levels, a significant correlation was found between petiole K^+ concentration (%dry wt) and the K^+ content of fresh berry juice.

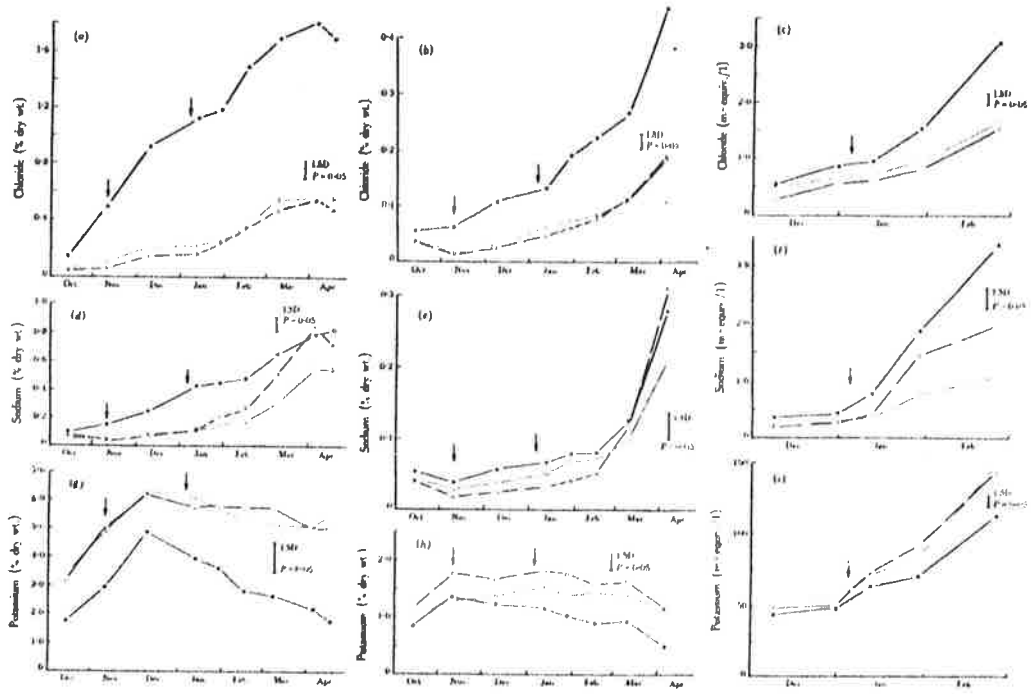

Fig 1.8 The chloride a,b,c; sodium d,e,f; and potassium g,h,i content of petioles, laminae and berries from 'Shiraz' scion growing on its own roots (o-o), on Harmony rootstock (o--o) and on Salt Creek rootstock (o...o). Vertical bars represent least significant differences between sample means at the P = 0.05 level. The onset of flowering and veraison is indicated by arrows. (a) (d) (g) Petioles. (b) (e) (h) Laminae. (c) (f) (i) Berries.

(From Downton, 1977).

Even though potassium status at veraison may somehow influence final potassium concentration of the berry, the ripening period initiated at veraison is still a time of significant potassium uptake. For the grape berry, potassium and sodium levels increase on both a concentration and

an absolute (per berry) basis throughout the ripening period (Hale, 1977, Peynaud and Ribéreau-Gayon 1971). The rate of K^+ accumulation in grapes with increasing level of soluble solids followed a sigmoidal pattern, showing rapid increase at above 17° Brix; above this sugar level [K^+] in grape berries was associated with increase in juice pH (Freeman and Kliewer 1983).

1.3.b.iv. Monovalent cations - distribution within the berry

The distribution of accumulated cations within the berry (Table 1.4) indicates that a considerable portion of the K^+ and Na^+ is localised in skin tissue. It appears that the rate of movement into skin and pulp is different; for the variety Merlot potassium increased 2 or 3 times in the skin but only 1.2 to 1.9 times in the pulp (Peynaud and Maurie 1953). Moskowitz and Hradzina (1981) have shown that potassium is a major component in the skin of 'De Chaunac' grapes. Large, possibly overestimated, concentrations were found in skin vacuoles (2.73M).

Table 1.4. Distribution of potassium and sodium between pulp and skin tissue of grape berries, as reported by various authors.

Reference	Grape Variety	Compound	% of total* pulp	skin
Peynaud and Maurie (1953)	'Merlot'	K^{+b}	78	22
	'Cabarnet Sauvignon'	K^{+b}	62	38
Hale (1979)	'Sultana'	K^+	63	27
Stella et al. (1978)		K^+	67	33
		Na^+	88	22

* the total is the sum of the amount of compound present in the pulp plus the amount present in the skin
b deduced from alkalinity of ash data.

1.3.c. TOTAL ANTHOCYANIN AND "TOTAL PHENOL" CONTENT

Phenolic compounds of black grapes include the anthocyanin pigments and colourless phenolic material, including catechins, proanthocyanidins, and polymeric material which is often considered synonymous with tannins. Investigations by Ribéreau-Gayon (1974) have identified five anthocyanidins in the grape: delphinidin, petunidin, malvidin, cyanidin and peonidin. V. vinifera species are characterised by the 3-monoglucosides of four or five of the above anthocyanidins. Acylated anthocyanins may also be formed due to esterification of the -OH group in the sixth position of the glucose molecule by caffeic or acetic acid. Examples of the above compounds are shown in Fig. 1.9.

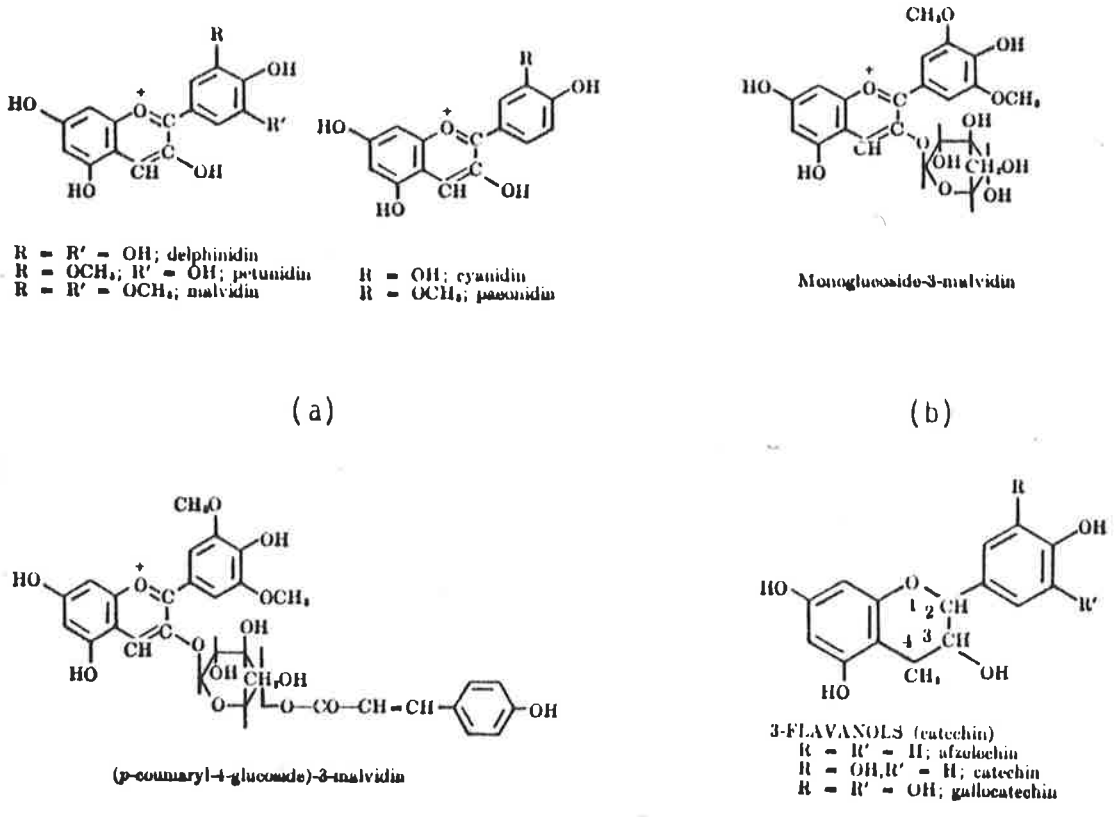

Fig 1.9. Examples of (a) The anthocyanidins (b) monoglucoside (c) acylated anthocyanin and (d) the monomers contributing to tannin formation.

The pigments from skin of 'Shiraz' grapes grown in the Barossa Valley of South Australia, were identified as the 3-monoglucosides of delphinidin, petunidin, malvidin and peonidin (cyanidin was not found) together with a complex mixture of probably twelve acylated derivatives involving both p-coumaric and caffeic acid (Somers 1966). Malvidin-3-glucoside was responsible for approximately 70% of the total colour.

Application of gel column chromatography with spectrophotometric detection (Abs_{520}) enabled Somers (1968, 1976) to obtain profiles of grape skin extracts from which measures of tannin, acylated anthocyanins and anthocyanins could be calculated. Obvious differences in tannin content and ratio of acylated to non-acylated anthocyanins were apparent between varieties and for a individual variety at various stages of ripeness (Fig 1.10).

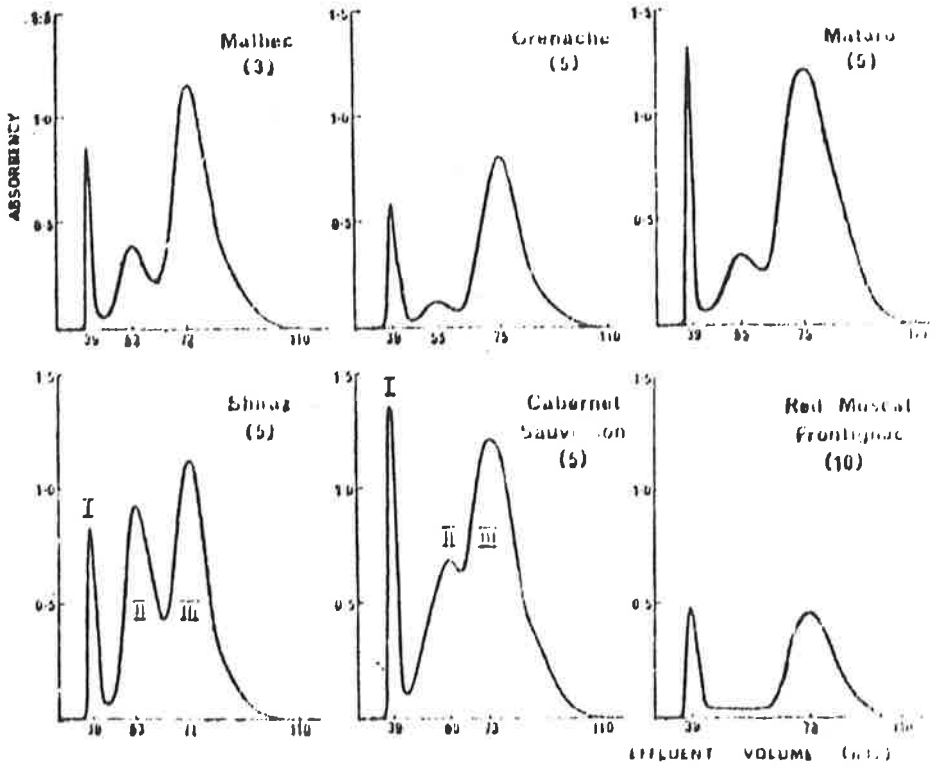

Fig. 1.10. Pigment profiles of wine grape varieties sampled from the Barossa Valley, 1967. (fraction I = tannin, fraction II = acylated anthocyanins and fraction III = parent anthocyanins). (from Somers, T.C., 1968).

This apparent tannin contribution to pigment content was later shown (Somers 1971) to be an artifact of the extraction procedure since the use of acidified extracting solvents may induce polymeric pigment formation from catechins, and pro-cyanidins interacting with the anthocyanins *in vitro* during extraction. When grape skin was extracted with 50% aqueous methanol, and subsequently chromatographed, the immobile red brown tannin spot, normally seen when extraction was performed with acidified solvents, was absent. This suggests that *in vivo* the pigmentation in the skin of black grapes is largely due to anthocyanin content, with little contribution from polymeric material (Somers 1971).

A likely explanation of colour type in skin tissue involves co-pigmentation of anthocyanins and other flavanoid material which enhances and stabilises pigments. So called black grapes then do not appear red (the characteristic colour of the major anthocyanin malvidin-3-glucoside) but take on a purple hue due to *in vivo* association of the anthocyanins and tannins present in the skin. However this explanation is disputed by Hrazdina and Moskowitz (1982) who attribute the bluish colour of dark grapes to high concentrations of anthocyanins in the vacuoles, the light scattering effects of the vacuoles, and the presence of chlorophyll in the subepidermal layers. Even though cation concentration in skin vacuole cells is large, it is thought that the cations K^+, Na^+, Ca^{++} and Mg^{++} do not form complexes with anthocyanins and have no influence on colour expression in skin tissue (Hrazdina and Moskowitz 1982).

The total phenol content includes all anthocyanin and tannin like material. The catechin and pro-cyanidin contents of grape skin are relatively low, these compounds being concentrated in the seeds.

Tannins are water-soluble phenolic compounds having molecular weights between 500 and 3000 and apart from typical reactions characterising the phenol moiety, have special properties such as the ability to precipitate gelatin and other proteins (Ribéreau-Gayon 1974). Tannins are formed by the polymerization of elementary phenolic compounds; those found in grapes are condensed tannins.

Depending on ripening conditions different condensed phenolic compounds are synthesised in the grape berry. The nature of these compounds is more important than their concentration, since taste concepts in the resultant wine may be influenced by tannin type more significantly than their amounts (Ribéreau-Gayon and Glories 1980).

To characterise tannins in the grape identification of both total tannin and degree of polymerisation are necessary. The latter however is difficult to evaluate as isolated fractions are molecular mixtures, being diverse in both structural type and molecular dimensions; in most studies only the total tannin has been measured.

Methods of extraction and analysis of phenolic compounds are summarised by both Ribéreau-Gayon (1982) and Singleton and Esau (1969). Extraction of phenolic compounds from grape skin is possible with aqueous, alcoholic (ethanol, methanol and isoamyl alcohol) or acidic (e.g. 0.1N HCl) solvent; extraction conditions (time, temperature and selectivity) varying largely between solvents. A major factor in extraction appears to be disruption or killing of the skin cells to allow diffusion of compounds from the vacuole, and thus techniques involving heating, autoclaving, ultrasonics and use of solvents are applicable. In fermentation on skins, extraction of phenolic material occurs naturally as ethanol concentration increases during the course of the ferment.

For this thesis, total anthocyanin and "total phenolic" content in the skin of grape samples, were determined by extraction with methanolic 0.1% HCl solvent, followed by appropriate dilution with 1M HCl and measurement of absorbance at 520nm and 280nm respectively (Somers and Evans 1977).

Anthocyanin values were based on $E^{1\%}_{1cm} = 500$ for the mixture of acylated and non-acylated anthocyanins (Somers and Evans 1974). The contribution of polymeric pigments and non-anthocyanin pigments to the reading of A_{520} is negligible (Pirie and Mullins 1976). A_{280} values were converted to concentration units, determined from a standard curve of gallic acid in 1M HCl solution; total phenol then being

expressed as mg of gallic acid equivalents. Although the above procedure provides only a broad distinction between phenolic type, it is an appropriate method for quantitatively assessing and comparing colour and phenol response in grapes due to various viticultural treatments.

Anthocyanin and phenolic content of the berry show rapid increase at veraison, but then decrease at the end of berry ripening, indicating that no advantage in terms of colour development is achieved by delaying harvest (Fig 1.11) (Somers 1976). The tannin content follows a similar pattern to that of the anthocyanins but at the onset of ripening when anthocyanin level is low, the tannin content can be relatively high (Ribéreau-Gayon. P. 1980, 1982).

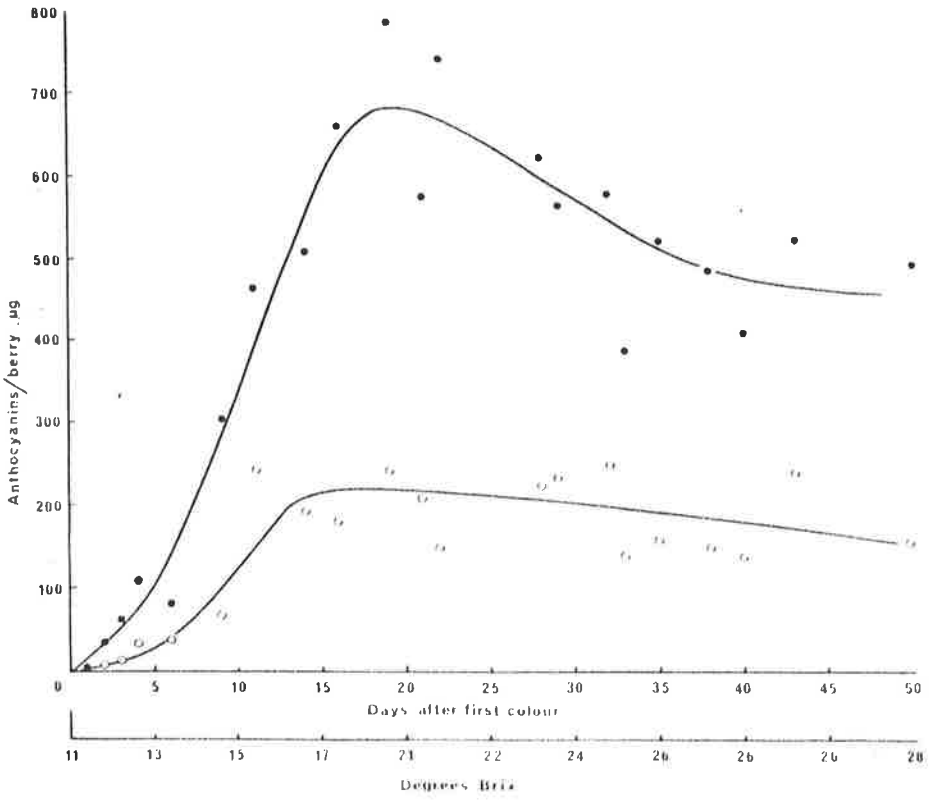

FIG 1.11. Change of anthocyanin contents of 'Shiraz' berries during ripening (from Somers 1976).

Levels of total anthocyanins and total phenols may show considerable variation depending on variety, location and environmental conditions.

1. 4. SOME FACTORS AFFECTING BERRY COMPOSITION

1.4.a. BERRY COMPOSITION - GENERAL CONSIDERATIONS

In this section the effect of the factors, temperature, light, and vine water status on the chemical composition of grapes, is discussed. The components of interest are the organic acids, cations and phenolic content of the grape berry.

For a given vine in a set locality and soil type, the chemical composition of the berry is largely determined by seasonal variation in climate, maturity level and cultural practices. Many investigations have demonstrated the effect of climatic and seasonal conditions on the development and composition of wine grapes both overseas (Amerine and Winkler 1944, Amerine 1956, Caldwell 1925, Du Plessis 1968, and Peynaud and Maurie 1956), and locally (Cirami 1973, Heinze 1977, and Rankine et al. 1971). Despite the vast number of investigations, few reports provide information on levels of tartrate, malate and cations in the one study. Compositional aspects relating to titratable acidity, pH and malic acid in juice samples have received most attention. Investigations on factors influencing phenolic or anthocyanin content have often only analysed for these specific components without correlating them with other components.

It is experimentally difficult to analyse the separate behaviour of each factor. Application of any one treatment may result in variation of others, e.g. irrigation may induce foliage growth which by limiting direct solar radiation will significantly affect not only light quality and quantity within the canopy but also berry temperature.

The climate in close proximity to the berry (microclimate) is thus an important factor in setting conditions that influence berry composition (Smart 1982). Individual berries at different positions on the grape vine do not experience the same temperature conditions (Smart and Sinclair 1976, Smart et al. 1977). Irrespective of position on the vine, berries experience temperature conditions greater than ambient during each day; berries exposed to sunlight will be hotter than shaded

berries, and the differential may reach extremes greater than 10°C (Millar 1972, Smart and Sinclair 1976). At night time, exposed berries are cooled by longwave radiation emission and may be 4°C cooler than air temperature, while protected berries will be close to air temperature (Smart et al. 1977). The temperature of the berry is not solely determined by air temperature but depends on compactness of the cluster, solar radiation and wind velocity, all of which not only establish the absolute amount of heat in the berry but also create a temperature gradient within individual berries.

Shading not only reduces solar radiation but alters the ratio of short:long wave radiation, lowers foliage- and cluster-temperature, lessens moisture stress, reduces wind velocity, and affects humidity, CO_2 availability and overall photosynthesis and metabolic activity of the whole plant (Radler 1965, Schultz and Lider 1964, Smart 1982, and VanZyl and Van Huyssteen 1980). Vine leaves are the major attenuator of solar radiation, strongly absorbing wavelengths in the region 400-700nm, but beyond 700nm little absorbance occurs. In dense 'Concord' canopies the attenuation of PAR (photosynthetically acitve radiation) was about 100:1, and the associated decrease in ratio of 660nm/730nm radiation was about 10:1, hence interior leaves and berries are exposed to radiation that is low in PAR but enriched in the far red region (Smart 1982).

Variations in levels of Red:Far Red radiation could influence phytochrome induced reactions. The backward reaction (Fig 1.12) which results in the destruction of the active form of phytochrome, is accelerated by excitation with far red light , hence shaded plant tissue may have limiting amounts of these specific light-controlled reactions. P_{FR} may also reverse thermally to P_R and high temperature conditions would also be expected to decrease metabolic reactions mediated by phytochrome.

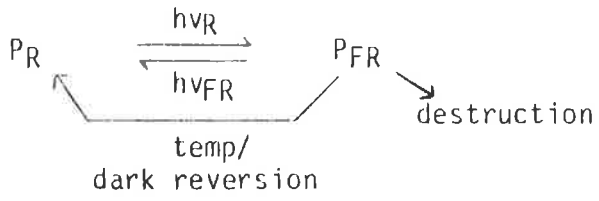

Fig 1.12. Scheme for inter-conversion of phytochrome forms (from Mitrakos and Shropshire 1972).

An index of shading in grape vine canopies can be simply obtained by the use of a point quadrat technique (Fig 1.13), which essentailly gives the spatial distribution of foliage and clusters in relation to direct solar radiation interception (Smart 1982).

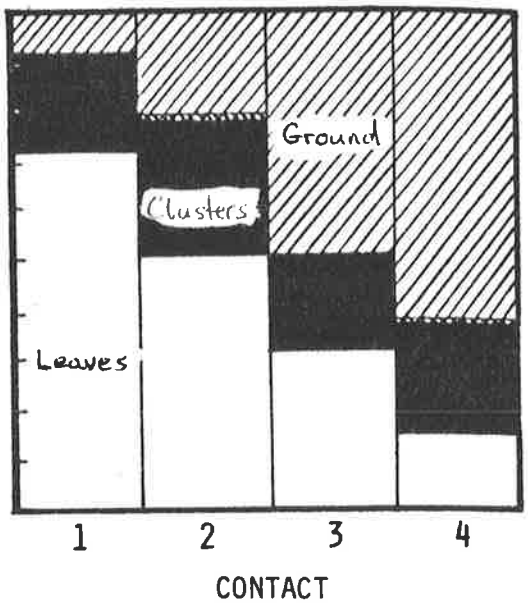

Fig 1.13 An example of results from the point quadrat technique (from Smart 1982).

Smart (1982) further emphasises that results from experiments obtained by manipulation of leaf or berry environment (by either artificial shading or using growth cabinets) may not be extrapolated to conditions naturally present in vine canopies. This is particularly so for naturally shaded vine parts where phytochrome-mediated reactions are modified.

Environmental conditions and vine water status may also influence berry size either positively or negatively and this may complicate comparative compositional data if berry size is not uniform between treatments.

1.4.b. BERRY COMPOSITION - EFFECT OF TEMPERATURE AND LIGHT

In grapes grown in cool regions or in cool seasons malic acid generally comprises a greater percentage of total acidity than it does in the same variety grown in warmer regions or seasons (Kliewer 1965). Rankine et al. (1971) indicated that vintages can be classified into 'high' and 'low' malic acid years. Robinson et al. (1959) found a negative correlation between total heat accumulation (from bloom to fruit maturity) and the amount of malic acid present in the fruits of V. labrusca 'Concord' grapes. However, as noted by Kliewer (1964), neither data by Amerine (1956) nor Peynaud (1947) indicated a uniform relationship between cool years and malate concentration. It is probable that modifications in microclimate, induced by seasonal and cultural variations compound the complexity of macro-climate influence on berry composition.

A variety of experimental techniques have been used to estimate the effect of temperature on organic acid level in the fruit of the grape during its development. The influence of temperature of treated shoots on incorporation of $^{14}CO_2$ into organic acids in the berry was investigated by Kliewer (1964). At higher temperature less label was recovered in malate during the ripening period. The degree of labelling found in tartaric acid was not greatly influenced by temperature either in immature or ripe berries. When grapes were grown from veraison to fruit maturity in a phytotron the concentration of total soluble solids and tartrate in the berry juices were generally not significantly altered between different temperature regimes, however low temperature resulted in lower pH and increased titratable acidity and malate concentration (Kleiwer and Lider 1970, Kliewer 1968).

Both photo- and nycto-period temperatures play an important part in determining final concentrations at ripeness (Kliewer 1973). Similar studies by Buttrose et al. (1971) confirm that malic acid content is greatly enhanced by a decrease in the temperature environment of the berry. In a novel approach, Radler (1965), enclosed Sultana grapes in temperature- controlled heated cannisters (33 $\pm$ 2°C) to examine

temperature effects on acid metabolism. The temperature increase (the differential existing mainly at night) caused a reduction in berry size and induced a more rapid decrease in titratable acidity.

In the experiments of Smart et al. (1977) single berries, previously shaded, were uncovered from sunrise to sunset; analysis of top and bottom halves showed that day-time exposure resulted in higher temperatures and less malate was found in the top halves of these berries. Koblet et al. (1977) found that during maturation sun berries were always higher in sugar and lower in acid concentration than shade berries; the effect was primarily on malic acid levels (being lower in the sun berries), the differences in tartaric acid concentration being small.

Another important factor that influences the metabolism of organic acids in grapes is solar radiation. Ribéreau-Gayon (1959) observed that vines shaded with bamboo produced grapes which were higher in malic acid than grapes from unshaded vines. Kliewer and Lider (1968) found that berries on clusters that were shaded by foliage (shade fruit) were cooler (0-20°F differential) and had higher acidity and lower pH than fruit exposed to direct sunlight (sun fruit). In particular malate concentration was 2-3 times as great in mature shade fruit as in sun fruit, whereas tartrate level was little affected by exposure to sun. Differences in composition and ripening patterns were attributed largely to the higher and more variable temperatures in the sun fruit. In other experiments variation of solar radiation reaching grape vines was achieved by positioning of artificial shading (Kliewer et al. 1967, Schultz and Lider 1964). Environments representing 21, 30 and 100% full sunlight were achieved. The titratable acidity of sun fruit was less than that for the shaded treatments. There were some quantitative differences between varieties but essentially the malate concentration in mature fruit from shaded vines was greater than that in mature fruit from vines grown in full sunlight. For one variety ('White Riesling'), the amounts of tartaric acid and malic acid per g. fresh weight were higher in shaded berries than for 100% sun berries at both early ripening and later, however interpretation of these results is complicated not only by differences in fresh weight (heavily shaded

berries had slightly lower fresh weight than 30% or 100% treatments which were of similar weight) but also by variations in maturity level at time of sampling. For samples at the early stage the degree of maturity for 21, 30 and 100% treatments were respectively 7.5, 8.0 and 10.0° Brix. The lower level of malate in 100% treatment at this stage could be explained by greater breakdown of malate at the higher °Brix, since on a fresh weight basis malate breakdown is especially rapid at this early ripening stage. Again at the later stage of ripening maturity levels varied considerably with sampling time and were 15.3, 17.6 and 19.7 °Brix for 21, 30 and 100% sun treatments respectively. This confounding of sampling time and maturity level is difficult to avoid and commonly leads to some confusion in interpretation as shown not only in this particular experiment but in a number of investigations reported in the literature.

More recently, experiments conducted in a hot climate region of South Australia demonstrated the effect of vine canopy manipulation on must and wine quality (Smart 1982). Treatments which caused fruit and leaf shading produced musts with high pH, compared with treatments such as G.D.C., which creates conditions of high fruit and basal leaf exposure. The musts from G.D.C. plots had the highest titratable acidity but the pH, malate and potassium content were the lowest of the four treatments. Berry malate levels were correlated negatively with fruit exposure. Wines from the treatment causing most shade were of low quality, while the other three treatments were similar (although G.D.C. wines were preferred).

Van Zyl and Van Huyssteen (1980) investigated and compared micro-climate and grape composition of four different trellising systems in a vineyard in South Africa. The variations observed in malate content could be explained by temperature differences induced by growth patterns on the various trellises. High temperature conditions resulted in low malate concentration. Difference in tartrate concentrations for samples from the four trellising systems were insignificant. Overall the effect of differences in microclimate did not lead to dramatic differents in grape juice composition, nor in fact could wine quality be related to the must quality (the must with initially the highest pH was

judged as producing the better wine). These observations lead the authors to conclude that in very hot regions, small micro-climatic differences induced by trellising systems are overruled by the overwhelming effect of macro-climate, and that micro-climatic effects would probably be more pronounced in cooler climatic areas.

Metabolic patterns during fruit ripening are thought to be associated with changes in permeability of cellular membranes (Sacher 1973, Hale 1977) and light may be important in this respect because of its influence on membrane structure. The photo conversion of P_R to P_{FR} could be associated with conformational changes in membranes as a result of charge dislocations and charge polarization in specific sites of the membrane, inducing changes in membrane permeability, cellular energy metabolism, active transport and osmotic potential (Mitrakos and Shropshire 1972). Thus metabolism, in particular that of malic acid, in shaded environments may not only be influenced by lowered temperature conditions but also by modification in membrane permeability due to altered light regime.

In most of the above studies of temperature and light effects on the metabolism of organic acids it is regretable that the analysis of cation content was neglected, especially of potassium.

The importance of solar radiation attenuation by grapevine canopies is considered to be a critical factor in potassium distribution within the vine (Smart et al. 1981, Smart et al. 1984). Shaded conditions (dense canopies) caused high potassium levels in both the mature fruit, and in the leaves at veraison which appeared to be a principal source of potassium for the ripening fruit (Smart et al. 1981, Smart et al. 1984). A link between leaf photosynthetic activity and potassium transport has been suggested by Boulton (1980e) and Freeman et al. (1982). For Ricinus communis L., Smith and Milburn (1980) have shown that in extended dark periods, when sucrose is not available, potassium loading into the phloem is increased. Freeman et al. (1982) suggests that any conditions that reduce photosynthetic activity such as stomatal closure, could contribute to increased potassium levels in the phloem and subsequently the berries. Reduced solar radiation levels received by interior leaves could influence berry potassium uptake in this way.

Anthocyanin content of berries is widely variable and data by Ribéreau -Gayon (1982) show large yearly differences indicating the importance of climatic conditions on anthocyanin synthesis in the grape berry. Temperature and solar radiation are regarded as the most important climatic factors influencing anthocyanin synthesis (Kliewer and Torres 1972, Kliewer 1977, Pirie 1977).

Experiments by Kliewer (1977) with controlled temperature environments showed a) at day/night of 37/32°C no anthocyanin formation occurred in fruits under either high light (66.5% sunlight) or low light (9.5% sunlight), b) higher fruit colouration occurred under day temperatures between 15-25°C with night temperatures between 10-20°C and c) anthocyanin synthesis was positively influenced by increasing solar radiation. Temperature and light had a greater proportional effect on anthocyanin content than on sugar accumulation.

The optimum temperature range for anthocyanin synthesis appears to be about 17°C to 26°C (Pirie 1977). Pirie suggests that warm climates are likely to produce grapes more pigmented than those from hotter or colder climates since the ripening period occurs in the optimum temperature range for anthocyanin synthesis, provided all other factors influencing pigment synthesis are equal.

That light is an important external factor in grape colouration has been demonstrated by Kliewer (1970, 1977) and Pirie (1977). Both leaf and bunch shading influence anthocyanin formation; when leaves were shaded and berry microclimate held constant by wrapping in foil, the berries associated with unshaded leaves had higher anthocyanin levels (Pirie 1977). Possibly under the influence of light leaves produce a precursor for anthocyanin synthesis, which is then translocated where a further light reaction is required to bring about anthocyanin synthesis (Mitrakos and Shropshire 1972). Light is required in pathways producing carbohydrate, acetate and phenylalanine which are starting points for anthocyanin metabolism, while light, which also mediates phytochrome activity, regulates the synthesis of these intermediates towards anthocyanin and phenolic production. Smart (1982) suggested that altered ratios of 660nm/730nm in dense canopies may be responsible for

low levels of anthocyanin formation in berries in shaded environments. Thus for the grape berry both leaf and bunch exposure are important factors for the production of anthocyanins and phenolics.

Anthocyanin accumulation in the skin of 'Kyoho' berries was greater when fruit temperature was 20°C as opposed to 30°C where inhibition of anthocyanin synthesis occurred (Lee et al. 1979). A connection between ABA levels and anthocyanin accumulation was suggested since temperature had a direct effect on both these components, however no mechanism was proposed for such a regulation.

Under field conditions, depending on ambient temperatures during ripening, temperature and solar radiation may act in the same or opposite directions on anthocyanin and phenolic synthesis in the grape berry.

1.4.c. BERRY COMPOSITION - EFFECT OF VINE WATER STATUS

The effect of irrigation on compositional aspects of grape berries is complex, involving not only amounts of water applied but current climatic conditions, soil-water status and yield parameters. Compositional analyses have mainly concentrated on juice components (pH, titratable acidity and total soluble solids) and data on whole berries is limited.

A number of studies confirm that juice titratable acidity of grapes from irrigated vines were higher than from non irrigated vines (Coombe and Monk 1979, Freeman et al. 1980, Neja et al. 1977, Van Zyl 1977, Wildman et al. 1976), but despite this consistency of titratable acidity response to irrigation, the same studies present conflicting data for juice pH response. Juice of berries from irrigated vines may show increased pH (Freeman et al. 1980, Hardie 1981, Morris and Cawthorn 1983); no significant change (Van Zyl 1977, Wildman et al. 1976), or decreased pH (Coombe and Monk 1979, Neja et al. 1977), when compared to non irrigated vines.

One study that reported values for all four compounds, tartrate, malate, potassium and sodium, was that by Hardie (1981), who maintained 'Zinfandel' vines under three different water regimes during the period from flowering to harvest. The juice from grapes on vines which received maximum irrigation had highest levels for pH, and highest concentrations of potassium, sodium and malate, compared to vines receiving less irrigation. The main accumulation of minerals occurred after veraison. It was suggested that this mineral increase (mainly potassium) was attributed to both redistribution of potassium in the foliage to the fruit and the effect of water supply in providing a source and transport system for minerals from the roots to the fruit. The concentration of tartrate was usually higher in fruit where berry growth was restricted by water supply, except where stress was suddenly relieved by either irrigation or rainfall during ripening.

Irrigation treatment applied at fruit set (earlier than other

treatments) resulted in highest titratable acidity for juice samples at harvest (Van Zyl and Webber 1977). These conditions also induce vegetative growth and the resultant denser canopy of irrigated vines may indirectly influence malate metabolism. Under these conditions (lower temperature and reduced light) a higher retained level of malic acid within the berry is likely. Hardie (1981) also attributed high malate levels to this indirect effect of vine foliar growth. Freeman et al. (1980) suggest that the higher juice acidity and pH for irrigated vines, compared to non irrigated, was not a result of water stress or temperature during maturation as the treatment differences existed early in berry development. It would appear from the above studies that the period of vine growth up to veraison plays an important role in establishing the pattern for titratable acidity and pH in the developing berry.

When the vine is stressed, vine leaves respond by stomatal closure. Under hot conditions (e.g. Griffith, Australia) vines adapted to water stress photosynthesised for longer daily periods than irrigated vines, since the stomata of non irrigated vines closed at a lower water potential than those of irrigated vines (Freeman et al. 1980). They suggested that for certain situations atmospheric conditions may override soil moisture conditions. Differences obtained by varying irrigation regime may then not be as pronounced as expected, and will be dependent primarily on current atmospheric conditions. Marked seasonal variation for titratable acidity and pH may be due to this apparent interaction between temperature and soil moisture (Neja et al. 1977). The importance of seasonal variation is also emphasised by Morris and Cawthorn (1983) since in a year of normal rainfall, irrigation reduced wine quality; however in a year of severe water stress conditions, supplemental irrigation improved juice quality by preventing loss of photosynthetic leaf area of the vines.

Cation concentration in grape juice normally increases with improved vine water status (Hardie 1981, Freeman and Kliewer 1983, Morris and Cawthorn 1983), however this was normally also associated with altered canopy structure which may have affected potassium distribution within the vine.

Few studies have defined the effect of vine water status on phenolic content in the intact berry and most often this is inferred from wine colour and phenolic composition. However in interpreting this data, the effects of berry size, and wine pH and SO_2 levels should be separated. Additionally wine making technique may dramatically alter the extraction and stability of actual phenolic content that was present in the mature berry at crushing.

One study that reported both grape and wine colour (Freeman and Kliewer 1983) showed that anthocyanin concentration in the skin of fruit of non-irrigated 'Carignane' vines was 44% higher than fruit from irrigated vines and wine colour was higher in wines made from the non-irrigated grapes. The lower wine colour from irrigated fruit was due to both lower levels of total pigment concentration and to higher pH values in those wines which decreased the coloured form of the total pigments.

In France, vineyards grown under conditions of deficient water supply during ripening, generally produce grapes of higher phenolic concentrations, a condition usually associated with the production of quality grapes (Ribéreau-Gayon and Glories 1982).

While this view is widely held (Branas 1977, Tyrell 1981) the effect is dependent on the degree of stress experienced by the vine, since fruit that failed to mature due to severe water stress also had lower skin pigment content whether assessed on a per-berry or per-unit-surface area basis (Hardie and Considine 1976). This was probably due to inhibited leaf photosynthesis, resulting in inadequate supply of precursors for anthocyanin synthesis in the berry.

Effects of altered light and temperature regimes (as discussed previously) would also be implicated in irrigation experiments if leaf and fruit shading resulted from such treatments.

1.5. GENERAL DISCUSSION AND RESEARCH AIMS

For many years Australian winemakers have experienced difficulty with the variable and unbalanced composition of musts from black winegrapes used for making dry red table-wine. Most of the problems are attributable to variation in acidity and pH which in the grape berry are due mainly to changes in concentrations of malate, tartrate and potassium. These changes occur both in the pulp and skin of the berry, and components in both areas would contribute to acidity relationships either initially in the juice or subsequently in the wine. Because of the importance of pH in juice and wine, and further the significance of phenolic material in red wine, this thesis was based on the following aims:

1. TO DEFINE AND QUANTIFY THE CHANGE IN THE MAJOR ORGANIC ACIDS, CATIONS AND PHENOLIC CONTENT THAT OCCURS EITHER IN THE PULP OR SKIN OF BLACK GRAPES DURING RIPENING.

2. TO INVESTIGATE VITICULTURAL FACTORS THAT MAY INFLUENCE THE EXTENT OF CHANGE IN THESE COMPONENTS.

3. TO EXAMINE MEMBRANE PERMEABILITY ASPECTS THAT MAY BE INVOLVED IN RIPENING MECHANISMS FOR THESE ACIDS AND CATIONS.

4. TO DETERMINE THE EFFECT OF SKIN EXTRACTION ON RED WINE COMPOSITION.

5. TO INTERPRET ACIDITY MEASURES IN GRAPE JUICES.

CHAPTER 2

ORGANIC ACID, CATION AND PHENOLIC CONTENT OF PULP AND SKIN OF 'SHIRAZ' GRAPES DURING RIPENING.

CHAPTER 2

ORGANIC ACID, CATION AND PHENOLIC CONTENT OF PULP AND SKIN OF 'SHIRAZ' GRAPES DURING RIPENING.

Abstract

Analysis of 'Shiraz' grape berries at intervals during ripening after separation into pulp and skin showed distinctive and important differences in the concentration of several components: tartrate per berry remained relatively constant in both pulp and skin during development but malate per berry declined dramatically in the pulp, yet at the same time doubled in the skin; potassium concentration increased in the pulp and especially in the skin - in some samples 40% of the berry's potassium was contained in the skin.

Differential extraction with 80% ethanol showed that of the major organic acids in the grape only tartaric acid was converted to salt forms during ripening. At all stages of berry ripening, malate existed entirely as the free acid form in both pulp and skin tissue.

Large variations in acidity components were apparent in samples from different Australian viticultural areas, even though berry sugar levels were similar. These variations are the seat of several vinification problems for red wines.

INTRODUCTION

Compositional aspects of berry development have been extensively investigated in viticultural and oenological research, yet few studies have considered the distribution of some of the oenologically significant components between the fundamental tissues of the grape, the epicarp (the skin) and the fleshy mesocarp (the pulp). For black grapes particularly there are major differences between skin and pulp; the most obvious being the coloured anthocyanin pigments in the skin. Other

constituents that have been found in grape skin include sugars (glucose, fructose and sucrose), organic acids (tartaric, malic and citric), other anions (chloride, phosphate), monovalent cations (potassium and sodium) and various other metal cations (e.g. Ca^{++}, Mg^{++}, Fe^{++}, Fe^{+++}, Cu^{++}) (Coombe and Matile 1980, Hale 1977, McCarthy and Downton 1981, Moskowitz and Hrazdina 1981, Peynaud and Maurie 1953, Pirie and Mullins 1977).

By the time the berry is ripe, large amounts of potassium have been accumulated in both the pulp and the skin of the berry. Peynaud and Maurie (1953) report that during ripening of the variety 'Merlot', potassium levels increased 2 to 3 times in the skin but only 1.2 to 1.9 times in the pulp.

Recently fundamental relationships concerning pH in juice and wine have been explained using amounts and relative proportions of the organic acids and monovalent metal cations, especially potassium (Boulton 1980a,b,d,e). It would be expected that acids and cations derived from both pulp and skin would contribute to these relationships in juice and wine which involved skin extraction. In fact high pH in wines has been attributed, in part, to extraction of excessive amounts of cations (mainly potassium) from the skins during red wine vinification (Van Wjk 1977), and a connection between potassium levels in the harvest and relative quality in Australian red wines has been suggested by Somers (1977).

Because of the fundamental importance of pH in juice and wine, and because Australian winemakers often experience difficulty with unbalanced must composition (low acid, high pH) from black winegrapes, it was decided to study the distribution pattern of these pH factors in pulp and skin of black grapes during ripening. Additionally anthocyanin and phenolic content in the skin of the sample berries were also determined.

2.1. MATERIALS AND METHODS

2.1.a. Period of berry growth examined

The period of berry development examined is the ripening phase (Period III as described by Winkler et al. 1974).

2.1.b. Grape samples

Berries were sampled from 'Shiraz' vines from different locations in South Australia. Details of the experiments conducted are as follows:-

i) Experiment 1 (1979): From veraison to maturity, a 50-berry sample was taken weekly from each of four plots of six 'Shiraz' vines in the vineyards of Roseworthy Agricultural College, South Australia. The vines were 20 years old, cane pruned and trained on a T trellis. Irrigation was applied during the summer. Vine and row spacing was 1.5m and 3m respectively. This experiment was a preliminary investigation to establish methods and also define levels of compounds present. A severe hail storm in the spring of 1979 caused damage to the vines and precluded sampling in the following years.

ii) Experiment 2 (1980): For the 1980 season samples were taken from 20 year old 'Shiraz' vines in the Alverstoke vineyard at the Waite Agricultural Research Institute, Adelaide. Vines were trained on a bilateral cordon with T-foliage wires, and were spur pruned. Irrigation was applied during the summer. Vine and row spacing was 2.2m and 3.7m respectively. Seven vines, positioned alternately in one row, were used, and the development of the berries was monitored, by determining °Brix on juice extracted from a random berry sample taken, at intervals, from each vine. At three stages of ripening 20-berry samples were taken from each vine; the stages are termed beginning^{A} (7-9°Brix), mid^{B} (15-17°Brix) and ripe^{C} (22-24°Brix). Additionally a 40-berry sample was taken collectively from vines 1-4, and also from vines 5-7 at several

other stages of maturity.

iii) Experiment 3 (1981): The samplings for this year formed part of an irrigation trial on 'Shiraz' vines in a commercial vineyard at Virginia in South Australia. Vines were cane pruned and trained on a T trellis system. Vine and row spacing was 1.5m and 3.5m respectively. Vines were drip irrigated. Three plots of 4 vines were sampled (50 berries per plot) on a weekly basis during the ripening stage.

iv) Experiment 4: Berries from 'Shiraz' vines in different locations in Australia (mainly South Australia), were sampled in order to determine the variation for these components that may occur under a variety of field conditions. A 200-berry sample was randomly taken from each vineyard and then further sub sampled into 2 x 50 berry lots for processing and analysis. Samples were collected over a number of years.

2.1.c. Extraction procedure

A number of berries (between 20 and 50 depending on the particular experiment) was randomly sampled from the experimental vines and the sample weighed. Each berry was cut in half longitudinally with a scalpel and the halves were allocated to separate petri dishes, to provide two sub samples of half berries. The weight of each sub sample was taken as half the weight of the whole berries. These sub samples were analysed by two methods, whole and separated.

Whole:

For this sample of half berries the seeds were removed, and washed briefly in distilled water and the seed washings retained. The seeds were dried by blotting with filter paper, counted and weighed. The sample of half berries was then placed in a beaker, and crushed with a glass rod, after which the extract was centrifuged at 2000rpm for 3-5 minutes. The total soluble solids content of the supernatant was measured by refractometer to obtain an estimate of sugar content (°Brix) and hence of the maturity level of the berries; this in turn permitted samples from different treatments, which showed different ripening patterns with time, to be compared at the same development stage (based

on °Brix), rather than relying on sampling date.

Separated:

i) pulp portion: After removing the seeds as above, the pulp and skin were separated by carefully peeling the skin from the half berry. This was more difficult with green berries, and in these cases some adhering pulp had to be scraped off the skin. The pulp was placed in a 20mL autoclave bottle. When the pulp from all half berries in the sample had been placed in this bottle, the pulp was crushed with a glass rod. The distilled water that had been used a) to wash the petri dishes, on which the berries were cut, b) to wash the seeds and c) to wash the skins, were combined and placed in other similar autoclave bottles. The autoclave bottles containing the pulp and the washings were autoclaved at 15 psi for 15 mins. After autoclaving, the samples were cooled, filtered and when the pH had been measured on the pulp sample, then all autoclaved extracts (i.e. pulp plus washings) were combined, made to known volume with distilled water and stored frozen.

ii) skin portion: After the skin had been separated from the pulp it was washed briefly in distilled water and blotted lightly on filter paper. The sample of skin from all the half berries was weighed and then shredded into small slices with a scalpel, and the slices of skin randomly distributed to give two skin sub samples; one was either 1g or 50% of the total weight of all the skins and the other either 0.25 - 0.5g or 20% of the total weight. The larger sample was used for an aqueous extraction, for which the skin slices were placed in a 10 mL autoclave bottle and a volume of distilled water (10 x weight of skin) added. This aqueous sample was autoclaved at 15 psi for 15 minutes. The autoclaved skin extract was filtered and made to known volume with distilled water and stored frozen.

For colour extraction, the smaller sub sample of skins was placed in a test tube and extracted exhaustively with 8 x 5-10 mL portions of methanolic 0.1% HCl. During each extraction the skin was first crushed with a glass rod and the extraction mixture shaken for 5-10 minutes on a mechanical test tube shaker; the complete extraction took about two hours. The combined extracts were filtered and made to known volume

with methanolic 0.1% HCl. Aliquots of the extracts were diluted with 1 M HCl appropriately for measurements of total anthocyanins ($OD._{520}$) and total phenols ($OD._{280}$) (Somers and Evans 1977). Anthocyanin values were based on $E^{1\%}_{1cm}$ = 500 for the mixture of acylated and non-acylated anthocyanins (Somers and Evans 1977).

$O.D._{280}$ values were converted to concentration units determined from a standard curve of gallic acid in 1M HCl solution; total phenols then being expressed as mg of gallic acid equivalents.

2.1.d. Analytical methods.

Potassium and sodium were determined by flame photometry, pH by pH meter and tartrate and malate by ion exclusion chromatography (Monk and Iland, 1984). In the experiment to determine the proportion of each acid existing as salt forms, tartrate and malate were analysed by h.p.l.c.

2.1.e. Effectiveness of extraction methods.

i) Analysis of spiked samples indicated that there were no significant losses of tartrate, malate, potassium or sodium during the process of separation, autoclaving, filtering, etc. of either pulp or skin samples.

ii) Re-extraction with distilled water and analysis of the material that remained after the skin and pulp samples were filtered, indicated that one extraction was sufficient to remove all compounds from the skin; for the pulp, extraction efficiency was in the order of 95% for the acids and 100% for the cations.

2.1.f. Proportional weight of pulp and skin tissue of whole berry weight at various stages of maturity.

At each of the three previously defined ripening stages (A, B and C) a 100-berry sample was divided into seeds, pulp and skin portions; each portion was separately weighed, and percentage weight of total berry

weight was then calculated for the 3 portions of the berry. This enabled concentration of each compound to be converted from a gram per berry weight base to a concentration base in the individual tissues, i.e. amount of compound in the pulp per weight of pulp and amount of compound in the skin per weight of skin.

2.1.g. Expression of results

Results for each compound were expressed as follows:-

i) The amount (milligrams) of each component separately in either the pulp or the skin per berry.

- mg in the pulp/berry
- mg in the skin/berry

ii) The amount of each component separately in either the pulp or the skin per g berry weight.

- mg in pulp/g berry weight
- mg in skin/g berry weight

iii) the concentration of each component separately in either the pulp or the skin per g fresh weight of the individual tissue.

- mg in the pulp/g fresh weight of the pulp
- mg in the skin/g fresh weight of the skin

2.1.h. The proportion of each acid existing as its salt forms in pulp and skin tissue at various stages of ripeness.

For this experiment, berries were sampled from the three irrigated and the three non irrigated plots described in section 3.1.c.i At the three previously defined maturity stages, a 50-berry sample was taken from each of the six plots, and further sub sampled into 10 berries per plot. Each 6 x 10-berry sample was treated separately during the subsequent treatment. Each sample of 10 berries were divided in half as explained previously, and on one lot of half berries stage of development (°Brix) was assessed. The other half berries from each sample were divided into pulp and skin portions, and these were

separately extracted by the method shown in Fig 2.1. The extraction method is based on the fact that the salt forms of the organic acids are insoluble in ethanol (Table 1.2). The method is similar to that used by Saito and Kasai (1968).

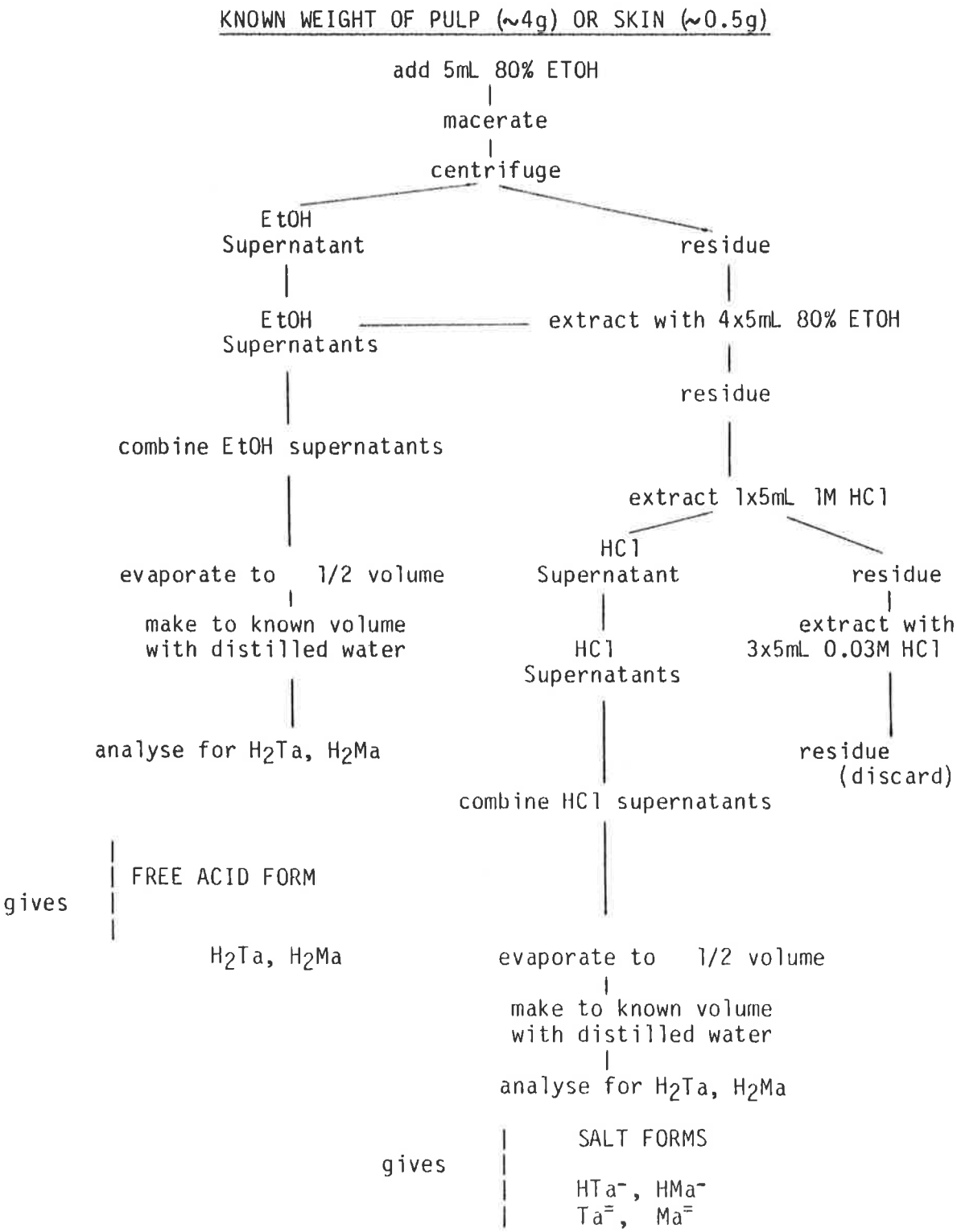

Fig. 2.1. Extraction procedure to determine the proportion of tartaric and malic acid existing as salt forms, in the pulp and skin of grape berries.

2.2. RESULTS

2.2.a. Proportional weight of pulp and skin at various stages of ripening

The total and percentage weight of the berry and its parts, at three stages of maturity are shown in Table 2.1. Proportions may vary slightly depending on berry size, so when this is a variable e.g. for samples from different areas or treatments, comparisons are best made on a fresh weight of whole berry rather than on an individual tissue weight basis.

Table 2.1. Weight of various parts of the grape berry and their proportion of the whole berry weight, at various stages of ripening. Each sample consisted of 100 berries from experiment 2.

	Degree of ripeness					
	Beginning		Mid		Ripe	
	weight(g)	%	weight(g)	%	weight(g)	%
Whole berry	1.24	100	1.48	100	1.52	100
pulp	1.04	84	1.29	87	1.29	85
skin	0.11	9	0.13	9	0.15	10
seeds	0.09	7	0.06	4	0.08	5

2.2.b. Changes during ripening.

Results for experiments 2 and 3 are reported in detail, while for the preliminary experiment (experiment 1) only part of the data is reported since the patterns were similar to those of the other experiments.

Typical patterns of berry growth and sugar accumulation were observed (Figs 2.2 and 2.3); initial berry growth being rapid, then slowing during the later stages of maturity. The decrease in berry weight shown in Expt. 2 in the ripest samples is common in 'Shiraz' and correlates with berry shrivelling apparently due to water loss. The ripening stages investigated in experiment 2 and 3 were 6-23 °Brix and

7-25 °Brix respectively. In Figs 2.4 on, the ripening parameters are plotted using °Brix as the abscissa, since this gives a better comparison between different experiments of the relationship with berry development.

The pH of both the pulp homogenate and the aqueous skin extract increased during ripening (Fig 2.4). Since methods of extraction and dilution of pulp and skin tissue differ, direct comparisons of pH values between these tissues are not appropriate.

The amount of malate in the pulp decreased on both a per berry and a per g berry weight base during the ripening stage, while in contrast accumulation of malate in the skin tissue was observed for both these expressions, during the same period (Fig 2.5, 2.6, Table 2.2).

Table 2.2. Change in amounts of tartrate, malate, potassium and sodium in the pulp and skin of 'Shiraz' grape berries during ripening.

	PULP mg berry^{-1}			SKIN mg berry^{-1}		
	veraison	maturity	change	veraison	maturity	change
experiment 2						
Tartrate	7.9	7.2	-9%	1.1	1.3	+18%
Malate	14.5	4.5	-69%	0.7	1.8	+157%
Potassium	0.9	1.8	+100%	0.4	1.5	+275%
Sodium	0.1	0.3	+200%	0.09	0.08	-11%
experiment 3						
Tartrate	5.9	6.6	+12%	1.0	1.6	+60%
Malate	8.5	1.6	-81%	0.6	1.4	+133%
Potassium	0.7	2.4	+243%	0.4	1.5	+275%
Sodium	0.05	0.10	+100%	0.02	0.04	+100%

The amount of tartrate in the pulp per berry remained relatively constant throughout the ripening period, (Fig 2.5, 2.6, Table 2.2); hence the decrease observed on per g berry weight base can be attributed to berry growth. In the skin, tartrate per g berry weight base decreased, while the amount per berry increased slightly as ripening progressed (Fig. 2.5, 2.6, Table 2.2). The different patterns are due to berry growth during ripening.

Potassium content increased throughout ripening in the pulp and the skin tissue on both a per g berry weight base and a per berry base (Fig 2.5, 2.6, Table 2.2). Sodium showed similar trends to potassium (Fig 2.5, 2.6, Table 2.2), but the magnitude of the values was considerably less. Sodium contributed only 15% (Expt 2) and 5% (Expt 3) of the total amount of potassium plus sodium in the pulp of mature berries. Corresponding values for sodium in skin samples were 5% (Expt 2) and 3% (Expt 3).

2.2.c. The proportion of each acid existing as its salt form in pulp and skin tissue at various stages of ripening.

The proportion of total tartrate present as the free acid form (H_2T) decreased in the pulp (increasing contribution of salt forms), and increased in the skin during ripening, while in both tissues malate existed entirely as the free acid (H_2M) at all stages of ripening (Fig 2.7).

2.2.d. Variation of organic acid, cation and phenolic contentin berry pulp and skin for samples taken from different viticultural locations.

The amount of tartrate, malate, potassium and sodium in pulp and skin tissue of a range of berry samples is given in Table 2.3. Stage of maturity (°Brix of juice sample) and the total anthocyanin and total phenolic content in the skin of the sample berries is also shown in this table.

TABLE 2.3. Amount (per berry) of organic acids, cations, total anthocyanins and total phenolics in pulp and skin of 'Shiraz' grape berries sampled from various viticultural areas. Sample sites may not necessarily be representative of that area.

SAMPLE NO.	VITICULTURAL AREA	MATURITY STAGE °BRIX	TARTRATE (mg)			MALATE (mg)			POTASSIUM (mg)			SODIUM (mg)			TOTAL ANTHOCYANINS in the skin (mg)	TOTAL PHENOLICS in the skin (mg of gallic acid equivalents
			PULP	SKIN	PULP & SKIN	PULP	SKIN	PULP & SKIN	PULP	SKIN	PULP & SKIN	PULP	SKIN	PULP & SKIN		
1	Eden Valley, S.A. 1979	26	5.57	1.10	6.67	1.86	0.68	2.50	1.45	0.89	2.30				1.60	2.27
2	Roseworthy College, double pruned 1979	25	5.79	1.42	7.21	3.84	1.28	5.12	1.64	0.91	2.55				2.04	2.44
3	Barossa (1) 1980	24	5.97	1.41	7.38	1.50	0.88	2.38	1.50	0.87	2.37				1.63	1.93
4	" (2) 1980	24	4.68	1.61	6.29	1.34	0.82	2.16	1.65	1.13	2.78				2.76	2.95
5	" (3) 1980	21	6.22	1.59	7.81	1.77	0.90	2.67	1.63	1.21	2.84				2.88	3.12
6	Hunter Valley, NSW, 1982	22	6.01	2.08	8.09	1.70	0.60	2.30	2.14	1.10	3.24				2.10	3.08
7	Roseworthy College (1) 1979	22	8.69	1.73	10.42	2.70	1.14	3.84	2.16	1.29	3.45				1.56	2.42
8	" " (2) 1979	26	10.13	2.00	12.13	1.92	0.99	2.91	2.55	1.12	3.67				1.68	2.48
9	Virginia (1) 1981	22	7.56	1.57	9.13	2.13	1.11	3.24	2.10	1.29	3.39	0.07	0.03	0.10	2.22	3.01
10	" (2) 1981	23	6.88	1.54	8.42	2.66	1.42	4.08	1.99	1.44	3.43	0.08	0.03	0.11	2.14	3.00
11	Adelaide, S.A. (1) 1980	24	6.01	1.23	7.24	1.89	0.81	2.70	2.08	1.13	3.20				1.60	2.04
12	" " (2) 1980	23	7.21	1.28	8.49	4.45	1.83	6.28	1.82	1.52	3.34	0.27	0.08	0.35	2.24	2.68
13	" " (3) 1980	23	6.77	1.41	8.18	3.31	1.36	4.67	1.46	1.30	2.76				2.87	2.96
14	" " (4) 1980	23	6.26	1.34	7.60	4.65	1.57	6.22	1.97	1.25	3.22				2.35	3.02
	RANGE LOW		4.68	1.10	6.29	1.34	0.68	2.16	1.45	0.87	2.30	.07	.03	0.10	1.60	1.93
	HIGH		10.13	2.08	12.13	4.65	1.83	6.22	2.55	1.52	3.67	0.27	.08	0.35	2.88	3.12

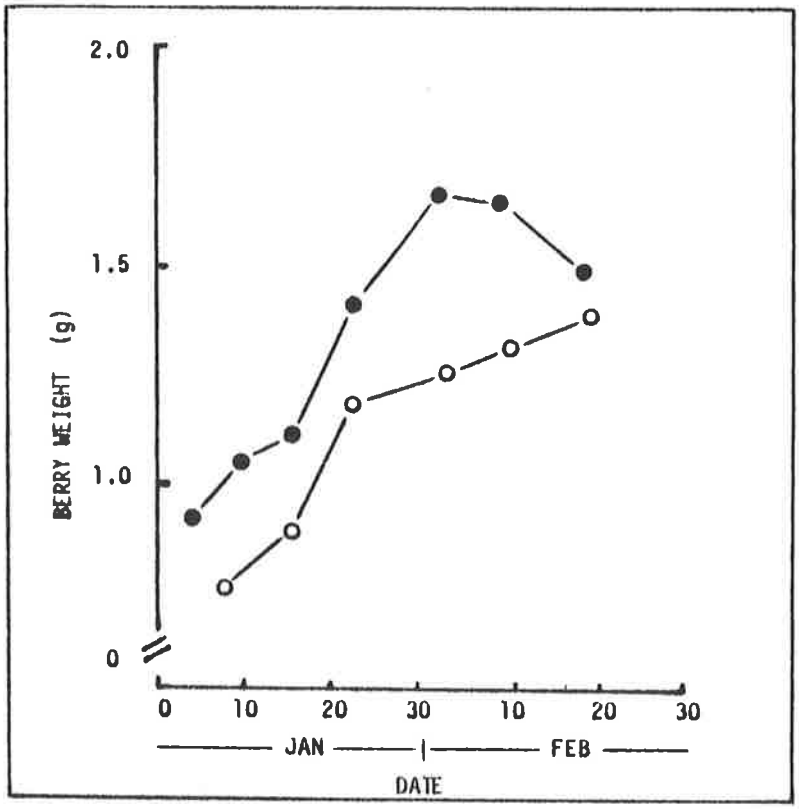

Fig. 2.2. Change in berry weight (g) of 'Shiraz' grapes during ripening (● expt 2, O expt 3).

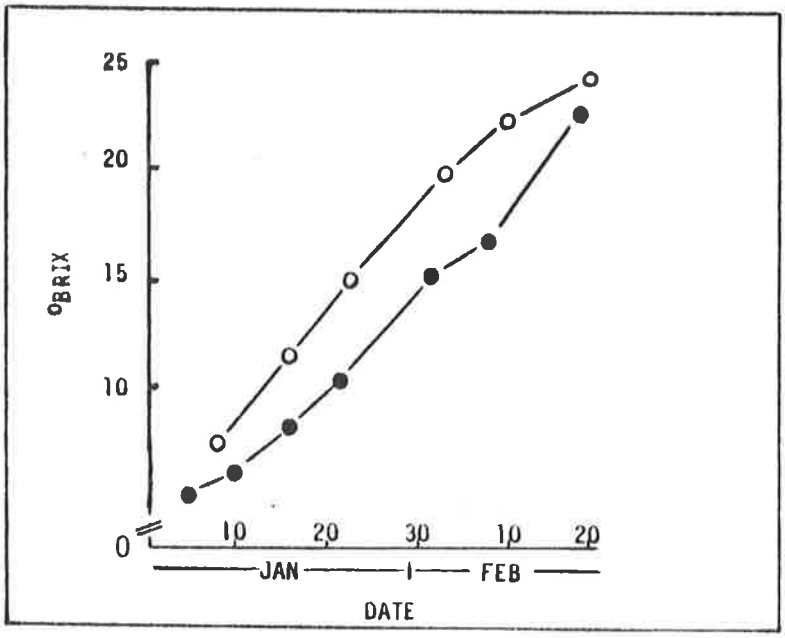

Fig. 2.3. Change in sugar concentration (°Brix) of 'Shiraz' grapes during ripening (● expt 2, O expt 3).

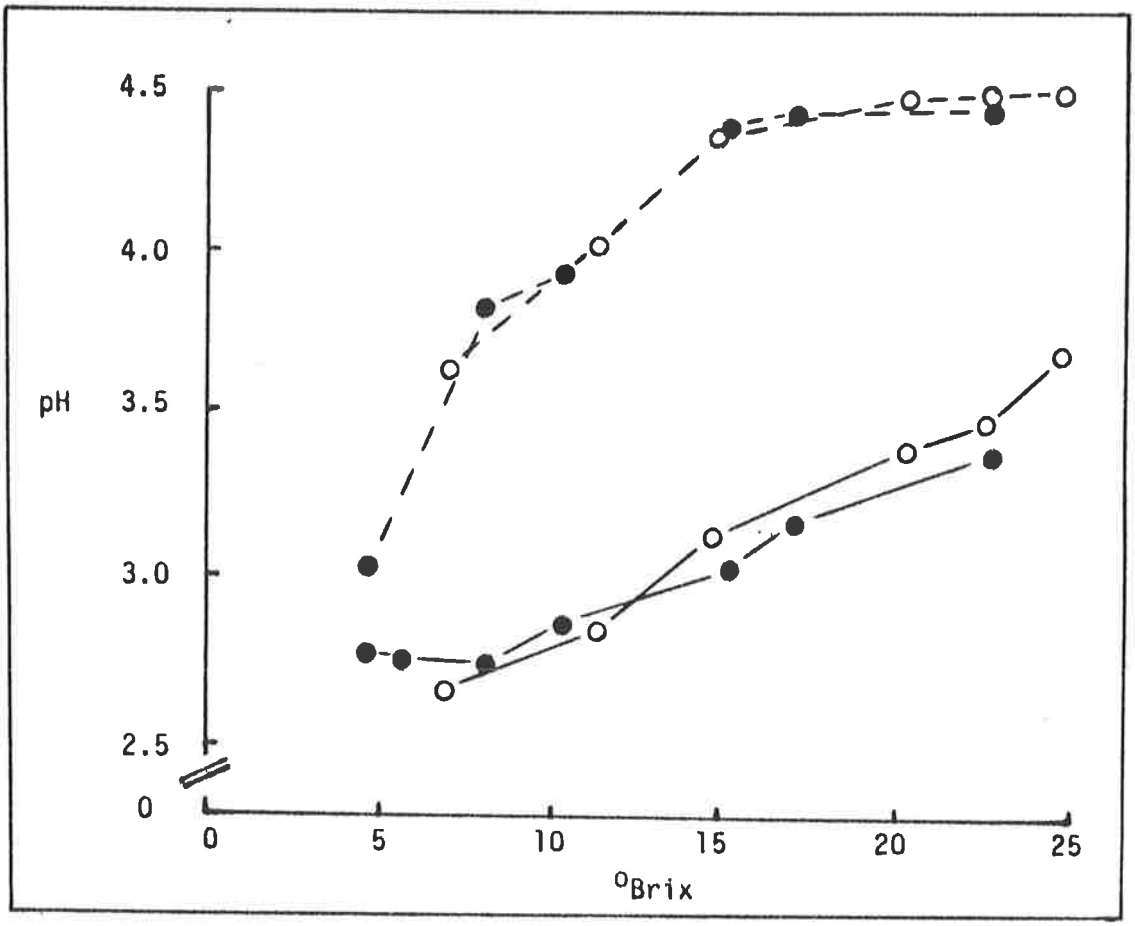

Fig. 2.4. Change in pH of the pulp homogenate (●—— ● expt 2, O——O expt 3) and of an aqueous skin extract (●--- ● expt 2, O---O expt 3) of 'Shiraz' grapes during ripening.

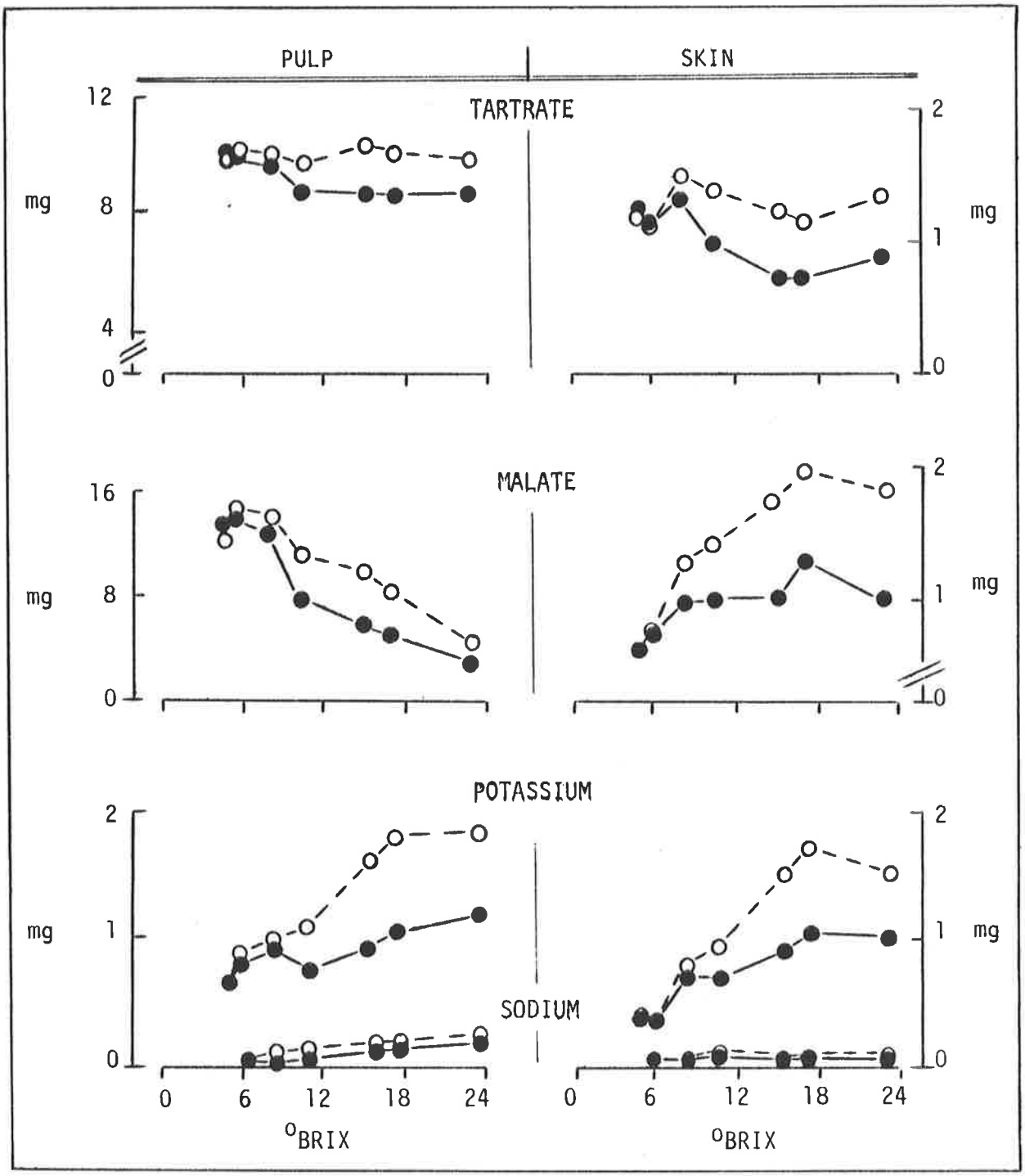

Fig. 2.5. Change in amount (mg/g berry weight ●——● and mg/berry O---O) of tartrate, malate, potassium and sodium in pulp and skin of 'Shiraz' grapes during ripening (Expt 2).

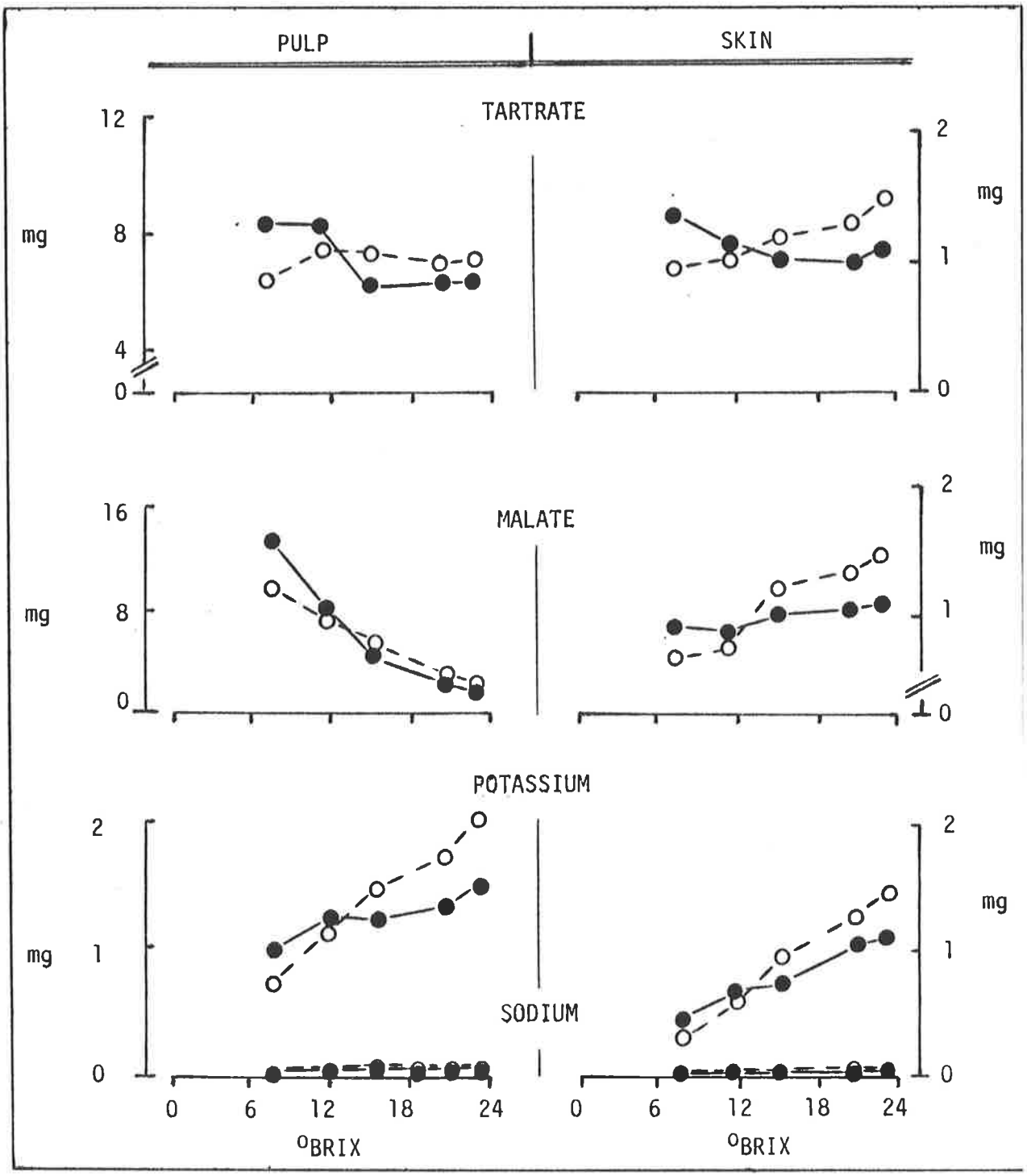

Fig. 2.6. Change in amount (mg/g berry weight ●——● and mg/berry O---O) of tartrate, malate, potassium and sodium in pulp and skin of 'Shiraz' grapes during ripening (Expt 3).

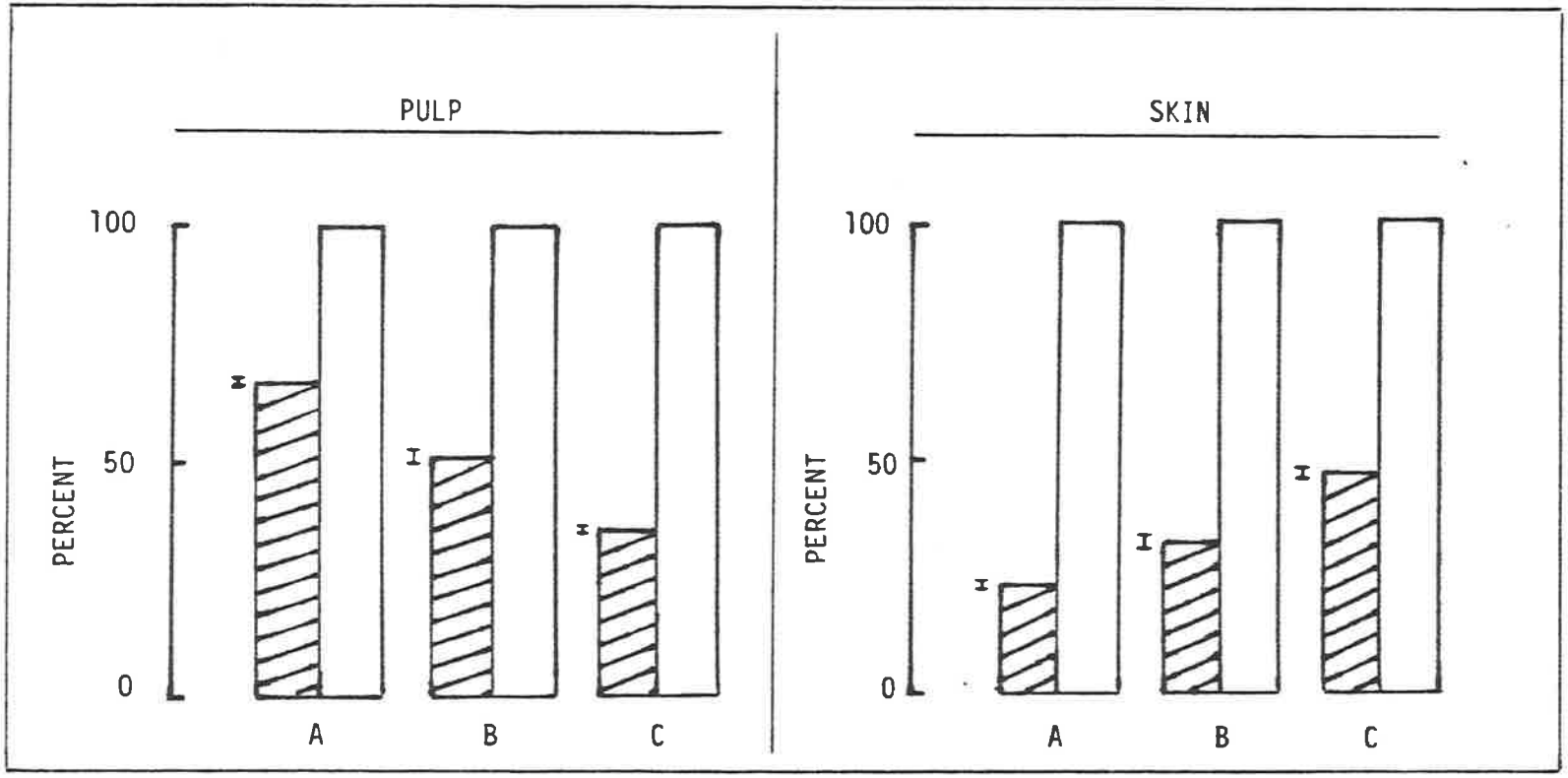

Fig. 2.7. Amount of tartrate ▨ and malate ▭ existing as the free acid form in the pulp and skin of 'Shiraz' grapes during ripening. The value corresponding to top of each bar represents the mean of three readings (I = SE of the mean). A, B, C = beginning, mid and ripe stages.

2.3. DISCUSSION

At veraison dramatic changes occurred in the course of both cation uptake and organic acid metabolism in 'Shiraz' grape berries. Results showed that, after veraison significant uptake of potassium occurred, of which a large proportion was directed towards the skin of the berry. Whether malic acid was respired or stored depended on where it was located within the berry.

Few reports have concentrated on this division between pulp and skin in relation to chemical composition, comparative data only being available for the varieties 'Semillon', 'Sauvignon', 'Merlot', 'Cabernet Sauvignon' (Peynaud & Maurie 1953), and 'Sultana' (Hale 1977).

The amount of tartrate in the berry was largely accumulated during the period of berry growth prior to veraison, and during ripening pulp tartrate remained relatively constant, while skin tartrate increased slightly. It follows then that after veraison variation in pulp tartrate concentration of berries is influenced predominantly by alteration in berry volume. Increase in skin tartrate levels on a per berry base during ripening was also observed for all varieties studied by Peynaud and Maurie (1953), however the same data provide varying responses for pulp tartrate:- pulp tartrate levels remained constant for white varieties, but decreased for the black varieties studied. Reports for other varieties, ('Sultana', Hale 1977; 'Zinfandel', Hardie 1981 and 'Muscadine' grapes, Carroll and Marcy 1982) confirm that on a whole berry base tartrate content is essentially constant during the ripening period.

For pulp malate both berry growth (which decreases concentration on a per g berry weight base) and remetabolism (which decreases absolute amounts) contribute to the overall decrease in this compound during ripening. This trend emphasises the different roles of the major organic acids in the ripening grape berry; while malate is an active intermediate in metabolic reactions, tartrate is essentially inert (Ruffner 1982a, 1982b).

In view of this characteristic decline of pulp malate with ripening, the accumulation of malate in the skin during the same period is somewhat surprising. In experiment 3, 80% of the malic acid present in the pulp at veraison was respired during ripening, while at the same time malic acid levels in the skin doubled. Data by Peynaud & Maurie (1953) suggest that there are varietal differences; for the varieties 'Semillon', 'Sauvignon' and 'Cabernet Sauvignon' there was an increase in amount of malate (expressed as milliequivalents per 100 berries) in the skin during ripening, and for the variety 'Merlot' a slight decrease. Hale (personal communication) found skin malate (mg per berry) decreased during ripening for the variety 'Sultana'.

Ruffner (1982b) suggests that compartmentation processes are involved in malate metabolism in the pulp of berry cells. He suggests that this acid is located in either of two distinct types of cells; those more centrally located in the berry structure which act as storage sites and those peripherally located termed "non-storage-type" cells. These peripherally located cells contain metabolically active compartments and a reserve of the acid in a transitory pool. Movement between (pulp) storage and metabolically active pools would control respiration rate of malic acid during berry ripening. During the day the temperature of grape berries is normally higher than ambient temperature (Smart and Sinclair 1976). Malate degradation is enhanced by increasing temperature (Lakso and Kliewer 1975), hence in the warm to hot climates in which these experiments were conducted, temperature effects on enzyme activity in both pulp and skin cells would be expected to influence acid levels negatively. Although a number of studies have shown the above effect of temperature on malic acid levels in grape berries, results here indicate that reactions favouring malic acid degradation are more active in pulp cells than those of the berry skin, where the balance in malate metabolism is towards accumulation. This would suggest that the compartmention of acids in skin cells might involve, in Ruffner's terminology, "true-storage-type cells". Differences in membrane permeability between cell type may explain this difference between the pulp and skin.

The ripening stage of berry growth is characterised by large increases in berry potassium; the accumulated potassium being

distributed into both pulp and skin. The differential accumulation observed for the variety 'Merlot' by Peynaud and Maurie (1953) is also evident in 'Shiraz' berries investigated in this study, potassium levels increasing 4 - fold in the skin (Exp 2 and 3) while corresponding increase in the pulp was in the order of 2-fold (Exp 2) and 3-fold (Exp 3), during the ripening period. For plants in general it is thought that potassium plays a physiological - biochemical role in enzyme activiation, membrane transport processes, anion neutralization and osmotic potential (Clarkson and Hanson 1980). However the specific role of potassium in pulp and skin tissue of grapes is uncertain. 50% of the chloride content of grape berries may be located in skin tissue (McCarthy and Downton 1981) and, since organic acid anions also accumulate in the skin during ripening, potassium movement into skin tissue may be associated with increased anion synthesis and uptake, as proposed by Hobson and Davies (1971) for ripening tomato fruit. This reasoning though does not apply for pulp alone or for the whole berry since for these the accumulation patterns for potassium and acid anions are opposite.

The low levels of sodium in both pulp and skin tissue are in line with the conclusion that potassium is the major univalent cation of the grape.

Concentrations based on fresh weight of individual tissue, show major differences between tissues. At maturity the concentration of tartrate, malate, potassium and sodium per fresh weight of skin may be in the order of 2, 8, 6 and 6 times greater than the respective value in the pulp (Table 2.4).

At maturity, the proportion of tartrate, malate, potassium and sodium in the skin of 'Shiraz' berries, sampled from various viticultural areas, was on average 19%, 31%, 38% and 27% respectively (Table 2.5). Extreme values indicate that about half of the berry's potassium may be contained in the skin tissue. In samples where pulp malate has been extensively remetabolised, the skin may then contribute up to 38% of this compound in the ripe berry.

Table 2.4. Concentration of tartrate, malate, potassium and sodium in either pulp or skin tissue of mature 'Shiraz' grapes, expressed on the basis, per fresh weight of individual tissue.

	PULP CONCENTRATION mg per g pulp	SKIN CONCENTRATION mg per g skin
Tartrate		
expt 2	5.7	8.7
expt 3	5.6	11.2
Malate		
expt 2	3.5	12.4
expt 3	1.3	10.3
Potassium		
expt 2	1.4	10.3
expt 3	2.0	11.0
Sodium		
expt 2	0.2	0.5
expt 3	0.05	0.3

Table 2.5. Proportion of the total amount of each compound (pulp plus skin content) that is located in either the pulp or skin separately.

	TARTRATE	MALATE	POTASSIUM	SODIUM
PULP Average	0.81	0.69	0.62	0.73
PULP Range	0.74 - 0.85	0.62 - 0.75	0.53 - 0.80	0.70 - 0.77
SKIN Average	0.19	0.31	0.38	0.27
SKIN Range	0.15 - 0.26	0.25 - 0.38	0.31 - 0.47	0.23 - 0.30

Samples from the different viticultural areas showed large variation in acid, cation and phenolic content, even though all were at approximately the same maturity level in terms of sugar concentration. This indicates that adequate sugar content in berry juice does not necessarily correlate with optimal levels for other berry components. Similar studies by Pirie (1977, 1979) showed that anthocyanin and phenolic content of berries were only related in a general way with juice sugar concentration. This variation in acidity components emphasises the problems experienced by Australian winemakers with unbalanced sugar : acid ratio in the raw material for winemaking.

Viticultural factors influencing the fate of the organic acids, cations and phenolic material in the ripening berry are reported in Chapter 3.

The proportion of these compounds, and additionally the degree of colouration and phenolic content in skin tissue of black grapes, has oenological implications since red wine is produced with skin contact where there is opportunity for the compounds present in the skin to enter and influence the chemical composition of the ferment and the resultant wine. The significance of this effect is discussed in Chapter 5.

The increase in pH of grape juice samples during berry ripening has been attributed, amongst other factors, to the conversion of the free acids to their salt forms (Winkler et al. 1974). Differential extraction with 80% ethanol showed that of the major organic acids in the 'Shiraz' grapes examined here only tartaric acid was converted to salt forms during ripening. In grape tissue the concentration of these tartrate salts may reach saturation, causing precipitation, particularly of the calcium salts (Ruffner, 1982a). The relative inertness of tartrate during berry ripening has been explained by this conversion to salt forms which are, it is thought, barely affected by metabolising enzymes (Saito and Kasai 1968). However, compartmentation into the vacuole during the acid accumulation stage, and subsequent storage there during ripening, may also account for the lack of remetabolism of tartaric acid after veraison. The increasing proportion of free tartaric acid in the skin tissue with ripening could be explained by additional acid synthesis in this tissue throughout the ripening

period. The ratio of salt form to free acid form of tartaric acid in the intact berry is important in setting the pH of juice expressed from that berry. The final juice pH depends on this ratio plus the amount of malic acid in berry cells. These aspects are discussed in Chapter 6.

CHAPTER 3

THE EFFECT OF SOME CULTURAL PRACTICES ON THE ACID, CATION AND PHENOLIC CONTENT OF PULP AND SKIN OF 'SHIRAZ' GRAPES DURING RIPENING.

CHAPTER 3

THE EFFECT OF SOME CULTURAL PRACTICES ON THE ACID, CATION AND PHENOLIC CONTENT OF PULP AND SKIN OF 'SHIRAZ' GRAPES DURING RIPENING

Abstract

Effects of canopy structure and vine water status on the amounts of the above components in the pulp and skin of ripening 'Shiraz' grapes were investigated. Ripe fruit from shaded canopy environments had higher potassium, malate and pH values in the berry pulp and decreased levels of total anthocyanins in the berry skin. Shaded conditions induced high potassium in the pulp of berries at veraison and this correlated positively with potassium levels in ripe berries. The interrelationship between improved vine water status and berry composition appeared dependent on the extent to which the applied treatment modified the canopy structure, possibly pre veraison.

CHAPTER 3

INTRODUCTION

The literature on the effects of cultural practices on grape composition is voluminous but difficult to interpret. The difficulties are of two types, a) those due to complexity of treatments where effects of variables may overlap e.g. improved water status leading to canopy shading and b) inadequate measures of the components of composition, e.g. without the individual organic acid and cation concentrations measurements of pH and titratable acidity are difficult to interpret.

A number of studies confirm that titratable acidity of juice of berries from irrigated vines was higher than from non-irrigated vines (Coombe and Monk 1979, Freeman et al. 1980, Freeman and Kliewer 1983, Hardie 1981, Neja et al. 1977, Van Zyl 1977, and Wildman et al. 1976), but despite the consistency of this response to irrigation, the same studies present conflicting data for juice pH. Juice of berries from irrigated vines may show increased pH (Freeman et al. 1980, Freeman and Kliewer 1983, and Hardie 1981), no significant change (Van Zyl 1977, Wildman et al. 1977) or decreased pH (Coombe and Monk 1979, Neja et al. 1977), when compared to non-irrigated vines. Irrigation responses are often complicated by differences in berry size and crop-load effects. The study of Coombe and Monk (1979) is of interest since increases in titratable acidity were achieved with no effect on berry volume, indicating that irrigation was influencing absolute amounts of the compounds studied.

Higher levels of malic acid are normally associated with improved water status, while tartaric acid content is only minimally affected (Hardie 1981, Seguin 1978). Irrigation management has been suggested as a means of regulating juice potassium levels, since imposed water stress delayed potassium accumulation in grape berries during the final period of ripening (Freeman and Kliewer 1983). In this work (Freeman and Kliewer 1983) a positive correlation between juice pH and potassium concentration was established. Similar results were obtained by Hardie (1981) who maintained 'Zinfandel' vines under three different water regimes during the period flowering to harvest and those vines receiving maximum irrigation showed highest levels of pH, potassium, sodium and malate in juice of berries from that treatment.

Temperature and solar radiation are the most important climatic factors influencing acid, cation and phenolic content of berries. Decreased concentrations of both malic acid and phenolic material are normally associated with high temperature conditions during berry ripening (Kliewer and Lider 1970, Kliewer 1968, Buttrose et al. 1971, Kliewer 1977). Shaded canopy conditions have been implicated in increased potassium uptake by the berry (Freeman 1982, Smart et al.1981, Smart et al.1984), and in one study these conditions also increased

juice pH, and malic acid concentrations (Smart 1982). Pirie (1977) has shown that both leaf and cluster exposure are important in promoting anthocyanin synthesis in grape berries.

As previously mentioned a problem in interpreting grape acidity studies, where either environmental conditions or vine water status have been adjusted, is that concentrations of all acidity components have rarely been determined in the one study; a notable exception was that by Hardie (1981). In this chapter the effects of canopy modification and vine water status on the organic acid, cation and phenolic content of 'Shiraz' grapes during ripening were investigated. Since these components are unevenly distributed between the pulp and skin of black grapes, measurements were made of treatment effects in these separate parts.

3.1. MATERIALS AND METHODS

3.1.a. Expt 1: Modification of canopy structure

3.1.a.i. Thinning treatment: A thinning treatment (both shoots and clusters) aimed at producing a more open canopy, was applied to 'Shiraz' vines in the Alverstoke vineyard at the Waite Agricultural Research Institute, Adelaide, during the 1980 season. Vine characteristics are described in section 2.1.b.ii.

Two treatments, normal and thinned, were allotted to single vine plots. The thinning treatment was applied just after berry set, when berry diameter was 5-6mm, shoot length 2.5-3 meters, with 18-24 internodes longer than 7mm. Thinning was achieved by removing every other shoot, then removing clusters until the remaining number equalled a third of the initial cluster number. The remaining shoots were then topped approximately 1 meter above the top cluster.

3.1.a.ii. Sampling procedure: A random sample of 20 berries was taken from each vine at three stages of maturity termed beginning (7-9°Brix), mid (15-17°Brix) and ripe (22-24°Brix). Additionally a collective 40-berry sample was taken for each treatment from pairs 1-4 and 5-7 at several other times during berry ripening.

3.1.b. Expt 11: Adjustment of vigour and vine water status

3.1.b.i. Treatments: Varying levels of irrigation were applied to 13 year old, spur-pruned 'Shiraz' vines in the Claremont vineyard at the Waite Agricultural Research Institute, Adelaide, during the 1980 season. Vines were trellised on a divided canopy with emphasis on upward shoot positioning (Coombe 1974). Advantage was taken of a gradient in vine water status which occurs along the rows in this vineyard, due to the presence of large gum trees at one end of the vineyard; vines closest to these gum trees have noticeably lower capacity, lower vigour and a more open canopy. Three blocks of six vines were established; one block being close to the trees (stressed treatment), another block in the middle of the row (normal treatment),

and the third farthest from the trees (irrigation treatment). To exaggerate the difference in vigour and water status between these blocks the following treatments were added: (1) the soil surface beneath the stressed block was covered with black plastic to disperse rain (2) the normal block was untreated and (3) a basin was dug around the vines in the irrigation block, and three basin irrigations of 60-80mm were applied on three occasions during ripening.

3.1.b.ii. Sampling procedures: In each plot three vines were selected as sample vines. Each vine was sampled (20-berry random sample) at the three stages described in Exp. 1.

3.1.c. Expt. 111: Adjustment of vine water status

3.1.c.i. Treatments: During the period veraison to maturity, two levels of irrigation, none and frequent, were applied to 'Shiraz' vines in a commercial vineyard at Virginia in South Australia during the 1981 season. Vineyard characteristics are described in Section 2.1.b.iii.

All vines were drip irrigated according to normal commercial practice prior to veraison. The non-irrigated (NI) treatment was established by blocking drippers over a section of the vineyard and then selecting three well barriered four-vine plots in this area. Adjacent to this area three four-vine plots were established in rows receiving normal drip irrigation treatment. Vines in these watered plots received water by the drip system four times during ripening. The rate varied between 100-150L per vine each time depending on the duration of irrigation cycle. Additional water was applied in basins at the rate of 45L per plot, twice weekly.

3.1.c.ii. Sampling procedure: Each plot was sampled (50-berry random sample) once weekly during the period veraison to maturity.

3.1.d. Analytical procedures (for all experiments)

Sample berries were divided into pulp (without seeds) and skin tissue and each portion analysed for organic acid, cation and phenolic

content. Extraction and analysis methods were the same as those described in Section 2.1.

3.1.e. Statistical analysis: Differences in amounts and concentration of individual components in either pulp or skin tissue were analysed by two way ANOVAR for Expt. 1 and 11 and one way ANOVAR for Expt. 111.

3.1.f. Measurements of vine water status: Leaf xylem water potential was measured with a pressure chamber, at three stages during ripening, (beginning, mid and ripe) for both Expt. 11 and Expt. 111. Diurnal fluctuation in leaf water potential for NI and I vines was determined for vines at the ripe stage during Expt. 111. Exposed, mature leaves were sampled and stored in plastic bags held in an esky containing ice, until measurement within ½ hour from sampling.

3.1.g. Defining canopy microclimate:

No quantitative comparison was made of canopy microclimate for Expt. 1 and Expt. 11 during the sampling period, but during a subsequent season, assessment of microclimate was carried out on the same vines used for the S, N, and I treatments in Expt. 11. Canopy structure was visually similar to that present during the 1980 season, and a check sampling of mature berries showed consistent results to those obtained for the 1980 season. Because of the nature of the trellis in the Claremont vineyard, the point quadrat system used by Smart (1982) was modified: an assessment of berry exposure was obtained by setting the probe at a cluster position and then pointing it vertically skywards and recording contacts with leaves. Percent frequency of contact for first and second contacts were constructed in the manner described by Smart (1982).

In Expt. 111 the unmodified point quadrat method was used to define canopy differences for the NI and I vines in Expt. 111 at two stages (beginning, and ripe) of ripening during the season that compositional measurements were taken i.e. the 1981 season.

3.2. RESULTS

3.2.a. Expt. 1: Modification of canopy structure.

Canopy structure was assessed visually during maturity and thinned vines had a more open canopy and greater cluster exposure. This effect was established from just after berry set, and maintained until ripeness. Some lateral growth occurred but it was not extensive enough to cause significant change in cluster exposure (assessed visually).

The yield of the thinned vines was, as expected, lower than that of normal vines (Table 3.1). This was mainly due to reduction in cluster number since berry weight was not significantly different between treatments. Berry number per cluster was not determined but this would not be expected to be a variable since treatments were applied after set and this yield component would have been already determined.

Table 3.1. Yield data for normal and thinned vines in Expt. 1.

	Normal		Thinned		L.S.D.
	mean	s.d.	Mean	s.d.	5%
Yield (per vine)	10.8 ±	4.4	4.0 ±	1.1	4.4
Berry weight (g)	1.52 ±	0.06	1.57 ±	0.11	n.s.

°Brix measurements indicated slight increase in sugar accumulation due to the thinning treatment (Fig 3.1), hence °Brix and not time was used as the independent variable in comparing treatments. The stages A, B and C described in the figures refer to 7-9, 15-17 and 22-24 °Brix respectively.

The pH of the pulp homogenate and potassium content (amount in the pulp/g berry weight) of ripe berries were both reduced ($p < 0.05$) by the thinning treatment (Fig 3.2 and 3.3). These differences would be

further increased if the measurements had been made at a slightly higher °Brix for the normal vines. There was no effect on tartrate or malate content in the pulp of ripe berries , and further, no effect on the acid or cation levels in the skin of ripe berries (Figs 3.3 and 3.4).

Thinning treatment significantly increased skin anthocyanin and phenolic content ($p < .01$ and $p < .001$ respectively), (Fig 3.4). Differences developed mainly during the second half of berry ripening. Total phenolics were similar at the mid ripening stage, although differences in anthocyanin content were apparent then.

3.2.b. Expt. 11: Adjustment of vine vigour and water status.

Vine leaf xylem potential decreased (increasing negative value) with ripening for all treatments, and at all stages of ripening the stressed vines had significantly more negative potentials than both the normal and irrigated vines (Fig 3.5). Differences were already established at the beginning of ripening. There was no difference between normal and irrigated vines.

Leaf density above clusters was lowest in the stressed vines (Fig 3.6). The sum of the frequency of the first two contacts for gaps was 112, 45 and 46 for the stressed, normal and irrigated vines respectively.

Berries from normal and irrigated vines reached the same level of ripeness (23° Brix) about 6 days later than those from the stressed vines. Final berry weight was not significantly different between treatments (Fig 3.7).

Berries from stressed vines had significantly lower levels of pulp potassium, expressed either as mg in the pulp/g berry wt. ($p < 0.05$) or mg in the pulp/berry ($p < 0.05$), than berries from either normal or irrigated treatments (Figs 3.8 and 3.9). Potassium levels of whole berries (pulp + skin) at veraison was correlated positively with levels in whole berries at maturity (Fig 3.10). Pulp pH was significantly lower in the stressed treatment ($p < 0.05$) (Fig 3.11).

Malate content (mg in the pulp/g berry wt.) of berries from irrigated vines was significantly higher ($p < 0.05$) than for berries from normal and stressed vines; however on a per berry basis this variable was significantly different only between irrigated and stressed vines (Fig 3.8 and 3.9). Decrease in absolute amounts of malic acid was delayed in the normal and irrigated treatments, and did not occur until about 17° Brix for the berries from irrigated vines (Fig 3.9).

Anthocyanin content of ripe berries from stressed vines was significantly higher than the other treatments, however there were no differences in total phenolic content of berries between treatments (Table 3.2).

Table 3.2. Amounts of anthocyanins and total phenols in the skin of ripe 'Shiraz' grapes.

	Anthocyanins		Total Phenols (gallic acid equivs)	
	mg/g berry wt	mg/berry	mg/g berry wt	mg/berry
Expt 11				
stressed	2.15	2.87	2.21	2.96
normal	1.83	2.38	2.07	2.69
irrigated	1.76	2.33	2.25	2.99
$LSD_{5\%}$	0.40	0.46	ns	ns
Expt 111				
non-watered	1.88	2.22	2.55	3.01
watered	1.61	2.14	2.25	3.00
	ns	ns	ns	ns

3.2.c. Experiment 111. Adjustment of vine water status.

Vine water status was monitored by measurement of leaf water potential at various times during maturity (Fig 3.12). No difference was apparent at the beginning of ripening, but then the vines receiving

no water became significantly more stressed (more negative water potentials) than vines receiving liberal water supply. The large negative water potential for vines from both treatments at the early stage of ripening were probably due to the fact that no water had been applied to either treatment for ten days prior to measurements being taken. During the previous two days before measurement of leaf water potential, temperature conditions were extreme, with daily maxima of 38.5°C and 39.5°C respectively.

Diurnal variation in leaf water potential (Fig 3.12) showed that non watered vines reached maximum water stress quicker and took longer to recover than well watered vines; however the maximum levels reached in both treatments indicate that stomatal closure may have occurred, probably limiting photosynthesis in both sets of vines for at least some period during the day.

Assessment of canopy shading by the point quadrat method showed no large difference between the non-waterd and watered vines either at veraison or at maturity (Fig 3.13). All vines had been topped at about veraison to facilitate vineyard management. This was carried out before any of the treatments for Expt. 111 were applied. The sum of the frequency of the first two contacts for clusters was 26 and 28 for non-watered and watered vines at veraison respectively. At maturity these measurements were 33 and 28 respectively.

There was no difference in ripening time between treatments (Fig 3.14) suggesting that non-watered vines were photosynthesising efficiently during this period; however at maturity leaves on the non-watered vines were showing stress symptoms, and leaf senescence was also greater in these vines. This is reflected by the higher value for cluster exposure of stressed vines at maturity.

Irrigation treatment significantly ($p < .05$) increased berry weight (Fig 3.15).

The only significant compositional differences occurred in the pulp and not the skin. Irrigation decreased both pulp potassium content,

(amount in the pulp/g berry weight) ($p < .05$) (Fig 3.16) and the pH of the pulp homogenate ($p < .05$) (Table 3.3). These differences reflect the variation in berry size, since the concentration of components on a per berry base were similar (Fig 3.17). The increase in skin tartrate was more regular in berries from well-watered vines (Fig 3.17).

Table 3.3 pH values of pulp homogenate of 'Shiraz' grapes from non-watered and watered vines (Expt 111)

	Pulp pH		
Replicate	non-watered	watered	L.S.D. 5°/°
1	3.63	3.51	
2	3.54	3.44	
3	3.57	3.49	
mean	3.58	3.48	0.09

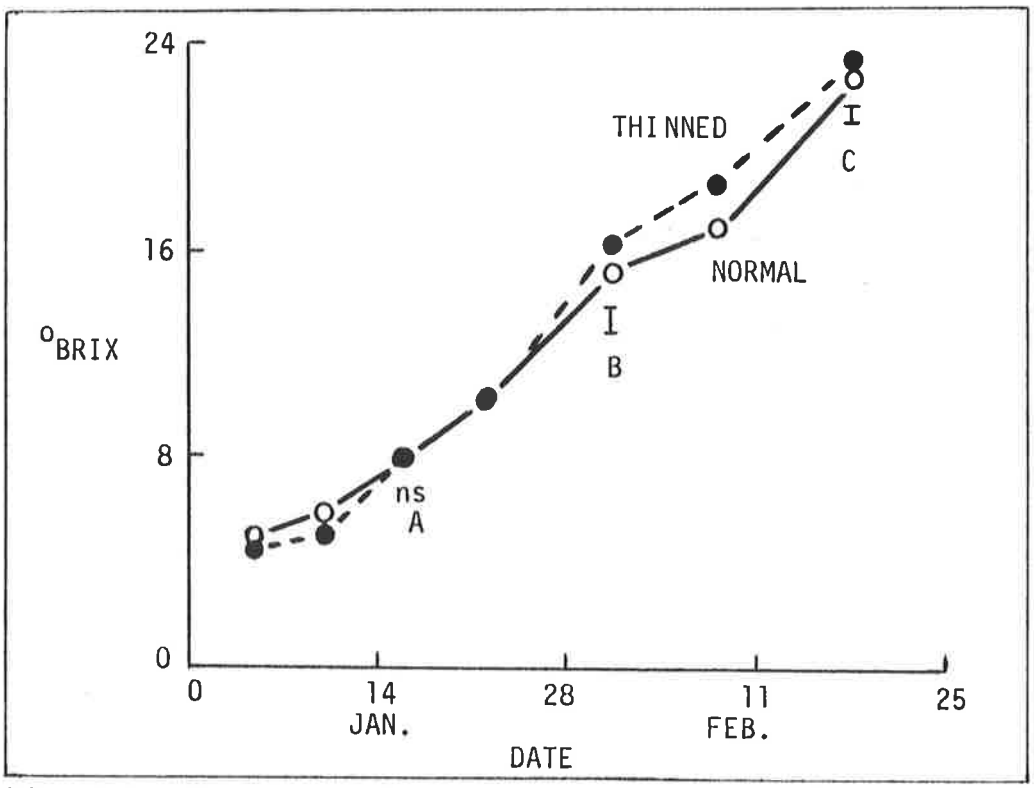

Fig. 3.1. Sugar accumulation of 'Shiraz' grapes from thinned (●) and normal (O) vines (Expt 1). A, B and C represent the beginning, mid and ripe stage of ripening and each of these points is the mean of 7, 7 and 6 individual vine measurements respectively. All other points are the average of two composite samplings. Vertical bars represent LSD values at the 5% level.

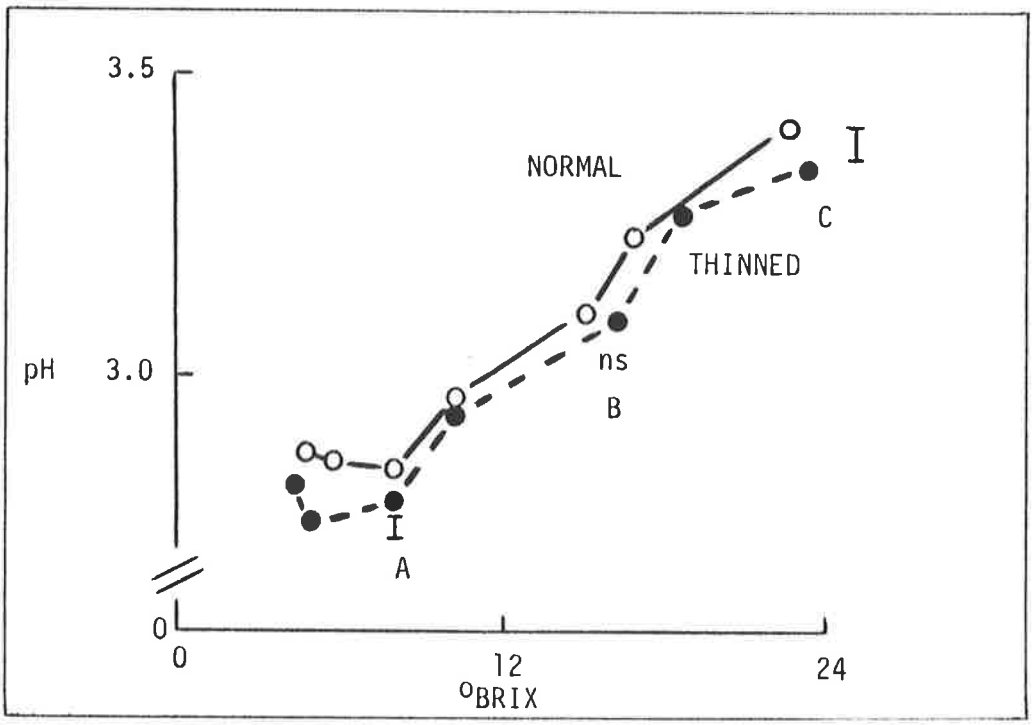

Fig. 3.2. Change in pulp pH of 'Shiraz' grapes from thinned (●) and normal (O) vines during ripening (Expt 1). Symbols are similar to those described in Fig. 3.1.

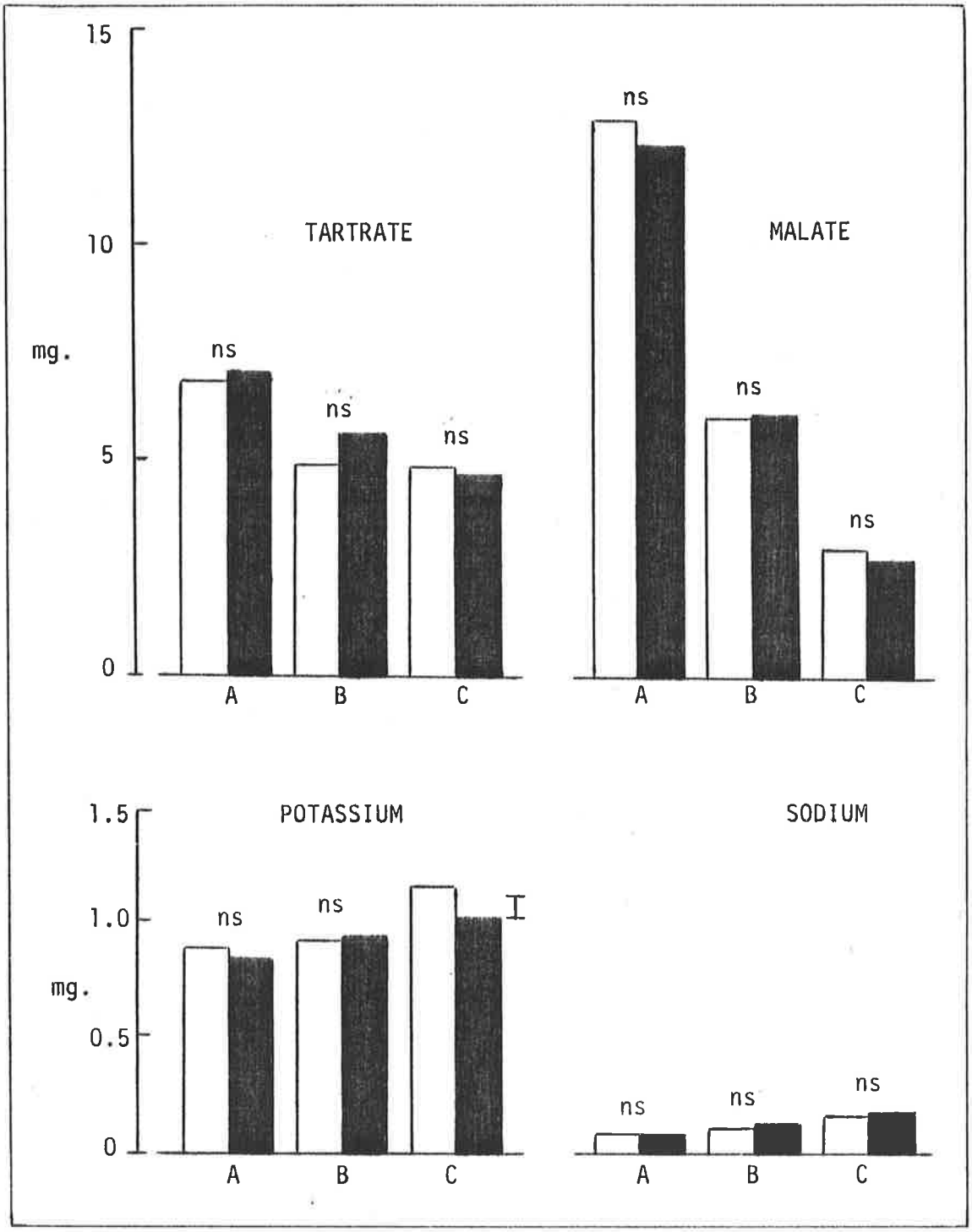

Fig. 3.3. Composition of the Pulp (mg. component in the pulp/g berry weight) of 'Shiraz' grapes from thinned ■ and normal ▭ vines at three stages of ripeness. A, B and C represent the beginning, mid and ripe stage of ripening and each of the mean values are from 7, 7 and 6 individual vine measurements respectively. Vertical bars represent LSD values at the 5% level.

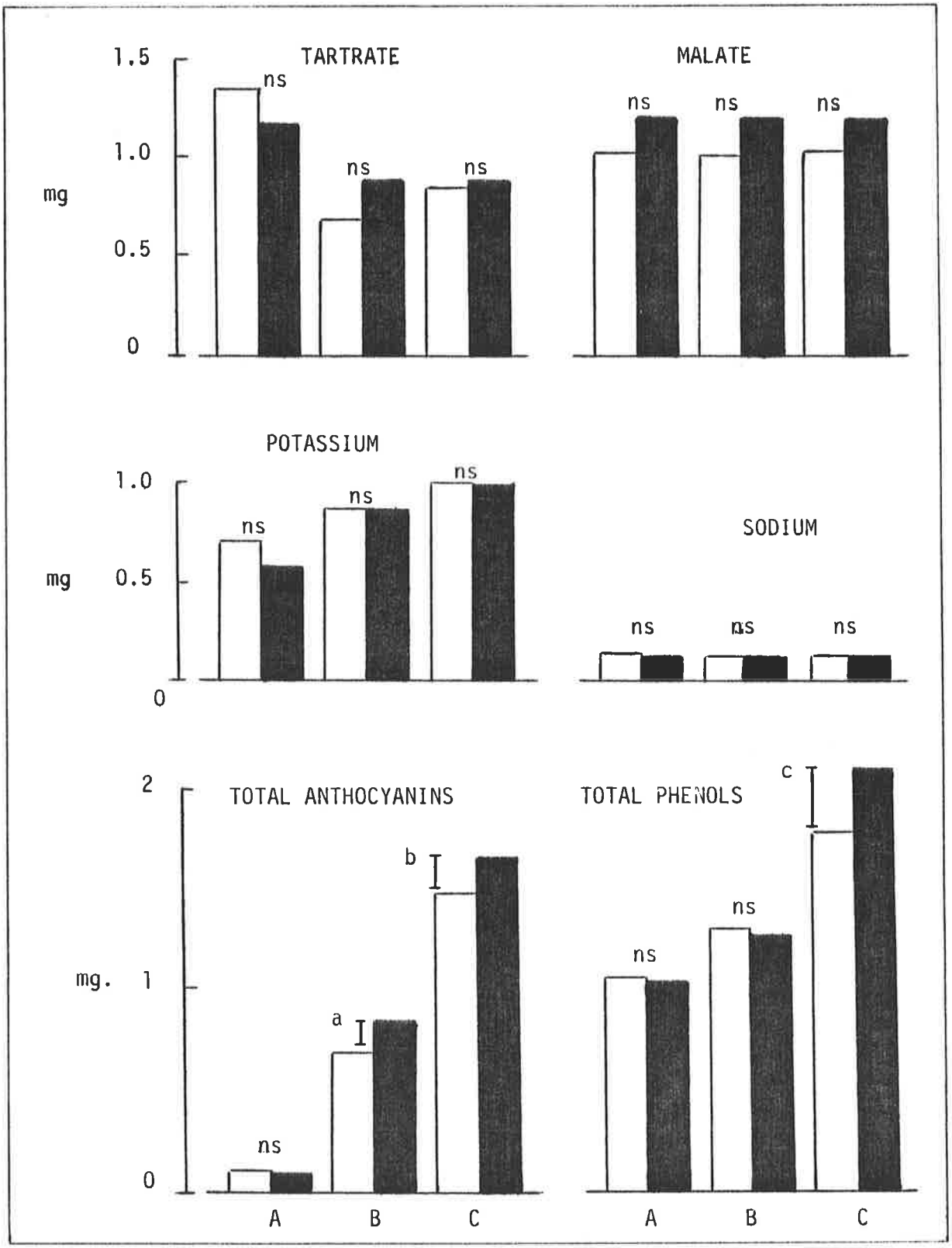

Fig. 3.4. Composition of the skin (mg. component in the skin/g. berry weight) of 'Shiraz' grapes from thinned ■ and normal □ vines at three stages or ripeness. Symbols are similar to those described in Fig. 3.3. Vertical bars represent LSD values at a) 5%, b) 1% and c) 0.1% levels respectively.

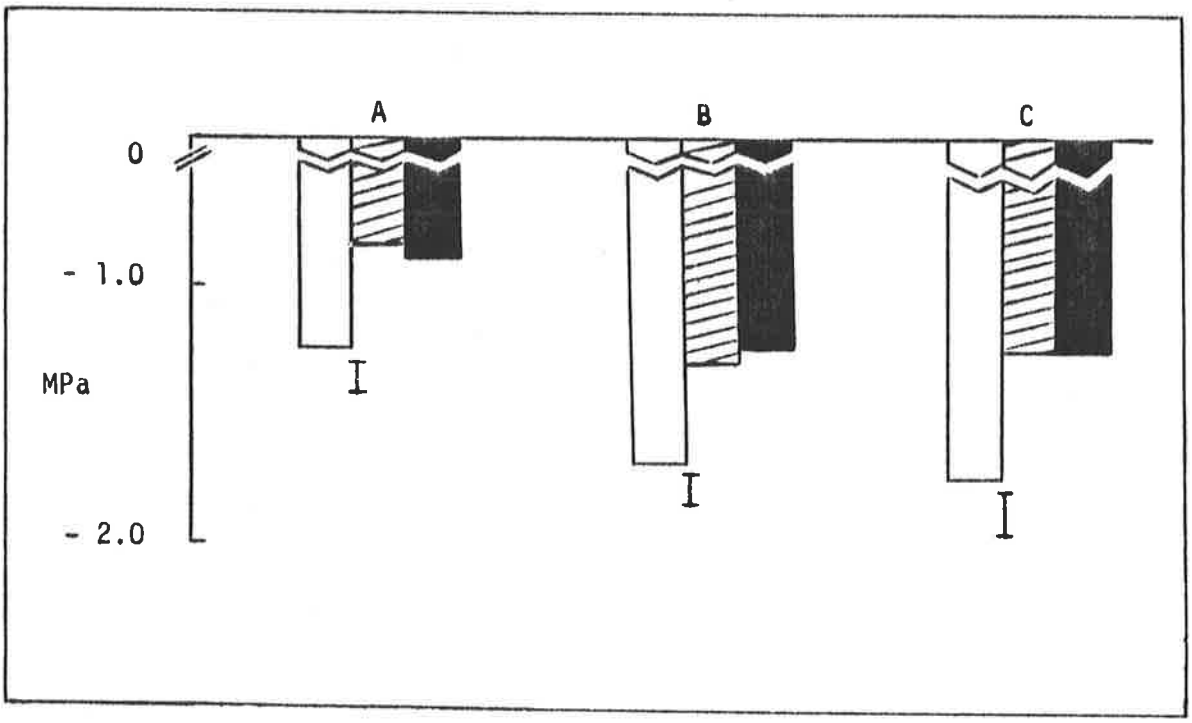

Fig. 3.5. Vine leaf xylem potential of stressed (S, □), normal (N, ▨) and irrigated (Ig, ■) 'Shiraz' vines at 3 stages of ripening (Expt. 11). A, B and C are the beginning, mid and ripe stage of ripening. Each value is the mean of six measurements (two per vine). Vertical bars represent LSD values at the 5% level. Measurements were taken at dusk.

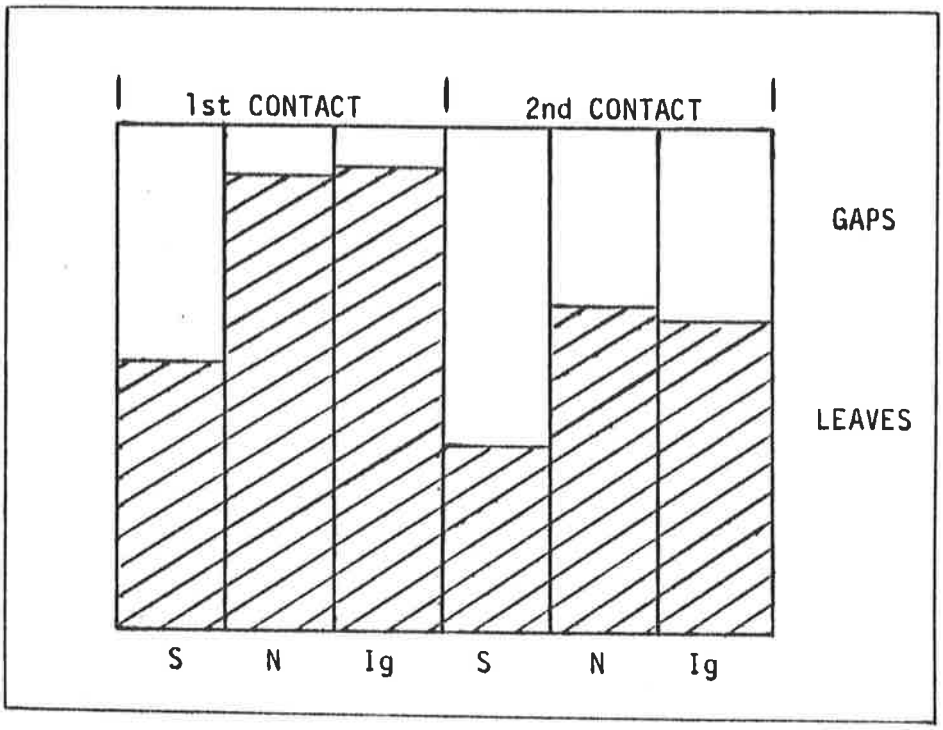

Fig. 3.6. Results of modified point quadrat analysis for 'Shiraz' vines used in stressed (S), normal (N) and irrigated (Ig) treatments (Expt. 11). Measurements taken at the ripe stage in the 1982 season.

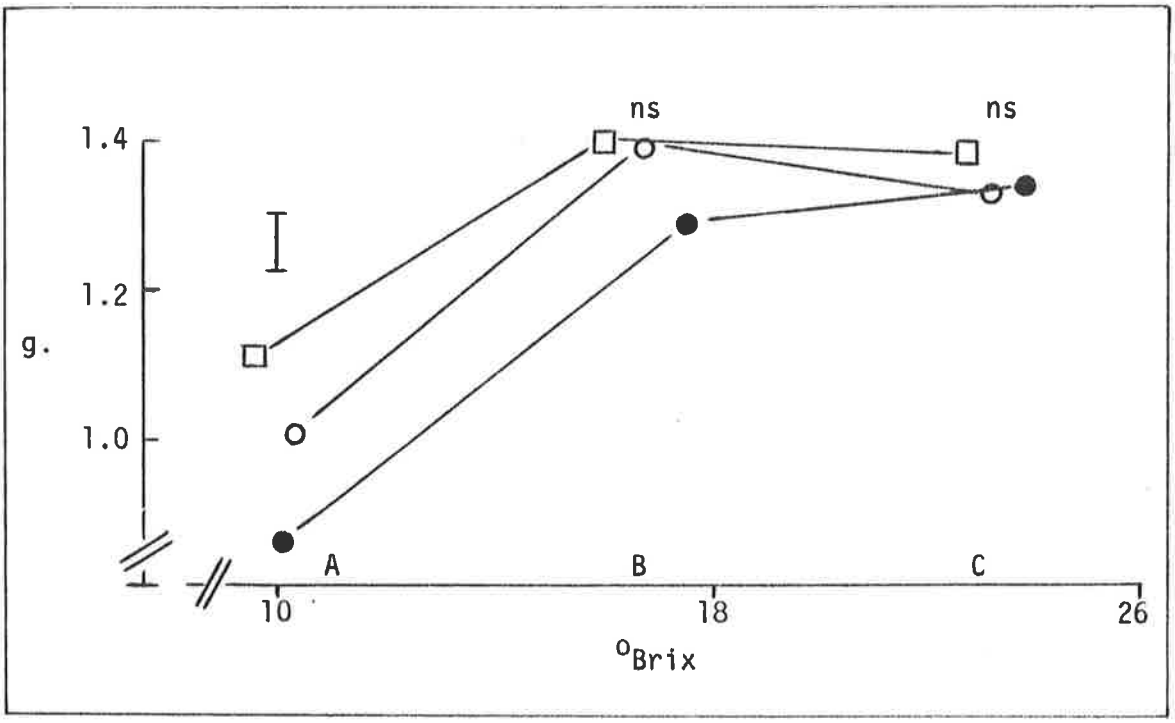

Fig. 3.7. Change in berry weight (g) of 'Shiraz' grapes from stressed (●), normal (□) and irrigated (○) vines (Expt. 11). Each point is the mean of measurements from 3 vines. A, B and C represent the beginning, mid and ripe stage of ripeness. Vertical bars represent LSD values at the 5% level.

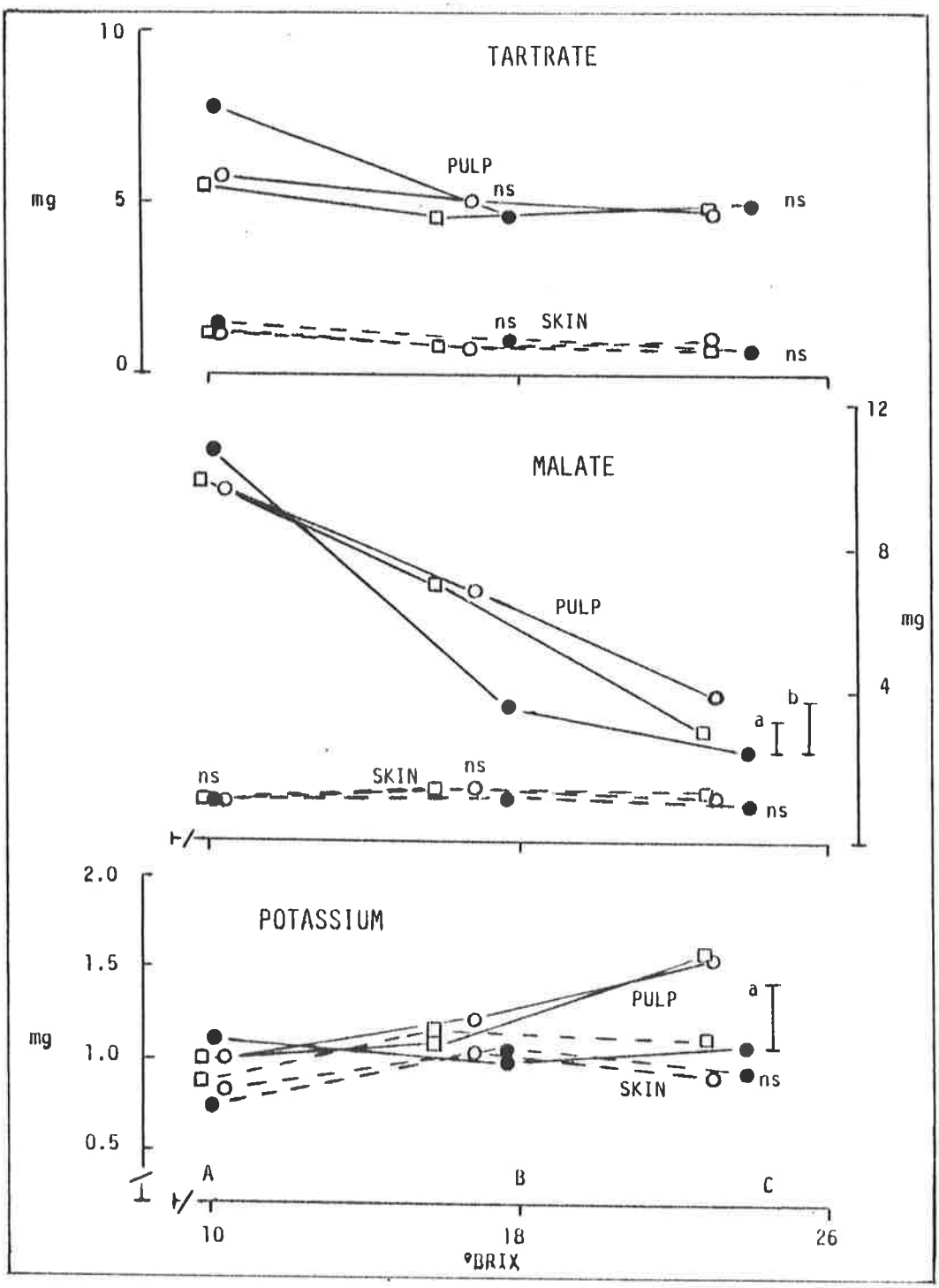

Fig. 3.8. Changes in amounts (mg/g berry weight) of tartrate, malate and potassium in pulp (_____) and skin (------) of 'Shiraz' grapes from stressed (●), normal (□) and irrigated (○) vines (Expt. 11). A, B and C represent the beginning, mid and ripe stage of ripeness. Each point is the mean of measurements from three vines. Vertical bars represent LSD at a) 5% and b) 1% levels respectively.

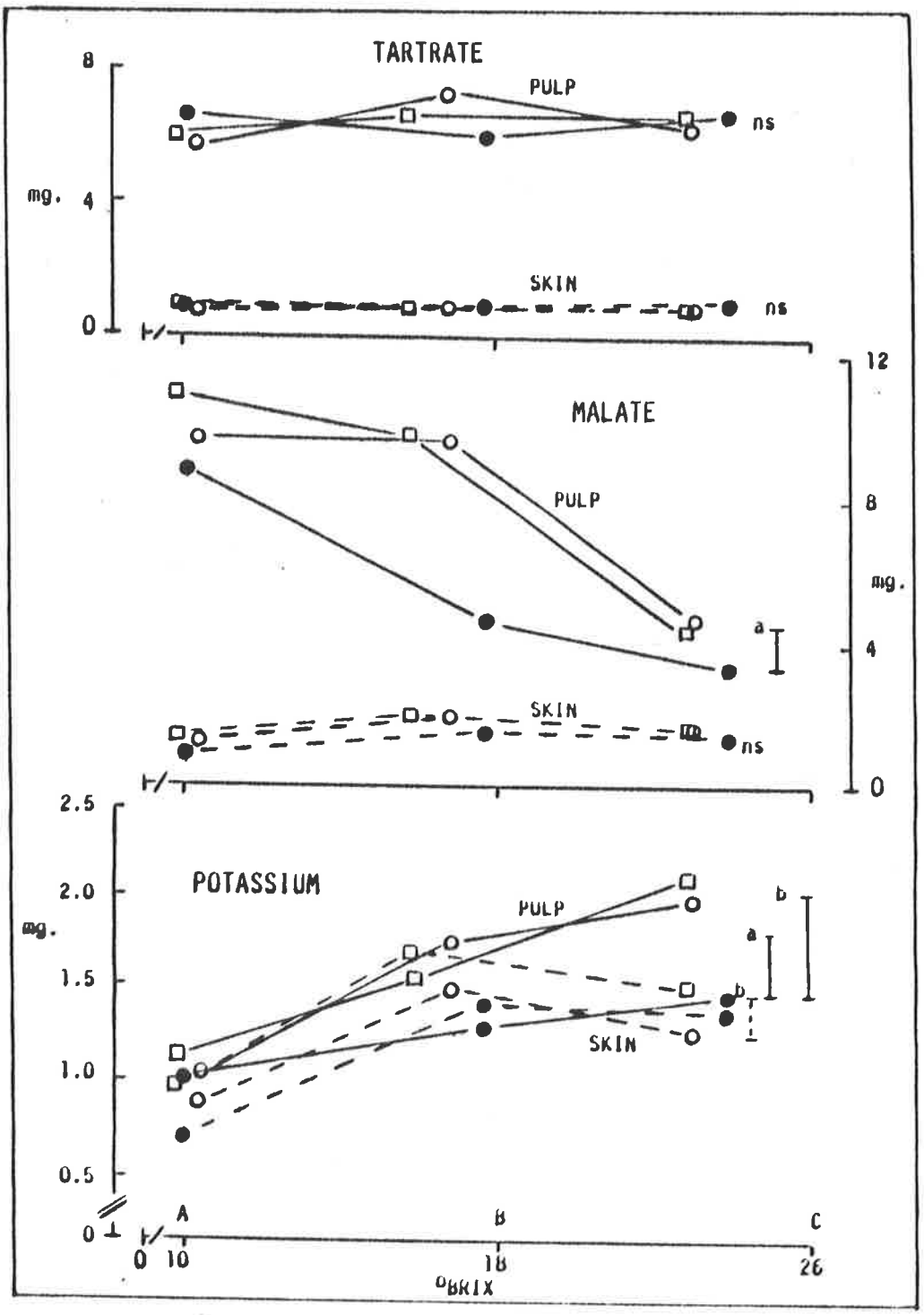

Fig. 3.9. Changes in amounts (mg/berry) of tartrate, malate and potassium in pulp (———) and skin (------) of 'Shiraz' grapes'(Expt 11). Symbols as for Fig. 3.8.

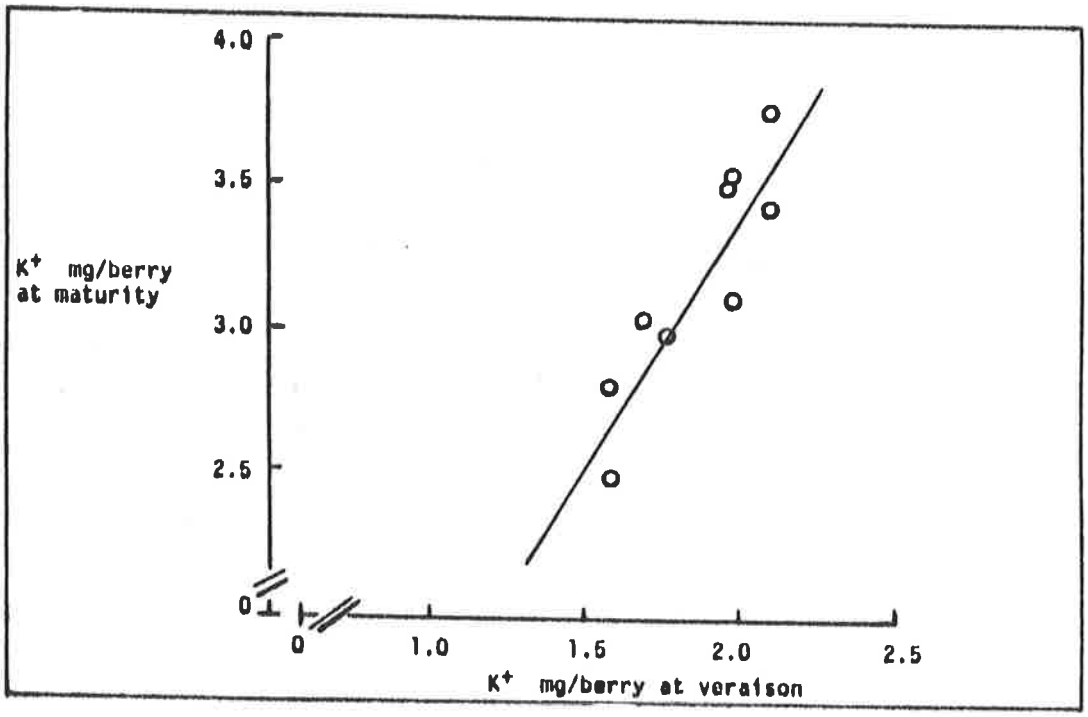

Fig. 3.10. Relationship between berry potassium content of 'Shiraz' grapes at veraison and at maturity.
($Y = 1.80X - 0.19$, $R^2 = 0.82$ ***)

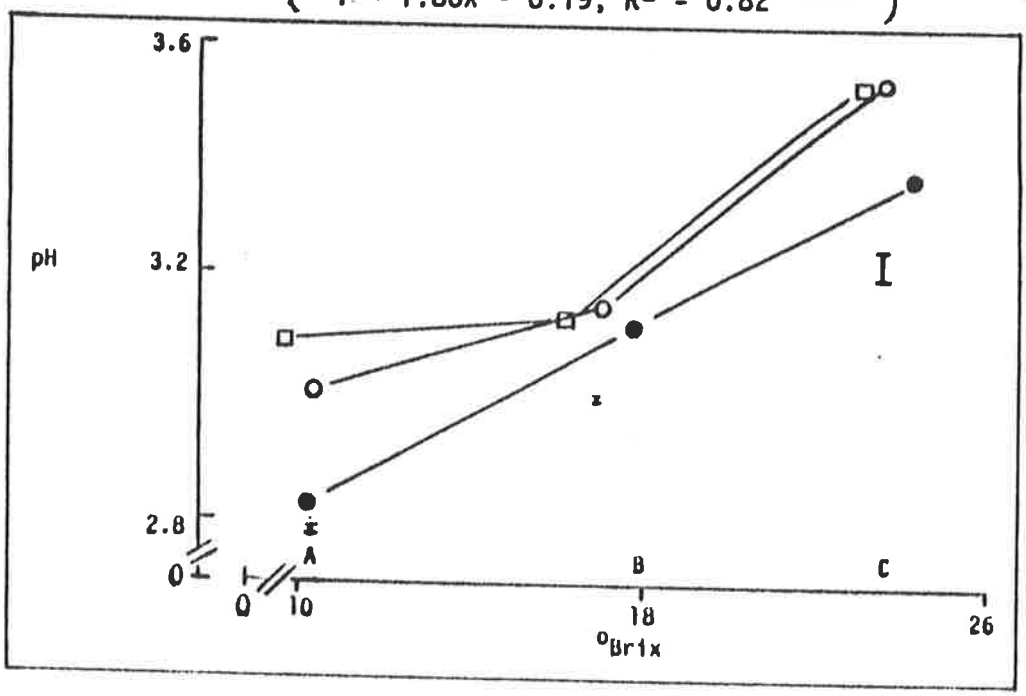

Fig. 3.11. Change in pulp pH of 'Shiraz' grapes from stressed (●), normal □ and irrigated (○) vines (Expt. III). Symbols as for Fig. 3.7.

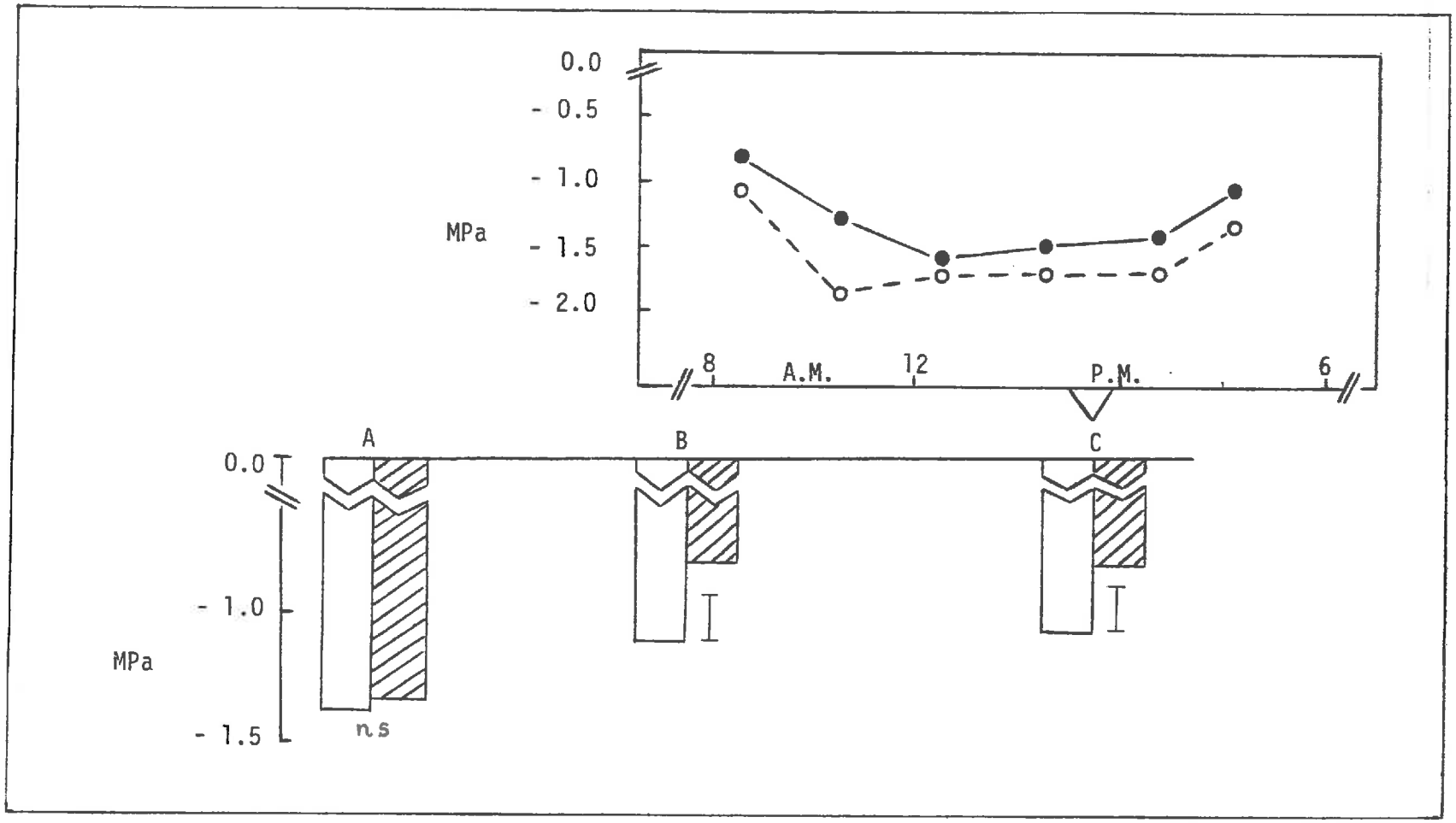

Fig. 3.12. Vine leaf xylem potential of non-watered (□ 0-0) and watered (▨ ●—●) 'Shiraz' vines at 3 stages of maturity. A, B and C are the beginning, mid and ripe stage or ripening. Diurnal variation for each set of vines is shown at the ripe stage. For both figures mean values are from 3 measurements. Vertical bars represent LSD values at the 5% level.

Measurements were taken between 7 am and 8 pm. The low ψ at stage A could be explained by the fact that both treatments had not been watered for one week, during which it was excessively hot (temp > 40°) and dry. Raintall occured between stage A and B.

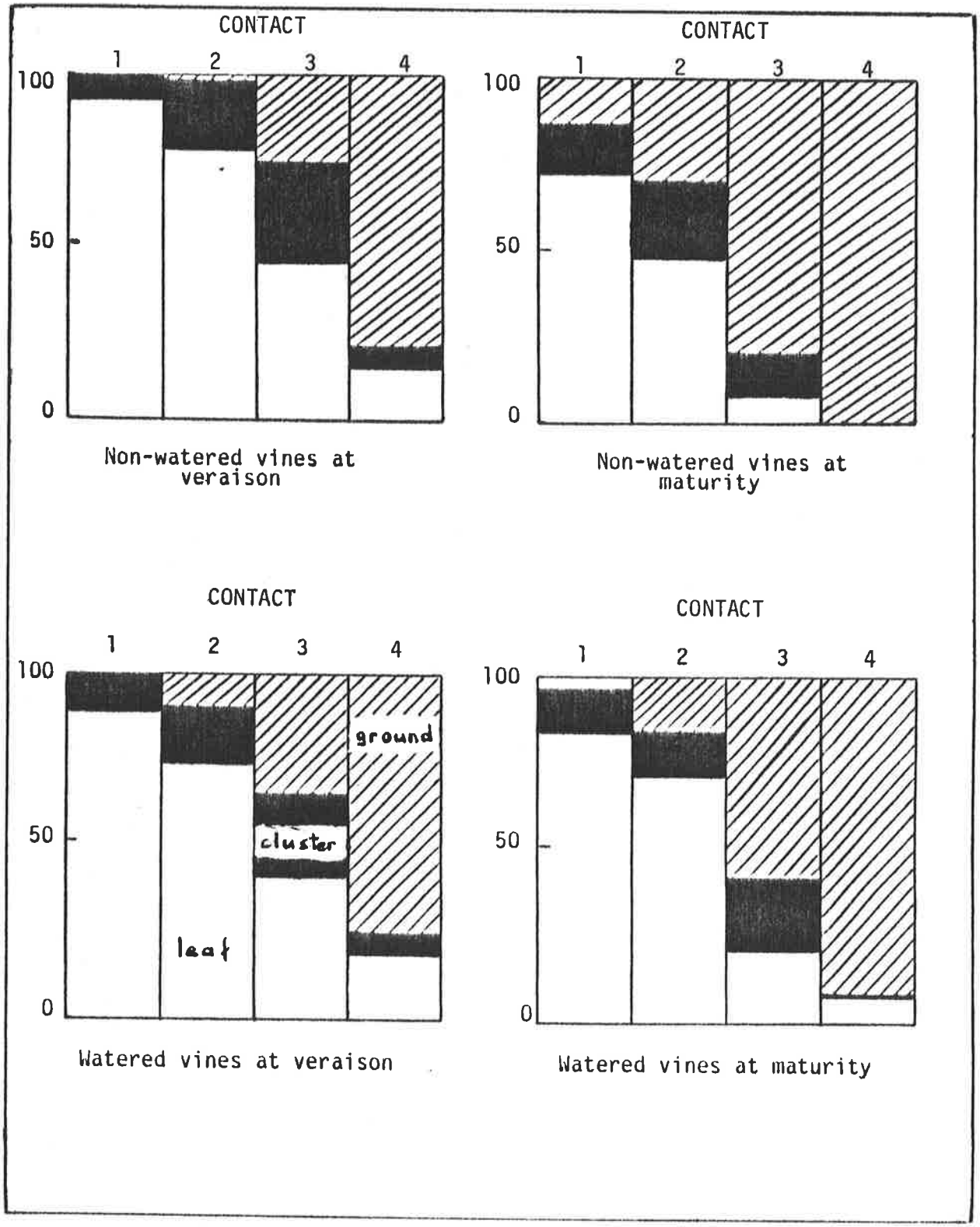

Fig. 3.13. Results of point quadrat measurements for Expt. 111.

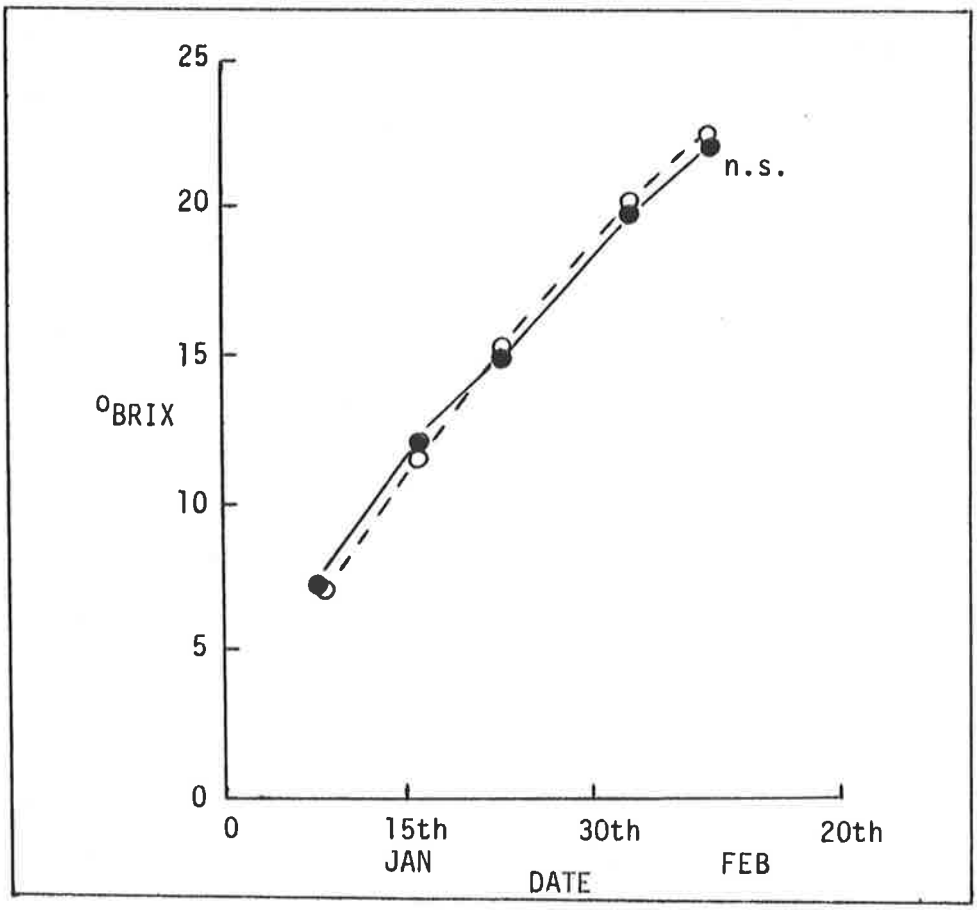

Fig. 3.14. Sugar accumulation (°Brix) for 'Shiraz' grapes from non-watered (-----) and water (———) vines (Expt.3). Each point is the mean of three values.

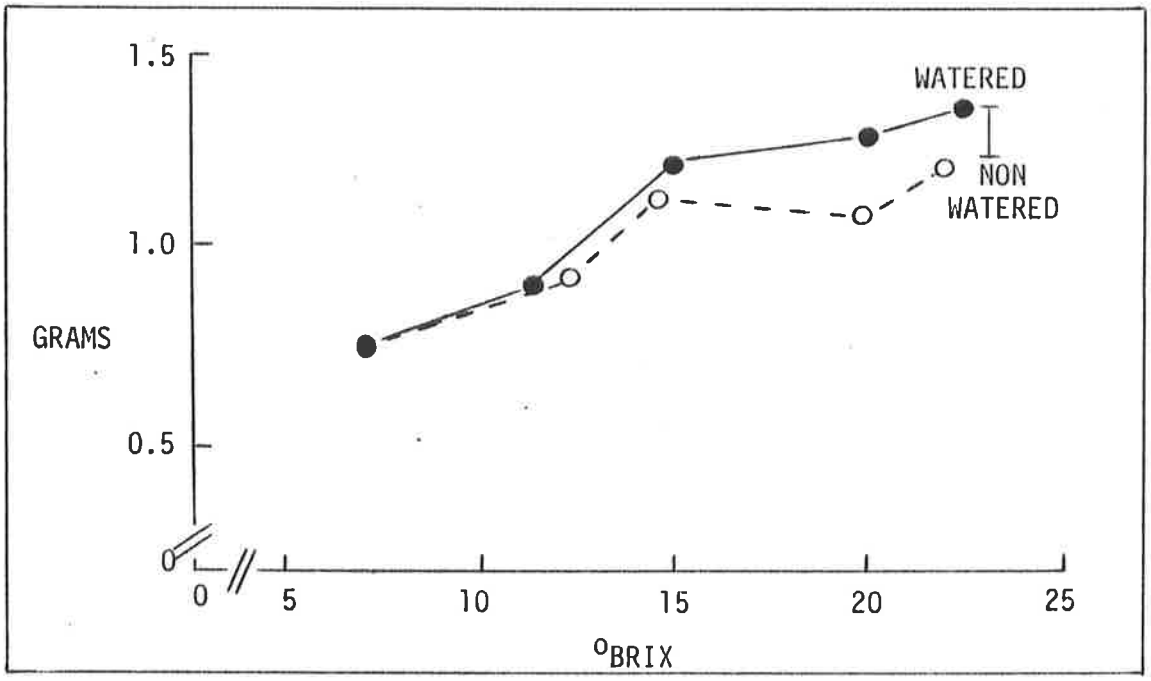

Fig. 3.15 Change in berry weight of 'Shiraz' grapes from non-watered (-----) and watered (———) vines. (Expt. 3). Each point is the mean of 3 values. Vertical bars represent LSD values at the 5% level.

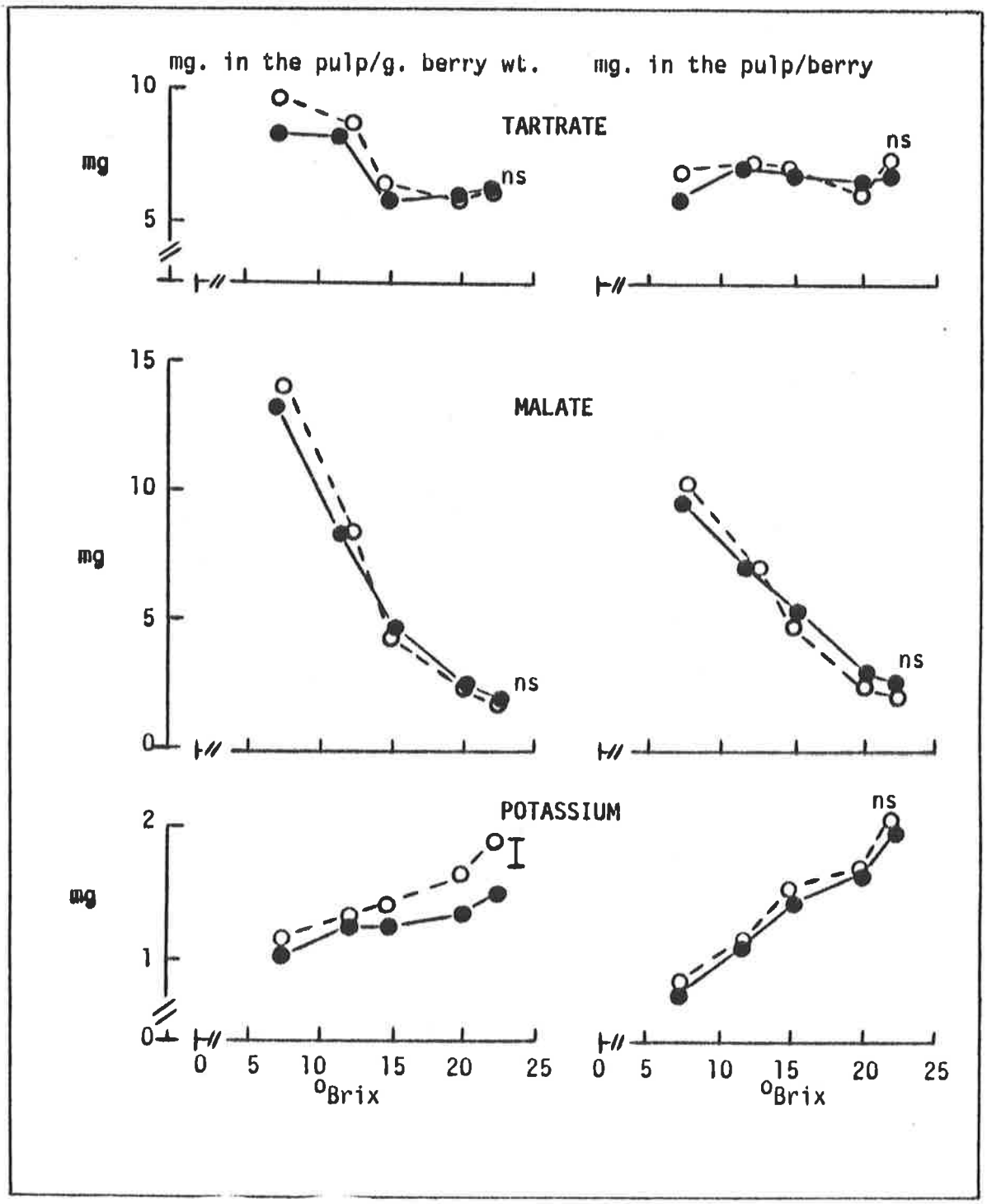

Fig. 3.16. Changes in amounts (left: mg. in the pulp/g. berry wt.; right: mg. in the pulp/berry) of tartrate, malate and potassium in the PULP of 'Shiraz' grapes from non watered (----) and water (——) vines during ripening (Expt. 3). Each point is the mean of three values. Vertical bars represent LSD values at the 5% level.

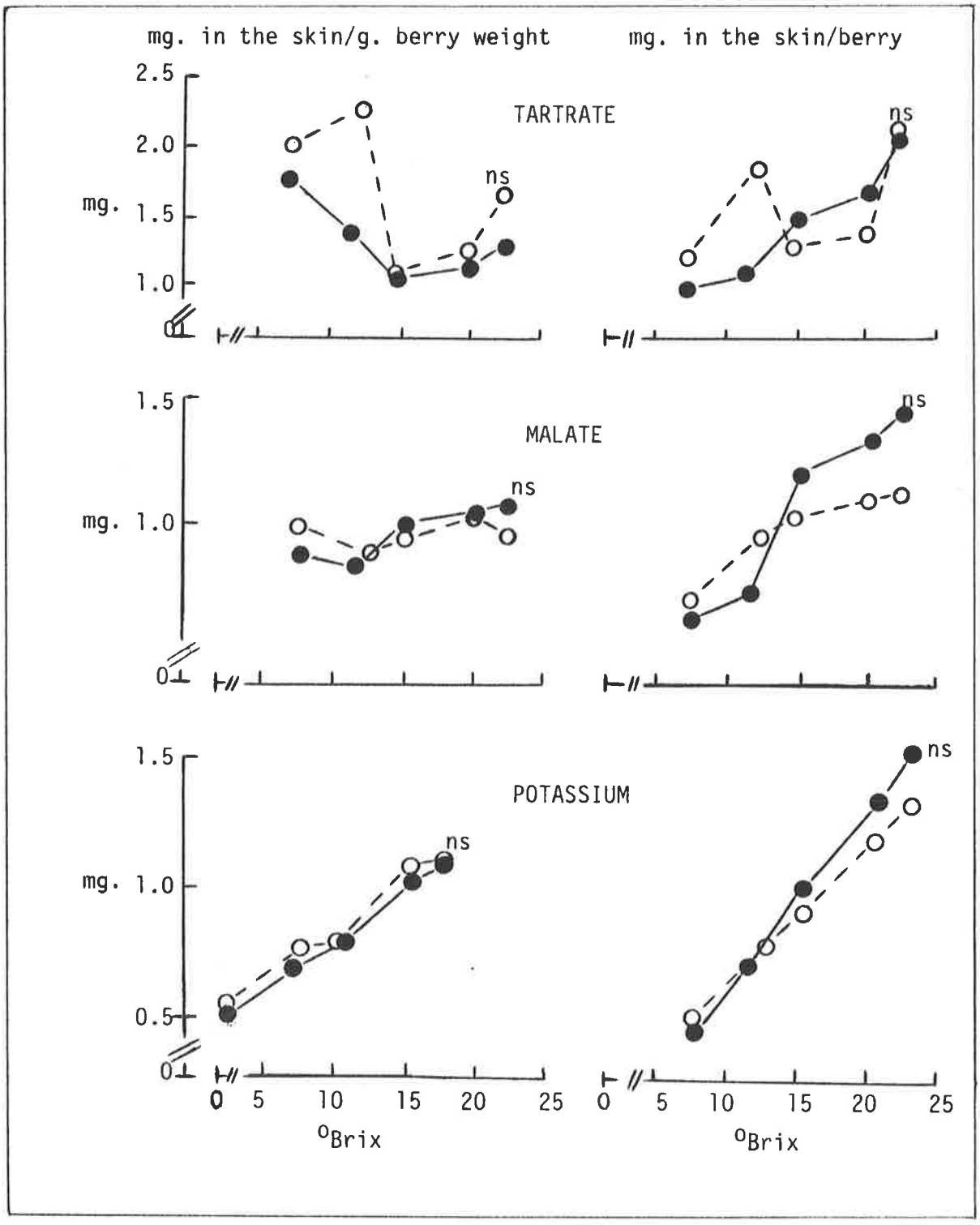

Fig. 3.17. Changes in amounts (left: mg. in the skin/g. berry weight; right: mg. in the skin/berry) of tartrate, malate and potassium in the SKIN of 'Shiraz' grapes during ripening (Expt. 3). Symbols are the same as for Fig. 3.16.

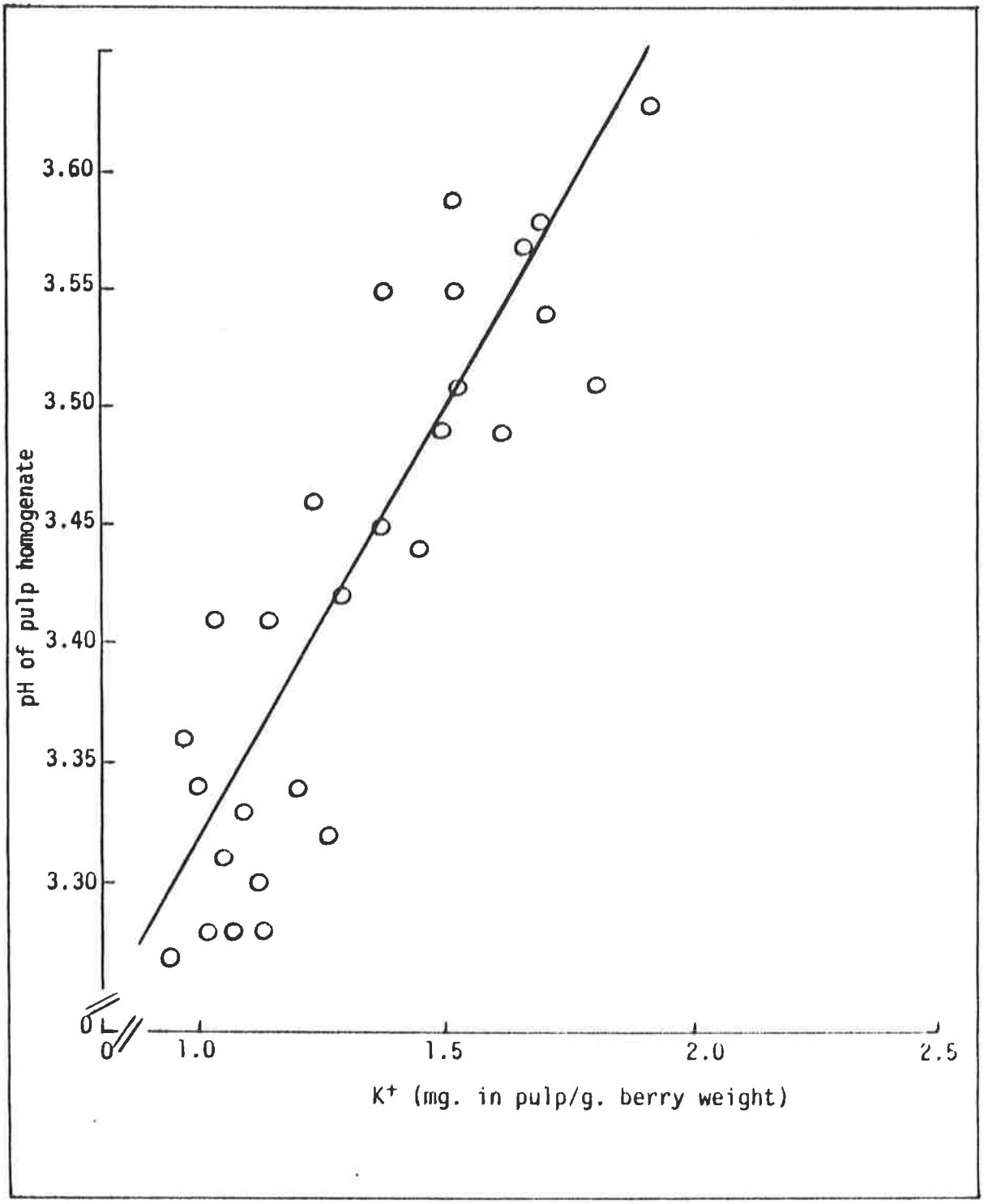

Fig. 3.18. Relationship between pH of pulp homogenate and pulp K^+ content. ($Y = 0,34 \times +2.98$, $R^2 = 0.78$ ***)

3.3 DISCUSSION

A variety of experiments were conducted to determine the effect of canopy structure and vine water status on fruit composition of 'Shiraz' vines. Results demonstrated that berry composition could be considerably altered by these treatments. Levels of malate, potassium and phenolics were most influenced, while the effect on berry tartrate content was small. Favourable adjustment in amounts of these compounds was achieved by creating a more open canopy structure. An important feature was that the major differences in both malate and potassium occurred in the pulp of berries rather than the skin. Since the pulp provides the principal constituents of the juice when the berry is crushed, any change occurring in the pulp will reflect in juice analysis.

Irrigation was shown to either increase (Expt II) or decrease (Expt III) pulp homogenate pH values with similar increases and decreases in potassium accumulation. This apparent contradiction may be explained by an examination of the influence of irrigation treatment on the respective vine canopies. In Expt II higher vigour and improved vine water status created denser and more shaded canopies in the irrigation treatment. Contrary to this, in Expt III the canopy structure of vines that were frequently watered was similar to that of vines that received no water after veraison, yet vine leaf water potential and berry water uptake had been significantly influenced. If canopy structure is a more important determinant of berry composition than soil water availability, major differences in irrigation experiments will not be seen unless there is also induced differences in canopy structure. Smart *et al*. (1981) have previously emphasised the importance of separating direct and indirect effects in experiments of this nature.

Shading of clusters lowers berry temperature (Smart 1982, Smart *et al*. 1982), which may result in decreased malic acid respiration maintaining higher levels of this acid during ripening. In Expt II shaded conditions (Fig 3.9) delayed the onset of nett respiration in the pulp. This did not occur until about 17° Brix. Whether malic acid was not being respired or whether continued synthesis was balancing

respiration loss is uncertain. If this late depletion of malic acid is coupled with continued potassium uptake, pulp pH should rise appreciably. Such was the case in the normal and irrigated vines. This feature may also explain the sudden loss of juice acidity and rise in pH sometimes observed in the field for irrigated vines approaching maturity. Hale (1977) reported a direct relationship between potassium and malate during berry ripening, and attributed this to an effect of potassium ion on membrane permeability. Possibly potassium acts in such a way in shaded fruits.

Potassium levels in ripe grapes were related to conditions present at veraison (Fig 3.10). Differences in vine water status also existed at this early stage of berry development which would suggest that the period of vine growth prior to veraison is important in setting the pattern of berry ripening. A similar correlation was reported by Downton (1977) when vines on rootstocks contained higher petiole, laminae and berry potassium both at veraison and maturity. In the experiments of Smart et al.(1984) shade at veraison caused high potassium concentrations in leaves and petioles, stems, and rachis and this was subsequently associated with higher must potassium levels. Other studies (Freeman et al.1980) suggested that the higher juice acidity and pH for irrigated vines, compared to non irrigation, was not necessarily a result of water stress or temperature during ripening as the treatment differences existed early in berry development. Similarly in Smart's (1982) studies, even though slashing prior to veraison improved exposure and fruit composition, the treatment that resulted in the most favourable composition was the G.D.C. trellis, a system that maintains good exposure of both clusters and basal leaves from berry set through to maturity. This treatment also had lower potassium concentrations in the inflorescence at flowering indicating that effects may occur very early in berry development (Smart et al. 1984). Thus it is important in future studies to examine the significance of open canopies not only during the final part of berry ripening, but also at the inception of, and early ripening period.

A number of studies (Downton 1977, Garoglio 1955, Freeman 1982, Smart et al. 1984) have shown that a large amount of the canopy

potassium is accumulated by veraison. After veraison redistribution of this previously accumulated potassium within canopy parts is important (Freeman 1982, Smart et al. 1981, Smart et al. 1984). It appears that regulation of potassium movement to the ripe berry involves mechanisms that achieve a low potassium status in both the leaves and the berries at the inception of berry growth and then restricting its transport to the berry at the later stages of ripening. Evidence to date suggests that open canopies accomplish both these effects. Trellising is a more acceptable means of creating openness in canopies than natural low vine vigour since yield is not sacrificed. An exception to this is if vine vigour is controlled by closer row spacing.

Higher levels of pulp potassium (mg in the pulp/g berry weight) were associated with increased pulp homogenate pH (Fig 3.18). This emphasises the importance of regulating berry pulp potassium uptake since high juice pH often leads to necessary adjustments and compromises during the subsequent vinification of those grapes.

Conditions that influence the tartaric acid accumulation stage need further investigation, since exposure prior to veraison (Expt 1) did not alter the maximum level of tartrate between treatments. Further there was no difference in tartrate content at the beginning of ripening (i.e. the end of acid accumulation) for the stressed, normal and irrigated vines of Expt 11. Differences in both berry exposure and vine water relationships would probably have existed between these treatments for at least some period of the acid accumulation stage.

The degree of water stress experienced by the vines may also account for the results obtained in Expt 111. Large negative leaf xylem water potentials were recorded for leaves from both NI and I vines, suggesting that vines in both treatments were exposed to high water stress during ripening. Comparison of diurnal variation for this parameter, showed that by midday both NI and I vines had reached critical levels (ψ_{leaf} xylem < -13 bars, Smart 1974), when stomatal closure would have restricted photosynthetic activity. Minimum leaf water potentials of -18 bars and -20 bars have been recorded by Freeman et al. (1980) and Hardie and Considine (1976) respectively.

Comparatively the minimum value reached by one of the NI vines in this experiment was -2073 kPA (-20.7 bars). In this situation, atmospheric conditions may overide soil moisture conditions. Such an effect was previously suggested from the response of irrigation trials in Griffith, also a hot viticultural area (Freeman et al. 1980). When photosynthetic activity is lowered potassium may be loaded into the phloem in preference to sugar, resulting in increased translocation of potassium to the ripening berry (Boulton 1980e, Freeman et al.1982). If the hot conditions influenced leaf photosynthesis equally in both treatments, then berry potassium levels should be similar for the NI and I vines, irrespective of soil moisture conditions, as was the case (Figs 3.16, 3.17).

Enhanced levels of total anthocyanins in the more open canopies (Expt 1 and Expt 11) are most likely due to increased light interception. Similar response was observed by Freeman and Kliewer (1983) who found that anthocyanin content in the skin of fruit from non-irrigated vines was 44°/₀ higher than from fruit of irrigated vines. In these experiments (Freeman and Kliewer 1983) vegetative growth was also promoted by the irrigation treatment and hence may have influenced anthocyanin levels indirectly by shading.

The studies add support to the concept that open grape vine canopies lead to improved berry composition, in particular lowered pulp pH and pulp potassium and increased anthocyanin concentration in the skin.

CHAPTER 4

COMPARTMENTATION OF ORGANIC ACIDS AND CATIONS IN PULP AND SKIN TISSUE OF 'SHIRAZ' GRAPES DURING RIPENING.

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COMPARTMENTATION OF ORGANIC ACIDS AND CATIONS IN PULP AND SKIN TISSUE OF 'SHIRAZ' GRAPES DURING RIPENING.

Abstract

Efflux studies were carried out using pulp and skin tissue of 'Shiraz' grapes at different stages of maturity. As ripeness progressed, the speed, with which tartrate, malate and potassium leached out of pulp tissue increased, indicating an increased membrane permeability with berry ripening. It was proposed that malate is contained in pulp cells that are separate to those storing tartrate and potassium. Large differences were found for efflux characteristics of pulp and skin cells.

INTRODUCTION

It has been suggested that ripening of fruit may be associated with changes in permeability of cell membranes (Sacher 1973) and various authors have explained ripening patterns of grape berries by such a mechanism (Hale 1977, Ruffner et al. 1976, Ruffner 1982b, Steffan and Rapp 1979).

It is likely that the large quantities of accumulated carbohydrates, organic acids, cations and phenolic material in grapes are compartmented in cell vacuoles. Investigations of the anion and cation content of skin extracts and vacuole lysates of ripe berries (cv 'De Chaunac') indicated that tartrate, malate and potassium were the major constituents (Moskowitz and Hrazdina 1981). The same authors reported a method for determining pH of intact skin vacuoles based on anthocyanin equilibrium within the vacuoles; this gave an average figure of pH 2.7

Ruffner (1982b) suggested that two distinct types of cells are associated with malic acid metabolism in grape tissue, those

peripherally located, termed "non-storage-type" cells and those more specialized and centrally positioned which act as true storage sites. He further suggested that, owing to an increase in membrane permeability, previously stored acid is radially transported to the berry periphery where it is dissimilated with berry ripening. The timing of the process whereby malate respiration is initiated may be crucial in determining final acid level of the mature fruit (Ruffner et al. 1976).

Temperature is thought to influence membrane permeability during fruit ripening, but this effect may be modified by potassium levels in grape tissue (Hale 1977).

Because large changes in malate and potassium content of berries does occur when ripening is initiated, it was thought important to see if these correlated with any changes in cell membrane permeability. In this study an attempt was made to quantify membrane characteristics of pulp and skin tissue of 'Shiraz' grapes taken at different stages during ripening. This was done by using the technique of efflux studies, where the speed with which components are leached from the tissue when it is immersed in a specific washing medium, is measured.

4.1. MATERIALS AND METHODS

4.1.a. Comments on the efflux technique for determining compartmentation and permeability of cell membranes in fruit tissue.

The study of fluxes (influx and efflux) of specific solutes in tissues provides information about the properties of its cell membranes and hence about compartmentation of that solute. The flux may be measured by following the amounts of radioactively-labelled solute or of the chemical itself.

After soaking a piece of tissue in a particular washing medium, compounds that are present in the free space and cytoplasm termed here as "readily diffusible portion", pass into the washing medium at a faster rate than compounds situated in the vacuole. A plot of the amount or the percentage of the total, for a particular compound remaining in the tissue after known time intervals, gives a curve of the form shown in Fig 4.1. The flattening of the curve indicates vacuolar diffusion. The amount of compound remaining in the tissue at this stage is an estimate of the amount of the compound remaining in the vacuole (compartmented) after that washing time. It is not an absolute measure of vacuolar compartmentation since slow movement out of the vacuole will occur concurrently with the faster movement out of 1) the free space and 2) the cytoplasm. The amount would also be affected slightly by the efflux of compounds of injured cells which would be included in the readily diffusible portion. Compounds that diffuse out of the vacuole have to pass through both the tonoplast and the plasmalemma.

The kinetics of the efflux may be expressed mathematically (Vickery and Bruinsma 1973):

$$K_t = K_1 \exp(k_1 t) + K_2 \exp(k_2 t) + \ldots \text{ etc.} \qquad \text{Eqt 4.1}$$

with from 2 to 4 terms, where

K_t = amount or percentage of total, of compound remaining in

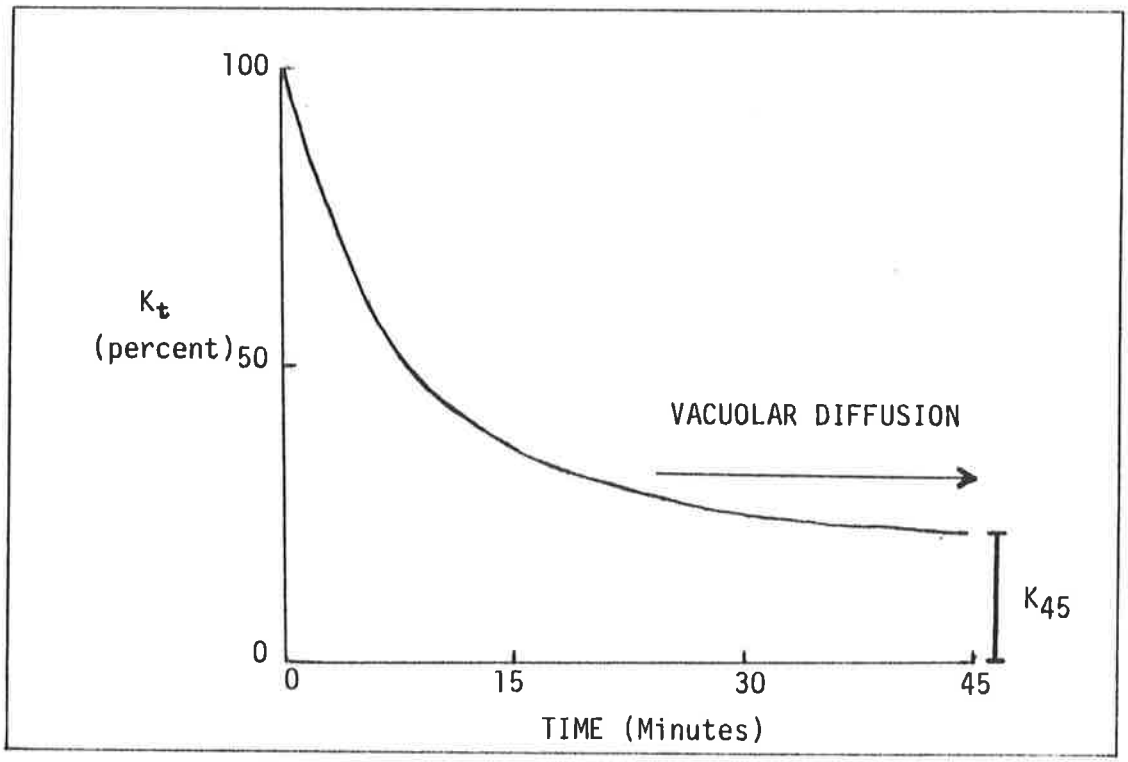

Fig. 4.1. Example of an efflux curve. The amount of compound remaining in the tissue at any time during the washing period, expressed as a percentage of the original amount (K_t).

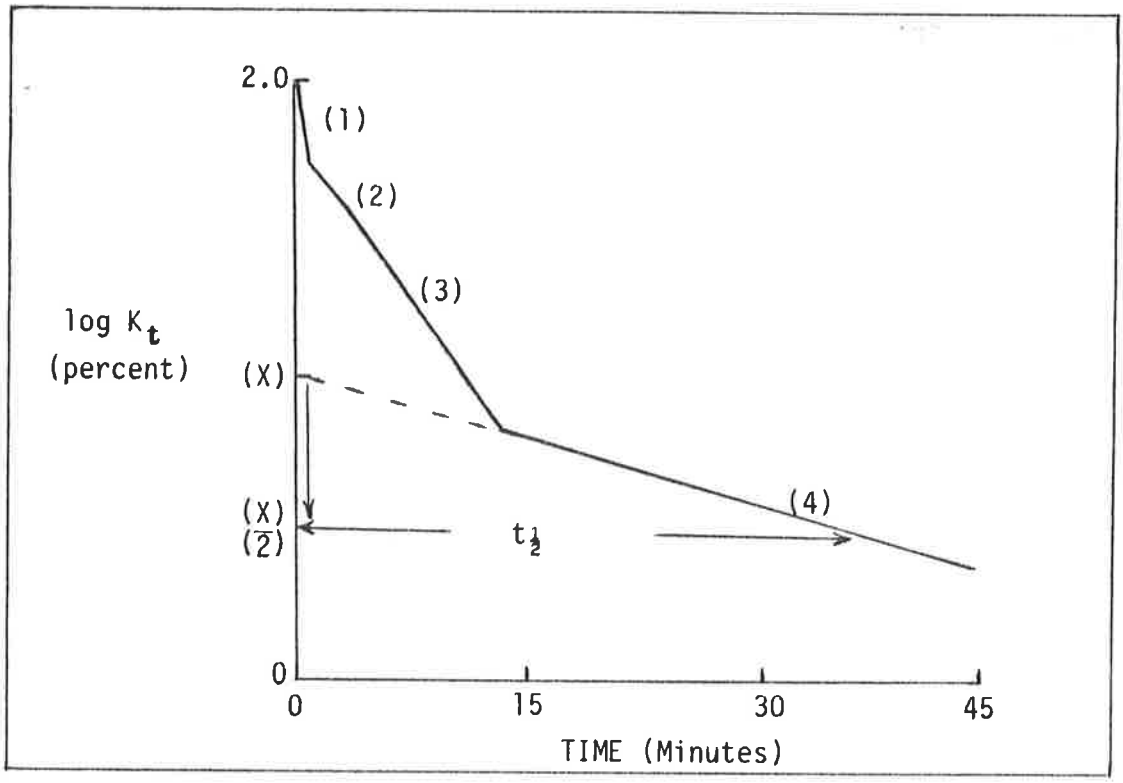

Fig. 4.2. Example of an efflux plot. The log of K_t (percent) at various times during the washing period. (1) (2) (3) (4) are explained in the text.

the tissue after time t.

K_1, k_1 = constants

t = time from start of efflux.

The simplest relationship for each term in equation 4.1 is given by

$$k = \frac{-2.303}{t} \cdot \log K_t \qquad \text{Equation 4.2}$$

When $\log K_t$ is plotted against time and if all 4 terms observe this simple relationship then the plot shown in Fig 4.2 is obtained.

Each section 1, 2, 3, 4 defines the rate of movement for:

1. fast movement out of the free space
2. slow movement out of the free space
3. movement out of the cytoplasm across the plasmalemma
4. movement out of the vacuole, across both the tonoplast and the plasmalemma.

The dotted line indicates vacuolar diffusion contribution to diffusion from 1, 2, and 3.

The relationship is not necessarily linear and each section 1-4 may exhibit more complex fuctionality. Curvature of a "linear" section with increasing time is indicative of membrane breakdown during the experiment. When a simple relationship is observed according to equation 4.2, the kinetic characteristics k (the rate constant) and $t^1/2$ (the time taken for half the amount of the compound in that compartment to diffuse) may be calculated.

4.1.b. Grape samples

Berries, cv. 'Shiraz' were from a 6-berry subsample of the 50-berry lots described in 3.1.c; again samples were taken for the three selected ripening stages - beginning, mid, and ripe. The berries in each subsample were cut in half; °Brix was measured on one lot of half berries, while the other lot was used for the washing experiments as follows; after removing the skin from the pulp, the pulp was cut into approximately 3mm cubes from which about 1g of pulp was taken at

random. The skin was blotted lightly, cut into slices and a weight of about 100mg was taken at random. These pulp and skin samples were used in the washing experiments. The number of replicates used for these experiments at each maturity stage is shown in Tables 4.1 and 4.2.

4.1.c. Washing technique

The pulp sample was soaked in 2.5mL washing medium (see next section) held in an ice bath, and aliquots of medium (either 0.25mL or 0.5mL) removed at 15, 30, and 45 minutes from the start of the washing period. At the end of this period, the remaining solution and tissue was autoclaved at 15 p.s.i. for 15 minutes. The aliquots and autoclaved extracts were analysed for tartrate, malate, and potassium.

Skin samples were treated similarly but, rather than periodic sampling of aliquots, the washing medium (again 2.5mL) was poured off every 15 minutes and retained; after blotting the skin lightly, a new portion of washing medium (2.5mL) was added back to the same skin sample. Finally the skin sample was placed in a fresh 2.5mL washing medium and autoclaved.

4.1.d. Composition of washing medium

The washing medium contained 2mM Carbowax 4000, 50mM Mes buffer pH 6.5, 10mM dithiothreitol, 2mM $MgSO_4.7H_2O$, 0.2mM $Ca(NO_3)_2.4H_2O$ and varying concentrations of mannitol and sorbitol. The concentrations of mannitol and sorbitol were adjusted to maintain an increasing total osmolality in accordance with the estimated in vivo situation in pulp and skin tissue of grapes for each ripening stage as follows: beginning (600 milliosmoles), mid and ripe (800 milliosmoles). This washing medium was used for both pulp and skin samples.

4.1.e. Expression of results

From the analysis of tartrate, malate and potassium in the aliquots and autoclaved extracts, the amount of each compound initially in the tissue, and the amount and percentage of each compound remaining in the

tissue at each sampling time were calculated. Percentage and the log of the percentage of each compound remaining in the tissue at a particular time, were plotted against time, and measures made of (1) the percentage of compound remaining in the tissue after 45 minutes of washing, K_{45}, taken as an estimate of vacuolar content and (2) the time for half of the compound to wash out of the vacuole ($t^{1}/2$ mins).

4.2 RESULTS

The samples were from the two treatment sets (watered and not watered) but there was no significant differences in flux between samples from these treatments, hence the data were combined.

Pulp

The results (Fig 4.3, Table 4.1) showed that for the pulp, the value for K_{45} decreased during ripening for all three components; the change from the beginning of ripening to the ripe stage was 44% to 11%, 46% to 4% and 38% to 15% for tartrate, malate and potassium respectively. Most of the decrease occurred during the earlier period (Fig 4.5a). These changes in K_{45} are also reflected by $t^{1}/2$ values (Table 4.1). Larger $t^{1}/2$ values are associated with higher percentage compartmented.

Skin

Efflux studies on skin (Fig 4.4, Table 4.2) showed that, unlike the pulp, there was not a similar trend for all compounds investigated. K_{45} values increased for tartrate and malate but remained constant for potassium during the earlier ripening stage (A - B); from the mid to ripe stage K_{45} values decreased for all compounds (Table 4.2 and Fig. 4.5.b). The changes in the parameters K_{45} and $t^{1}/2$ were smaller with skin than pulp tissue.

TABLE 4.1. Efflux parameters for tartrate, malate, and potassium in pulp cells of 'Shiraz' grapes during ripening.

	TARTRATE			MALATE			POTASSIUM		
	Beginning	Mid-Ripening	Ripe	Beginning	Mid-Ripening	Ripe	Beginning	Mid-Ripening	Ripe
Number of samples contributing to mean	6	2	5	6	2	6	6	2	5
Mean for percentage of compound remaining in the tissue after 45 mins. efflux time. (K_{45})	44	17	11	46	15	4	38	16	15
$t_{1/2}$ (mins.)	190	90	53	250	95	32	216	120	101

TABLE 4.2. Efflux parameters for tartrate, malate, and potassium in skin cells of 'Shiraz' grapes during ripening.

	TARTRATE			MALATE			POTASSIUM		
	Beginning	Mid-Ripening	Ripe	Beginning	Mid-Ripening	Ripe	Beginning	Mid-Ripening	Ripe
Number of samples contributing to mean	6	4	6	6	6	6	5	5	6
Mean for percentage of compound remaining in the tissue after 45 mins. efflux time. (K_{45})	14	27	23	6	27	16	25	25	17
$t_{1/2}$ (mins.)	98	190	94	76	245	73	95	95	71

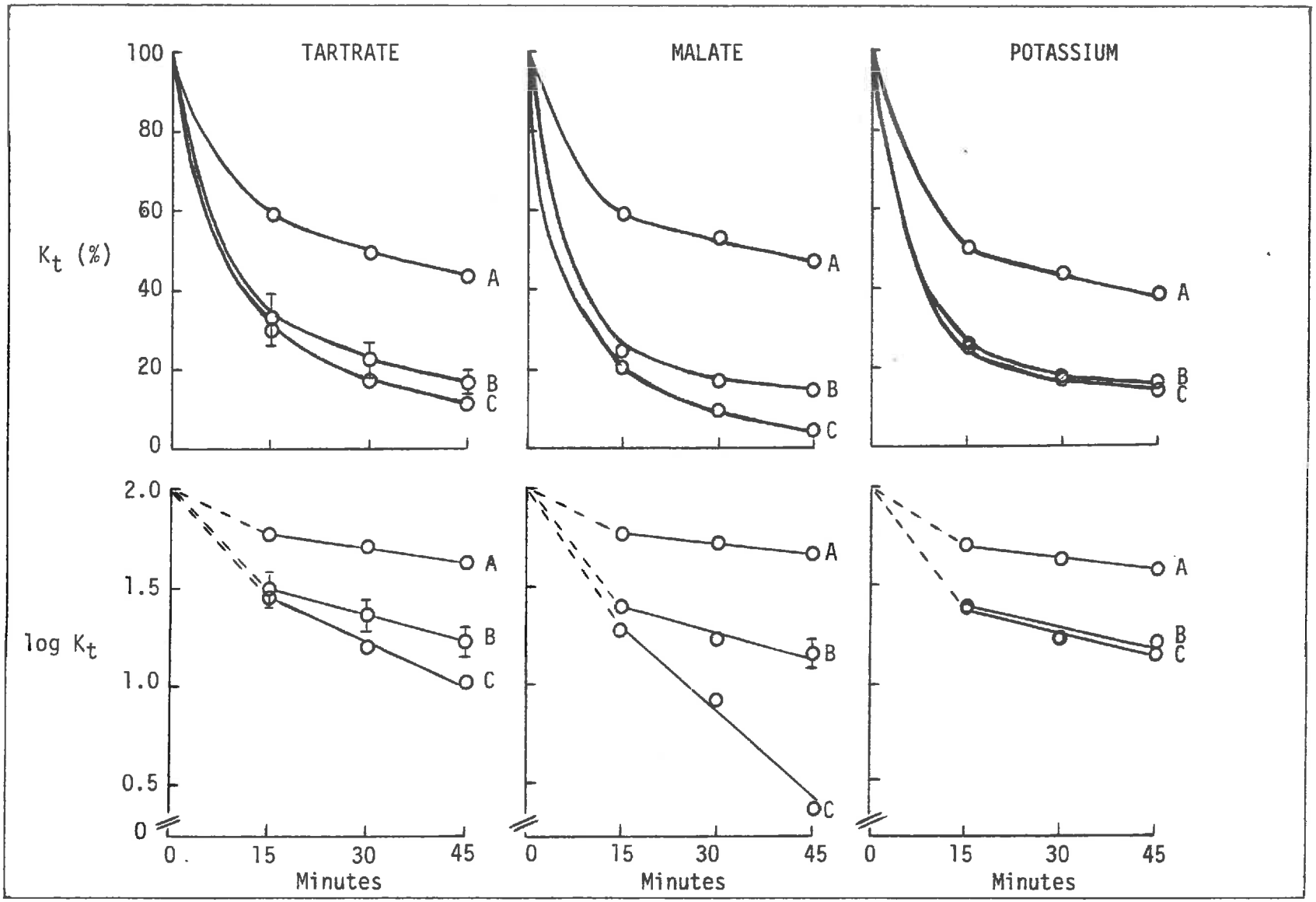

Fig. 4.3. (Top) The amount of compound (% of amount originally in the tissue) remaining in pulp tissue of 'Shiraz' grapes at varying times during the washing experiment. (Bottom) The log of this percentage at the same time intervals. A, B, C represent the beginning, mid and ripe stage of ripening. Vertical bars represent S.E. of the mean. When not shown these are smaller than the indicated symbol.

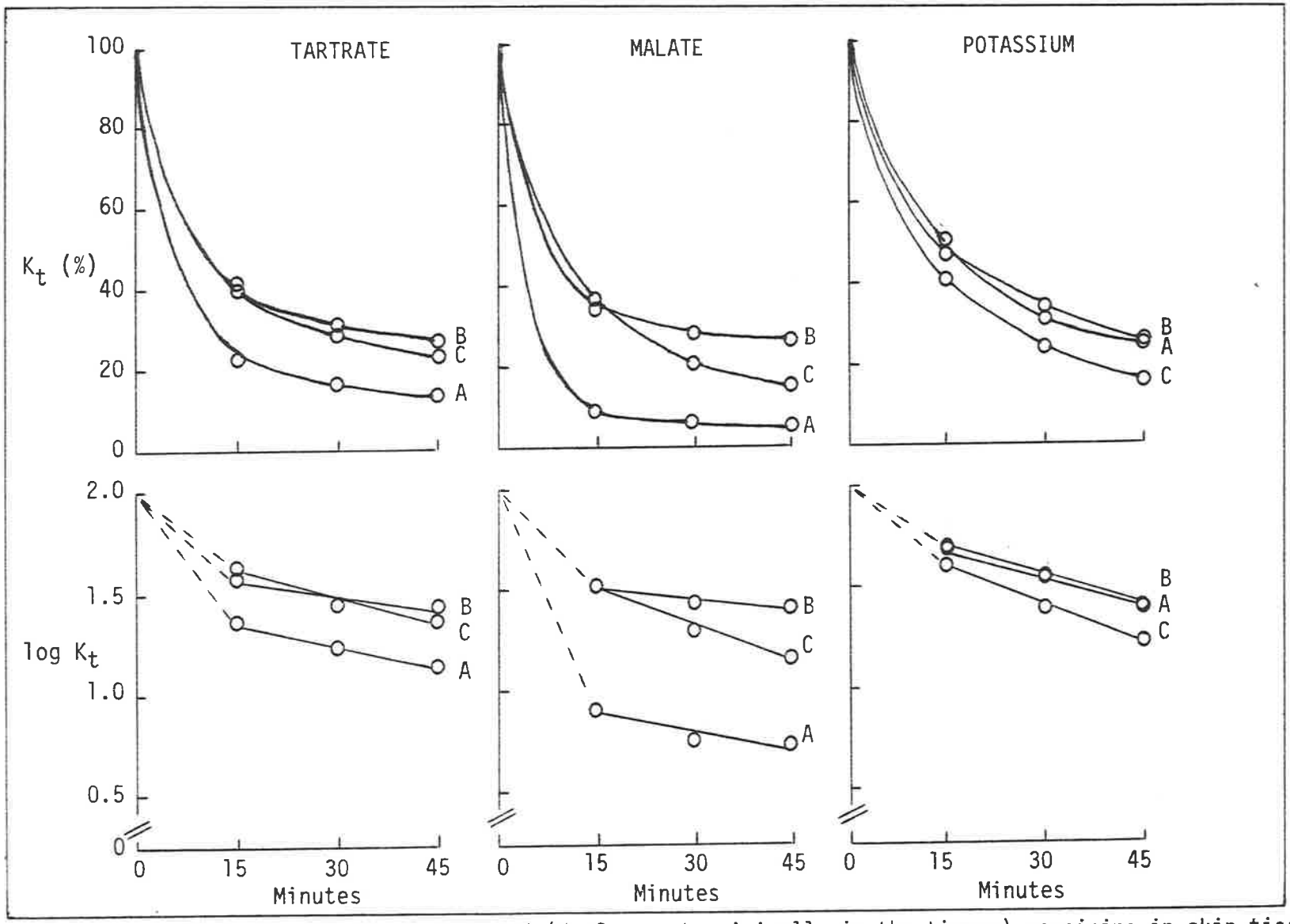

Fig. 4.4. (Top) The amount of compound (% of amount originally in the tissue) remaining in skin tissue of 'Shiraz' grapes at varying times during the washing experiment. (Bottom) The log of this percentage at the same time intervals. A, B, C represent the beginning, mid and ripe stage of ripening. Vertical bars represent S.E. of the mean. When not shown these are smaller than the indicated symbol.

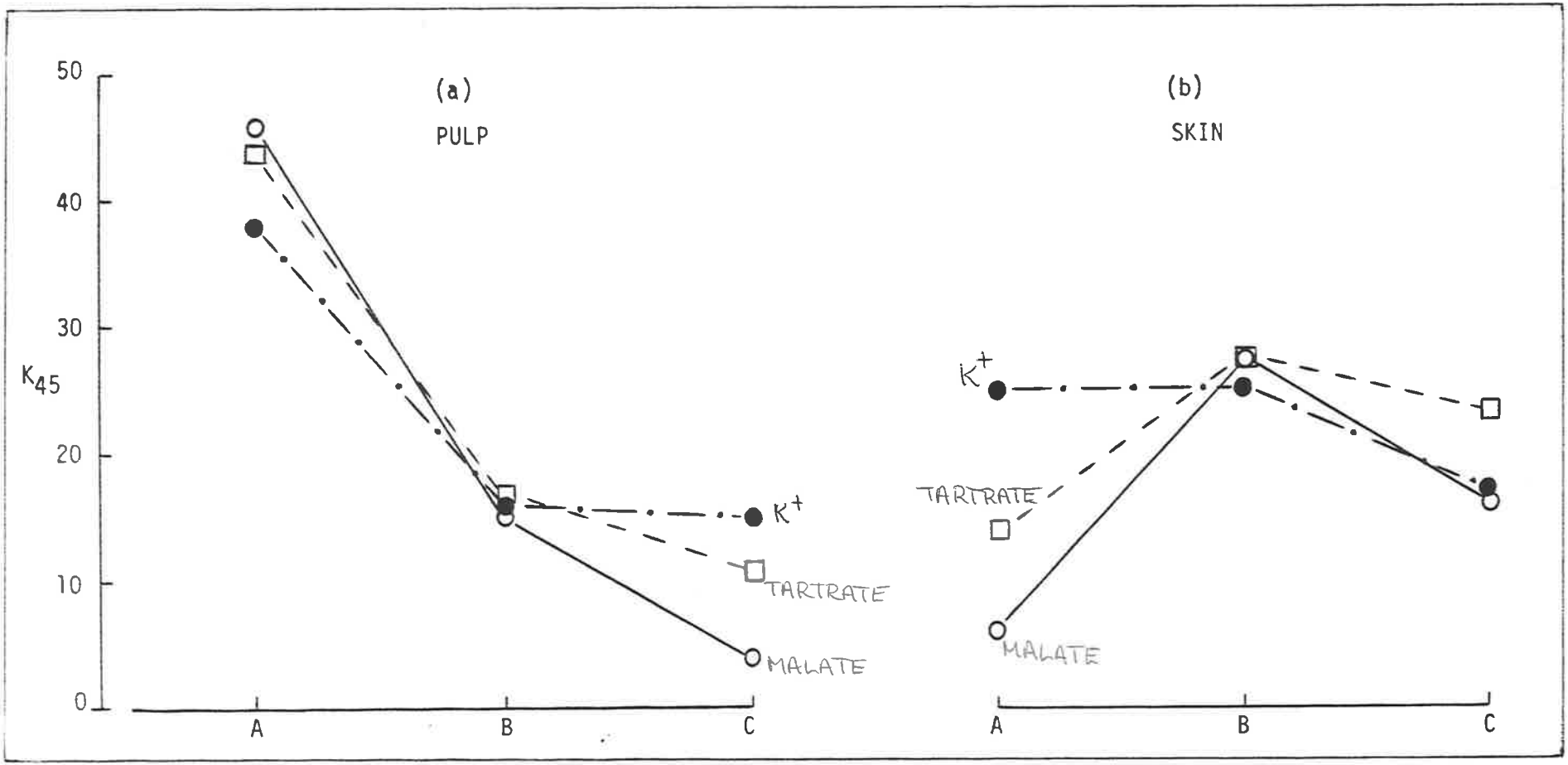

Fig. 4.5. Change in compartmentation (after 45 minutes immersion in the washing medium) for tartrate, malate and potassium in pulp and skin tissue of 'Shiraz' grapes during ripening. A, B, C represent the beginning, mid, and ripe stage of ripening.

4.3. DISCUSSION

Pulp studies

The permeabilities measured here are for the passive fluxes across membranes. Leakage from pulp cells became more rapid during the course of berry ripening, indicating that the properties of membranes change through the ripening phase. In ripe berries malate leached out quicker than tartrate or potassium, suggesting that the cellular membranes display selective permeabilities towards this particular compound or that malate is stored within a separate internal compartment within the vacuole. However another interpretation is that malate is compartmented in cells separate to those storing tartrate and potassium. This would explain the difference in passive fluxes for malate compared to tartrate and potassium since it would be leaching across different membranes. This conclusion is also consistent with the finding that malate existed entirely as the free acid form in berry cells (Chapter 2). If it was compartmented in the same vacuoles as tartrate and potassium, expected pH values (sometimes as high as pH 4.1) would necessitate the presence of salt forms of malate. (provided that vacuoles have no internal compartments)

It has been suggested that dissimilation of malate in pulp cells in vivo occurs after the stored acid has been transported to cells located peripherally within the berry (Ruffner 1982b). Increased membrane permeability with berry ripening could explain the way in which malate is moved throughout the berry. The tendency for tartrate and potassium to leach out of pulp cells more easily with berry ripening does not reflect the endogenous pattern of tartrate metabolism or potassium accumulation in the pulp of the ripening grape berry. Perhaps this efflux trend is indicative of general breakdown or disorganisation in membrane structure with ripening. If this occurs in vivo and tartrate is exposed to metabolising enzymes in the cytoplasm, it is unlikely that remetabolisation would occur, since in the pulp this acid exists largely as salt forms, which Saito and Kasai (1968) suggest are scarcely attacked by such enzymes.

Ripe pulp tissue is difficult to handle when cut into regular cubes and it is likely that leakage occurred in sample preparation, and the

possibility of leakage from damaged cells contributing to enhanced efflux cannot be discarded.

Skin studies

These studies provide further evidence of the different properties of pulp and skin tissue of the grape berry. Skin results (increasing or constant K_{45} during the early ripening stage) suggest increased compartmentation with ripening. This is consistent with _in vivo_ ripening pattern since all of these solutes are accumulated in skin tissue during the ripening phase (Chapter 2). Decreased values for K_{45} and $t^{1}/2$ at the ripe stage may be associated with changes in properties of cell membranes to facilitate berry shrivelling, which is common in 'Shiraz' grapes at the later stages of maturity.

Moskowitz and Hrazdina (1981) report values for tartrate, malate and potassium in the skin of 'De Chaunac' grapes on both a moles g^{-1} fresh wt and moles $vacuole^{-1}$ basis. Comparative data calculated from various sources (Table 4.3) show differences, but more interesting is the anomaly between determined vacuolar molar concentration and calculated molar concentration in compartmented solution, for the data of Moskowitz and Hrazdina (1981).

Either Coombe and Matile's (1980) assumed compartmented volume is too large (in the order of 30 fold for tartrate !!), or Moskowitz and Hrazdina's (1981) values are overestimated. Further irregularities are apparent when the ratio of moles g^{-1} fresh wt to moles $vacuole^{-1}$ (Table IV in Moskowitz and Hrazdina 1981) are calculated; if all of the compound present on a fresh weight basis is compartmented in the vacuole, then this ratio should be constant for all compounds, simply being the number of vacuoles per unit weight of skin; this however is not the case. An alternative interpretation which would validate different ratios for varying components, is that some compounds express only partial compartmentation. However this argument invokes unrealistic cytoplasmic concentrations eg potassium. Problems arising from a large range of yield of vacuoles and of vacuolar volumes must contribute to the above anomalies.

Table 4.3 : Tartrate, malate and potassium concentrations in skin tissue of grapes.

Source	Concentration								
	mg g^{-1} fresh wt			*molar conc in compartmented space			molar conc in vacuole		
	Tartaric	Malic	K	Tartaric	Malic	K	Tartaric	Malic	K
Mature 'Shiraz'									
Experiment 2	8.7	12.4	11.2	0.07	0.12	0.36			
Experiment 3 [This thesis Chapter 2]	13.0	12.0	10.0	0.11	0.11	0.32			
Mature 'Sultana' Hale (1977) (approx values)	13.0	4.0	4.0	0.11	0.04	0.13			
Mature 'De Chaunac' Moskowitz and Hrazdina (1981)	4.0	2.3	3.4	0.03	0.02	0.11	1.09	0.31	2.73
Green 'Pinot Noir' Coombe and Matile (1980)					0.005-0.02				

*Calculated on the basis that the volume of water within the compartmented space was 0.8mL g^{-1} fresh wt (Coombe and Matile 1980)

The average pH of skin vacuoles determined from anthocyanin equilibrium was 2.7 (Moskowitz and Hrazdina 1981). This was justified by high concentrations of tartaric acid but took no account of large concentration of vacuolar potassium. In another paper (Hrazdina and Moskowitz 1982) these authors address this problem and quote likely vacuolar pH values in the range 2.3 to 4.0, and further suggest that vacuolar K^+, Na^+, Ca^{++} and Mg^{++} are most likely to be present as salts in the vacuolar solution.

General

Recently Ehwald et al. (1980) have questioned whether washability determined in thin tissue sections can be related to the localization of the compounds in cell or tissue compartments in the intact organ. They found turgor-dependent effluxes or plasmolysis enhanced initial leakage in hypotonic solutions and the composition of the washing medium, in particular high osmolality and calcium concentrations, could dramatically reduce efflux rates.

For the washing experiments reported here it was hoped to adjust the osmality of the washing medium in accordance with an estimated value for the intact tissue (Table 4.4)

Table 4.4 : Estimated osmolality of pulp and skin grape tissue at different ripening stages and osmolality of the washing medium used at that sample stage

		Ripening stage		
		Beginning	Mid	Ripe
*Estimated tissue osmolality (milliosmoles)	pulp	523	834	956
	skin	626	1158	1652
+Osmolality of the medium (milliosmoles)		600	800	800

* estimated from the sum of molar concentration in compartmented space for glucose, fructose, tartrate, malate and potassium in respective tissue.
\+ same medium used for both pulp and skin tissue

The osmolality of the washing media was considered appropriate for all sampling stages except for ripe skin tissue, where hypertonic condition may have influenced efflux and could account for the decline in K_{45} for malate and potassium in mature skin tissue. The extent of this influence is uncertain and needs to be tested in future experiments of this nature.

CHAPTER 5

THE EFFECTS OF SKIN COMPONENTS ON RED WINE COMPOSITION.

CHAPTER 5

THE EFFECTS OF SKIN COMPONENTS ON RED WINE COMPOSITION

Abstract

The effects of skin contact on red wine composition were investigated. Potassium, total anthocyanins and total phenols were the major components extracted from the skins of 'Shiraz' and 'Cabernet Sauvignon' grapes during vinification. Higher amounts of extractable potassium were associated with initially natural low pH and low potassium levels in the juice. Reasons for the change in pH during fermentation of black grapes are discussed.

INTRODUCTION

In the fermentation of black grapes, musts are held in contact with the pomace. During this period there is opportunity for components of the skin to enter and alter both the nature and concentration of compounds in the ferment, causing large diversity of style and character in red wines. Some period of skin contact is necessary for achieving satisfactory extraction of pigments, the most obvious characteristic of red wines, yet at the same time it may be essential to limit extraction of other materials e.g. excessive extraction of potassium has been suggested as a partial cause for high wine pH which is often typical of warm regions (Van Wyk 1977, Somers 1975). Previous studies have shown that with extended skin contact time, tannin, extract, pH, potassium and colour increases, while titratable acidity decreases (Noble 1982, Schmidt and Noble 1983, Singleton 1972).

In this investigation, the effects of some skin components of 'Shiraz' and 'Cabernet-Sauvignon' grapes on the subsequent wine composition are discussed.

5.1. MATERIALS AND METHODS

5.1. Small and large scale fermentations

For some winemaking lots the composition of the juice and the wine after pressing were determined and compared. After crushing, a representative sample of the must was taken, centrifuged at 2000 rpm for about 5 minutes and the clear supernatant juice sample analysed for pH, tartrate, malate and potassium. After pressing, a representative sample of the wine was taken, centrifuged at 10,000 rpm for about 20 minutes and the clear supernatant wine sample was analysed in the same way. Colour parameters were also assessed on the wine samples. One commercial fermentation was assessed in more detail by periodic sampling of the ferment during the skin contact period.

In the above experiments the amount of potassium extracted from solids was calculated from equation 5.1, all concentrations being expressed as g L^{-1}.

equation 5.1

$$[K^+] \text{ extracted} = \frac{[\text{Tartrate}]_{\text{juice}} - [\text{Tartrate}]_{\text{wine}}}{3.85} - \left[[K^+]_{\text{juice}} - [K^+]_{\text{wine}}\right]$$

Micro-fermentations

In addition to the above approaches, the effects of skin components during vinification was determined directly by conducting micro-fermentations of berries with either skin attached or removed. A sample of 250 berries was randomly divided into five 50-berry lots and processed as follows: two lots were analysed for amounts of each component in the pulp and skin, three lots were used for the micro-fermentations. For each micro-fermentation lot, the berries were halved longitudinally. Skins were removed from one lot of half berries. In all, this provided three lots of 50 half berries with skins attached and three lots of 50 half berries with skins removed. The samples were placed in 30 mL glass bottles. The berries were crushed

with a glass rod and then 0.5 mL of activated dry wine yeast preparation was added to each bottle. Bottles were capped with cotton wool and held at room temperature during the fermentations. Ferments were taken to dryness, pressed and the fermentation medium was spun down at 10,000 rpm for 15 minutes. A sample of skins, removed at the end of the ferment was counted, rinsed with distilled water, briefly blotted dry and weighed. These skins were then shredded and divided into two weighed portions for aqueous extraction (addition of 10mL of distilled water and autoclaved) and residual colour extraction (methanolic 0.1% HCl extraction). Extractions were made to volume and analysed. These analyses gave a direct measure of the amounts of each component remaining in the skin after fermentation.

Compounds analysed

Samples of berries, juice and wine were analysed for tartrate, malate, potassium, pH and total anthocyanins and total phenolics. Methods of analysis were similar to those described previously (Section 2.1). Colour parameters in the wine were assessed by the methods of Somers and Evans (1977).

5.2. RESULTS

Small and large scale fermentations

The composition of a range of juices and the resultant wines after pressing are shown in Table 5.1.a. and 5.1.b.

Results obtained by periodic sampling of a commercial fermentation during time on skins are shown in Fig. 5.1. There was a large initial increase in pH and potassium concentration. Throughout the fermentation patterns of change for pH and potassium were similar. Tartrate concentration decreased immediately and continued in this pattern for the complete period of skin contact. As the fermentation progressed and the alcohol level of the ferment increased, potassium loss due to KHT precipitation exceeded potassium extraction from the solids, and potassium concentration decreased suddenly, but then rose slightly later in the fermentation. Malate levels increased slightly during the initial period and then decreased.

The amount of potassium extracted from the solids (pulp + skin) during the period of skin contact for each fermentation was calculated using Equation 5.1, and is shown in Table 5.1.b.

Micro-fermentations

The composition of the berries, the juice and the wine used in the pulp only and the pulp + skin fermentations are shown in Table 5.2. The wine from pulp + skin fermentations had higher pH, potassium, malate total anthocyanins and total phenol levels than those of pulp only. Calculations based on Equation 5.1 showed that there was 240 ppm potassium extracted from the solids in the pulp only and 1052 ppm potassium from the pulp + skin; this indicates that the skin is the major source of extractable potassium. The amount of each compound in the skin before and after fermentation is shown in Table 5.3. These samples were taken from the ferment just before it was pressed to avoid contamination from precipitated potassium bitartrate in the ferment

adhering to the skins during pressing. It is likely that further extraction of these compounds would occur during the pressing operation.

Table 5.1.a. Composition of a range of grape juice samples

	pH	TARTRATE gL^{-1}	MALATE gL^{-1}	POTASSIUM (ppm)
Small-scale fermentations				
1	3.53	4.22	2.22	1470
2	3.50	4.43	3.39	1330
3	3.48	4.70	3.50	1390
4	3.46	4.41	2.71	1290
5	3.31	4.50	5.36	1438
6	3.29	5.23	4.95	1431
7	3.30	4.54	5.39	1550
8	3.38	4.60	5.66	1600
9	3.68	4.15	2.40	1450
10	3.41	5.17	2.40	1075
11	3.52	4.82	3.01	1225
12	3.97	4.41	4.19	2125
13	3.92	3.90	3.78	1988
14	3.99	4.34	4.00	2163
Large-scale fermentations				
*1	3.30	5.6	4.2	1788
*2	3.20	4.2	4.0	1288
3	3.54	5.25	3.65	2414
4	3.45	6.32	3.20	2118

* 'Cabernet Sauvignon', all other figures are 'Shiraz'

Table 5.1.b. Composition of wines made from the grape juices shown in Table 5.1.a. The analyses were of 14 small scale and 4 large scale fermentations.

	pH	TARTRATE gL^{-1}	MALATE gL^{-1}	POTASSIUM (ppm)	POTASSIUM EXTRACTED FROM THE SOLIDS (ppm)
Small-scale fermentations					
1	3.78	4.42	1.50	2125	650
2	3.75	4.04	3.60	2075	845
3	3.73	3.83	3.25	2150	986
4	3.68	4.19	3.11	1925	694
5	3.70	3.24	5.23	2250	1139
6	3.72	3.81	4.94	2400	1338
7	3.71	3.30	5.59	2500	1272
8	3.79	3.07	5.50	2580	1377
9	3.84	2.44	3.39	1750	744
10	3.65	3.62	3.02	1600	929
11	3.79	3.29	3.24	1830	1002
12	4.19	1.70	3.62	1145	869
13	4.07	1.82	3.61	1000	552
14	4.12	1.67	2.88	1040	694
Large-scale fermentations					
*1	3.68	2.40	3.40	1875	2950
*2	3.80	1.92	2.70	1392	704
3 Free run	3.54	4.81	3.83	2424	124
Pressings	3.78	4.81	4.05	2671	371
4 Free run	3.57	3.72	1.27	1656	213
Pressings	3.82	3.89	1.41	2475	988

* 'Cabernet Sauvignon', all other figures are 'Shiraz'

Table 5.2. Results of micro-fermentations of 'Cabernet Sauvignon' grapes with and without skins. This data describes sample 3 of Table 5.4.

BERRY COMPOSITION

amount/g berry weight

	Tartrate (mg)	Malate (mg)	K^+ (mg)	Na^+ (mg)	Total anthocyanins (mg)	Total Phenols (mg gallic acid equivs)
Pulp	6.4	2.3	2.25	0.09	-	-
Skin	1.6	0.7	0.86	0.04	0.94	1.90
Total	8.0	3.0	3.10	0.13	-	-
amount/berry						
Pulp	3.9	1.4	1.37	0.06	-	-
Skin	1.0	0.4	0.51	0.02	0.55	1.20
Total	4.9	1.8	1.88	0.08	-	-

JUICE COMPOSITION

pH	Tartrate (gL^{-1})	Malate (gL^{-1})	K^+ (ppm)	Na^+ (ppm)	Total anthocyanins (mgL^{-1})	Total Phenols (mgL^{-1} gallic acid equivs)
3.30	5.50	4.20	1825	120	-	-

WINE COMPOSITION

	pH	Tartrate (gL^{-1})	Malate (gL^{-1})	K^+ (ppm)	Na^+ (ppm)	Total anthocyanins (mgL^{-1})	Total Phenols (mgL^{-1} gallic acid equivs)
*Pulp only ferment	3.58	2.47	2.77	1283	130	0	16
*Pulp + skin ferment	3.78	1.97	3.13	1967	111	362	52

*average of 3 micro-fermentations.

Table 5.3. The amount (mg/skin) of each compound in the skin of 'Cabernet Sauvignon' grapes before and after fermentation.

	Tartrate (mg)	Malate (mg)	K^+ (mg)	Na^+ (mg)	Total antho-cyanins (mg)	Total Phenols (mg gallic acid equivs)
Before fermentation	1.00	0.40	0.51	0.02	0.55	1.20
After fermentation	0.72	0.15	0.36	0.01	0.12	0.50
Percentage remaining in the skin after fermentation	72	38	71	50	22	42

Table 5.4. pH values for fermentations with and without skins. More detailed data for sample 3 is given in Table 5.2.

Sample	Juice pH	Wine pH	pH Change (wine-juice)	Change in $[H^+]$
1 Pulp only	3.45	3.66	+0.21	-38%
Pulp + skin	3.50	3.82	+0.32	-52%
2 Pulp only	3.96	4.02	+0.06	-13%
Pulp + skin	3.96	4.18	+0.22	-40%
3 Pulp only	3.30	3.58	+0.28	-48%
Pulp + skin	3.30	3.78	+0.48	-66%

Table 5.5 'Retention indices' for anthocyanins in red wines immediately after pressing.

*Sample	**Potential total anthocyanin concentration mg L^{-1}	***Measured total anthocyanin concentration mg L^{-1}	Retention index Measured / Potential (%)
1	3876	976	25
2	2329	818	35
3	3944	1172	30
4	2142	416	19
5	1768	455	26
6	2380	598	25
7	1615	472	29
8	1513	494	38
9	1547	476	31
10	1496	466	31
11	3043	1087	36
12	3009	1040	35
13	3247	1000	31
14	2788	1064	38

*Sample numbers do not correspond to Table 5.1.a. or 5.1.b.

**Calculated from analysed anthocyanin content in the skin (mg per g berry weight) and then assuming that 1g berry weight yields 0.6mL of wine.

***These measures are for the wine immediately after pressing.

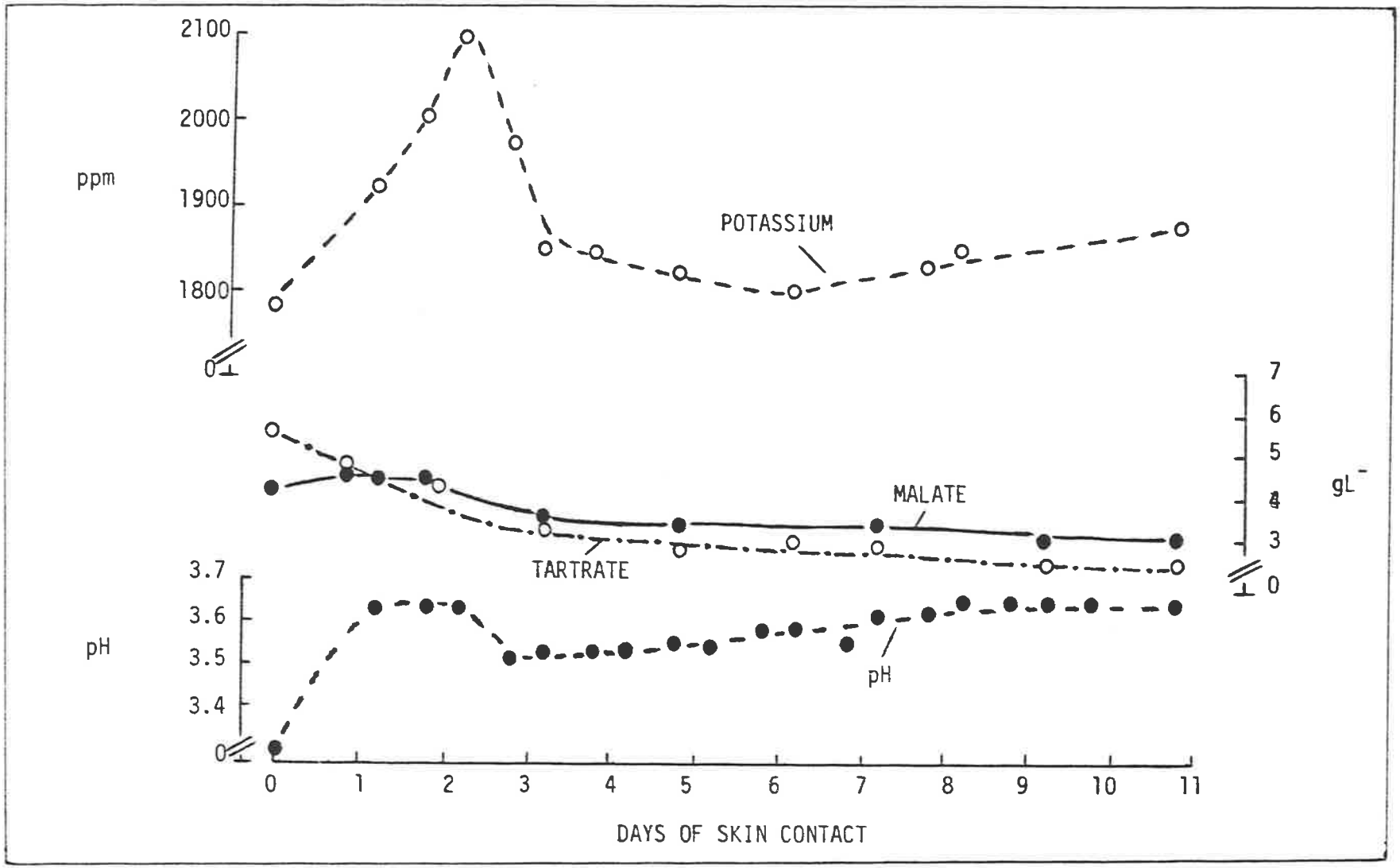

Fig. 5.1. Change in pH, and the concentration of tartrate, malate and potassium during the period of skin contact of a commercial fermentation of 'Cabernet Sauvignon' grapes.

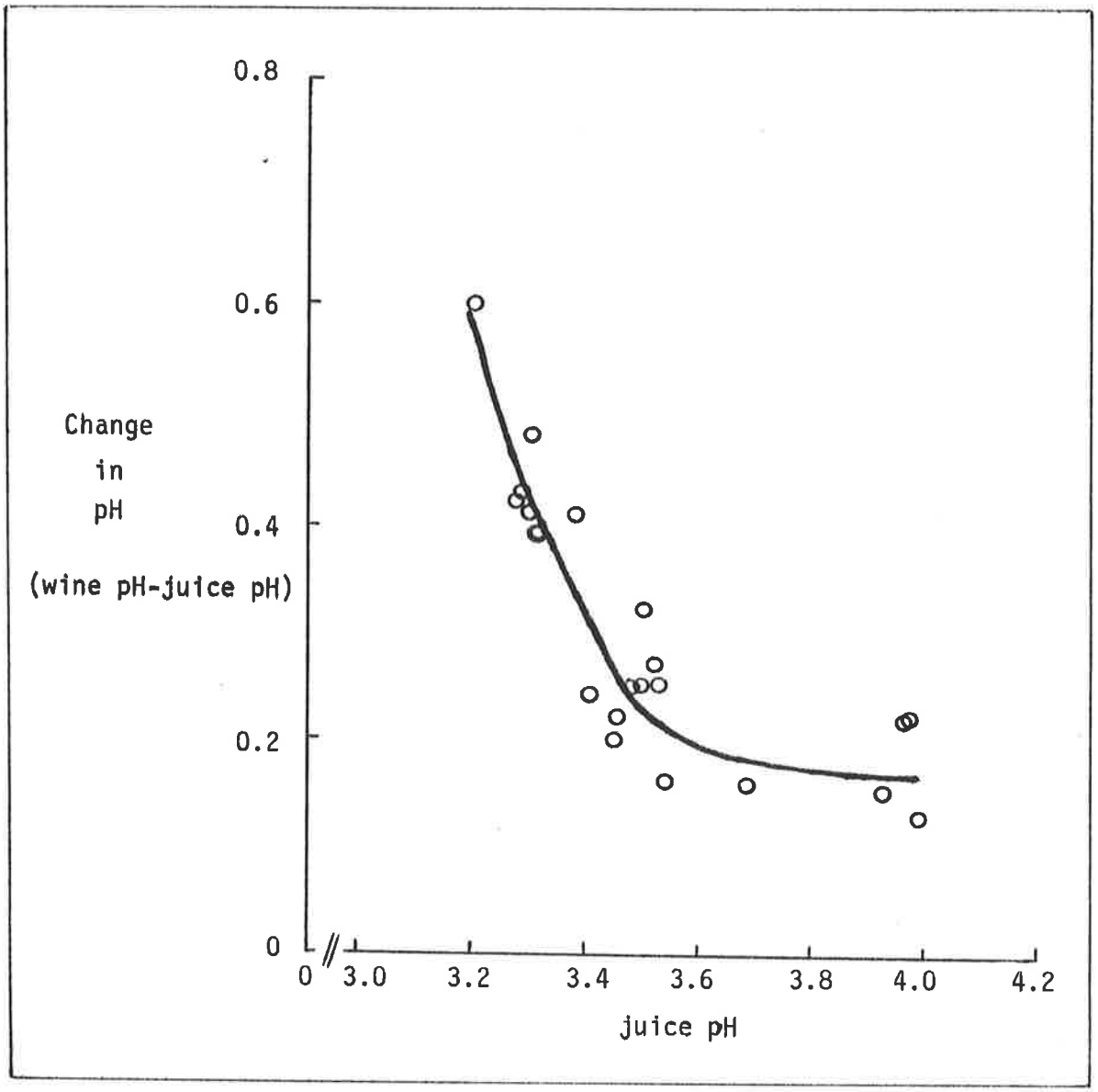

Fig. 5.2. The relationship between natural juice pH and the change that occurred during the skin contact period.

5.3. DISCUSSION

The large variability found in quality and style of red wines is due to variation in both grape composition and winemaking practice. Juice composition is of prime importance but, during vinification many compounds including flavours, cations, anthocyanins and phenolics are easily extracted from the grape skins. Oenological practices must aim at achieving a balance of these extractable materials, and excessive extraction of some components is undesirable. However extraction from the skins is not complete and it would appear that the skin contains an oenological excess of some of the above components. Analysis of skin tissue showed that 71%, 22% and 42% of the available potassium, total anthocyanin and total phenolic content respectively may remain in the skin after the period of skin contact. It is likely that large variations in these figures would occur due to wine making procedure and further analysis of this type from a variety of fermentations is necessary.

The amount of potassium extracted during the period of skin contact varied greatly, ranging from 124 to 2950 ppm in the fermentations studied here. Higher amounts of extractable potassium were associated with initially natural low pH and low potassium levels in the must.

Potassium concentration of the ferment is increased by skin extraction and decreased by potassium hydrogen tartrate (KHT) precipitation. After the initial large uptake of potassium ions the rate of precipitation exceeds extraction. If KHT precipitation was the only factor influencing potassium and tartrate concentrations after this stage both compounds should exhibit similar patterns of decreasing concentration. The observed difference in these patterns (Fig 5.1) indicates that extraction of potassium from skins continues throughout the period of skin contact. The slight rise in potassium concentration near the end of the skin contact period may be due to greater extraction induced by higher temperature, at this stage the fermentation temperature was allowed to rise slightly to ensure completeness of fermentation.

For some vinification lots malate concentration in the pressed wine was higher than that in the must, indicating that as the fermentation progressed malate in the skin was further extracted. This feature may be obscured if malic acid is metabolised concurrently with the primary fermentation. Marc samples collected after pressing from a number of the fermentations contained no malic acid (unpublished results). It appeared that the action of pressing was necessary to recover malic acid completely from the skins.

The pH of the ferment rises during the time on skins. An important feature of this pH rise during fermentation is that the change in pH is greatest when the initial natural juice pH is low (Fig 5.2). Factors contributing to this effect are decreased ionisation of the organic acids due to increasing alcoholic strength of the medium, conversion of malic acid to weaker acids, and the extraction of K^+ from solids. It is difficult to separate the effects of these factors, however removing the skin prior to fermentation allows the effect of K^+ extraction to be isolated. The change in pH was higher when pulp was fermented in contact with skin (Table 5.4). An increase of 0.1 - 0.2 pH units could be attributed to the effect of skin contact, although any metabolism of malic acid would also contribute to this pH change. This contribution was negligible for sample 3 in Table 5.4 since malic acid levels (shown in Fig 5.2) were similar in the wines made from pulp only and pulp + skin techniques. Only pH values were determined for samples 1 and 2 of Table 5.4.

High juice pH is normally associated with excessive levels of potassium derived largely from the berry pulp. In addition to pH defects, levels of titratable acid are often insufficient, particularly in grapes grown in warm to hot environments. For quality red wine production, adjustment of these acidity values is critical. Decrease in pH and increase in titratable acidity can be conveniently achieved by tartaric acid addition to the must prior to fermentation. If pH and potassium levels are excessively high and decrease in pH cannot be

obtained without large acidification then ion exchange may be an appropriate alternative (Somers 1975, Van Wyk 1977). Some natural acidity can be retained by preventing malo-lactic fermentation (Somers 1978, 1979).

Low juice pH is often associated with lower potassium and/or higher organic acid content. With these juices often no acidity adjustment is necessary prior to fermentation. However during fermentation the pH condition may change considerably. This is due in part to potassium previously located in the berry skin now entering the ferment and influencing the acidity equilibrium and in part to metabolism of malic acid, which is often a major acidity component. The emphasis for acidity regulation now shifts to adjustment of the wine either during or after the period of skin contact and is best achieved by addition of tartaric acid.

In juices where malic acid constitutes a major portion of the grape acidity, a greater pH increase would be expected to occur when this acid is either metabolised to succinic acid by yeast (Boulton 1980e) or to lactic acid by bacteria during the malo-lactic conversion. Consideration should be given to adjustment of even these natural low pH juices with tartaric acid prior to fermentation, in order that cumulative effects of cation extraction, decreased organic acid ionization and conversion of malic acid to weaker acids do not elevate the pH excessively and optimally not above about pH 3.65. Below this critical value any precipitation of potassium bitartrate salt will act to decrease the pH of the medium, which can compensate partly for the increase in pH during primary fermentation and further for any increases incurred by malic acid metabolism (Van Wyk 1977).

Further studies are needed to relate juice composition to the expected changes in pH during the various vinification stages. More useful decisions on acid adjustments and the desired incidence of malo-lactic fermentation could then be made.

Since pressing increases cation extraction, press fractions require critical evaluation and adjustment of pH.

No anthocyanins were detected in the wines produced without skin contact confirming the well established belief that for most black grapes the pigments are located entirely in the skin tissue. 'Retention indices' which give the proportion of total anthocyanins and total phenolics retained in the cold stabilized wines against the level initially available in the grapes have recently been described by Somers (1984). For the fermentations studied here a similar calculation showed that only 19-38% of the available total anthocyanins in the skin were retained in the wine after pressing Table 5.5. The low values are due to cumulative effects of imcomplete extraction and precipitation of extracted pigments. Further progressive losses would occur between this period and stabilisation.

These low 'retention indices' which were apparent immediately after the extractive stage of the fermentation suggest that the following approaches are necessary to optimise anthocyanin and phenolic balance in the finished wine:

(a) assessment of viticultural practices which give high total anthocyanin : total phenol ratio in wine grapes

(b) examination of vinification techniques to determine factors affecting extraction and loss of pigments and phenolics. Since large losses occur during processing it appears that methods aimed at retaining pigments and phenolics may be more important than excessive extraction which often leads to high total phenolic levels in the finished wine. Earlier removal of skins to prevent excessive phenolic extraction has been suggested by Somers (1975), especially for grapes from hot areas.

(c) maintenance of low pH and SO_2 regimes in the wine for optimal expression of the extracted colour.

The significance of pH and SO_2 on anthocyanin equilibrium in wine has been clearly demonstrated (Somers and Evans 1977). The first two factors require further examination. The technique of directly determining anthocyanin and phenolic content remaining in the skin at various stages of the fermentation is suggested as a useful research method to separate effects of extraction and precipitation.

These studies have shown that the skin, due to the extractable nature of a number of its components, affects red wine composition predominantly by increasing the cation, pH, total anthocyanin and total phenol levels. The degree of extraction of these compounds may vary considerably. An important feature not determined in these studies is the contribution of the skin to the flavour profile of red wine.

CHAPTER 6

INTERPRETATIONS OF ACIDITY MEASURES IN GRAPE JUICES.

CHAPTER 6

INTERPRETATIONS OF ACIDITY MEASURES IN GRAPE JUICES cvs. 'Shiraz' and 'Cabernet Sauvignon'.

ABSTRACT

Acidity measures (pH and titratable acidity) and the concentration of tartrate, malate, potassium and sodium were determined for a range of grape juice samples obtained from various Australian viticultural areas. Grape juice consisted mainly of the pulp contents of berries. When the berry was crushed only about 55% and 66% of the pulp's tartrate and potassium content repectively was dissolved into the juice. Malate was fully recovered.

Grape juice samples contain varying amounts of H_2T, HT^-, $T^=$, H_2M, K^+ and Na^+. It is proposed that the relative amounts of these forms that actually dissolved in the juice at crushing set the pH and titratable acidity in the juice. Higher pH values were associated with higher levels of HT^- and $T^=$, which correspond also to higher potassium concentration in the juice.

The data was used to test acidity relationships that had been proposed previously (Boulton 1980b,d,e). The results of these tests are discussed.

INTRODUCTION

Ripeness of wine grapes is normally assessed by the sugar content, acidity levels and flavour intensity of juice samples. Low juice pH and high titratable acidity are recognised as important ripeness indices for grapes designed for the production of quality dry red table wine. Components that contribute to these acidity expressions are the tartrate, malate, potassium and sodium concentrations; juice pH values

have been explained by the extent to which protons from the organic acid pool have been exchanged by cations, principally potassium (Boulton 1980b,d).

For acidity expressions at least, the contributing components are unevenly distributed between the pulp and skin of the berry e.g. at ripeness about 35% of the berry's potassium is contained in the skin (Section 2.3). In view of this, juice composition would be expected to provide only limited information on amounts and relationships of these components in the whole berry. Mattick (1983) has shown that erratic and incomplete extraction of potassium and tartrate occurs when normal methods are used for obtaining juice samples. He describes an extraction procedure which quantitatively accounts for these components. Malic acid on the other hand was shown to be readily soluble and completely extracted by the aqueous medium of grape juice.

In this chapter the above concepts are explored using a range of grape and grape juice samples from various Australian viticultural areas.

6.1. MATERIALS AND METHODS

A sample of berries was taken from a variety of grape loads prior to crushing. Sampling was either 4 x 50-berry lots from loads used in large scale fermentations or 2 x 50-berry lots from loads processed by small scale winemaking procedures at Roseworthy Agricultural College pilot winery. Sample lots were processed and analysed separately and the data averaged. The amounts of tartrate, malate, potassium and sodium in the pulp and skin of the berries were determined using methods of analysis similar to those described previously (Section 2.1.d).

After crushing, a representative sample of the juice was taken, centrifuged at 2000 rpm for about 5 minutes and the clear supernatant juice sample analysed for pH, tartrate, malate and potassium.

For each component a recovery index was calculated: this permits comparison of the actual concentrations in the juice to the potential concentration that would be achieved if all the component in the pulp were dissolved in the juice on crushing (Recovery Index I); or, the potential concentration that would be achieved if all the component in the pulp plus skin were dissolved in the juice on crushing (Recovery Index II). The factor used for converting amount per g berry weight to potential juice content was 0.6. This figure was the average calculated from berry weight/juice volume measurements of 6 wine making lots. Since this factor may vary for individual lots, calculations based on it will not be exact, but it does provide an estimate for interpretations.

The pH, titratable acidity, tartrate, malate, potassium and sodium concentrations in juice samples from various Australian viticultural areas were determined. Methods of analysis were similar to those described previously (Section 2.1.d).

Definition of terms

The terms tartrate and malate refer to the total amounts of each compound irrespective of form i.e. all undissociated (free) acid plus any of its dissociated or salt forms. Where the individual form is of

importance then the species is clearly identified.

The expected protons are the molar concentration of hydrogen ions that are associated with the organic acid anions when the concentration of these anions is expressed as if they existed entirely as the free acid form.

The titratable protons are the molar concentration of hydrogen ions measured by titration with a strong base (0.1M NaOH) to a predetermined end point (pH 8.2).

6.2. RESULTS

6.2.1. Relationship between berry content and juice composition.

The recovery indices (Table 6.1) show that juice analysis not only underestimates berry composition but is also erratic. Less than 50% of the tartrate and potassium in whole berries is revealed by analysing the juice. Tartrate and potassium in berry pulp are also only partly extracted, recovery indices being on average 55% and 66% respectively. Insolubility of tartrate salts (KHT, CaT) may account for these low recoveries. Recovery of malate was considerably higher, the average for Index I and Index II being 112% and 76% respectively. This indicates that essentially all of the malate in the pulp of the berry was dissolved into the juice on crushing, while malate located in the skin was not fully recovered.

6.2.b. Interpretations of acidity measures in juice samples.

The composition of juice samples obtained from various Australian viticultural areas is shown in Table 6.2. The data from Table 6.2. were assessed according to the methods described by Boulton (1980b,d,e). The following relationships were investigated:

i: the titratable protons (millimolar) versus the expected protons (millimolar) from the tartrate and malate concentrations (Fig 6.1).

TABLE 6.1. The relationship between berry content and juice composition.

SAMPLE	POTENTIAL JUICE CONCENTRATION DERIVED FROM CONTENTS OF THE PULP ONLY			POTENTIAL JUICE CONCENTRATION DERIVED FROM CONTENTS OF THE PULP AND SKIN			ANALYSED CONCENTRATION IN THE MUST (gL^{-1})			RECOVERY INDEX I (%)			RECOVERY INDEX II (%)		
	TARTRATE	MALATE	K^+	TARTRATE	MALATE	K^+	TARTRATE	MALATE	K^+	TARTRATE	MALATE	K^+	TARTRATE	MALATE	K^+
1	8.78	2.92	2.29	10.52	4.01	3.71	3.39	3.13	1.12	39	107	49	32	78	30
2	8.38	5.56	2.37	10.47	7.42	3.69	4.72	5.34	1.51	56	96	64	45	72	41
3	8.42	2.10	2.12	10.42	3.34	3.34	5.17	2.40	1.08	61	114	51	50	72	32
4	8.72	2.49	2.29	10.99	3.64	3.87	4.82	3.01	1.23	55	121	53	44	83	32
5	6.20	1.77	2.19	8.30	2.96	3.79	4.15	2.40	1.45	70	136	66	50	81	38
6	7.45	2.14	2.64	9.87	2.89	3.32	6.49	1.69	2.04	87	79	77	66	58	61
7	8.43	2.64	2.20	10.12	3.74	3.47	4.44	2.96	1.37	53	112	62	44	79	40
8	11.84	2.24	2.97	14.18	3.39	4.29	4.12	2.33	1.57	35	104	53	29	69	36
9	10.62	2.99	2.94	12.83	4.58	4.76	4.36	3.17	2.25	41	106	76	34	69	47
10	10.12	3.36	2.49	12.04	5.13	4.29	4.81	3.09	2.28	48	92	92	40	60	53
11	7.72	2.42	2.67	9.35	3.51	4.18	4.22	3.99	2.09	55	165	78	45	114	50
MEAN										54.5	112	65.5	43.5	75.9	38.9

TABLE 6.2. Composition of a range of juice samples from various Australian viticultural areas of 'Shiraz' and 'Cabernet Sauvignon' grapes.

SAMPLE	pH	TITRATABLE ACIDITY $g\ L^{-1}$ (as H_2T)	TARTRATE $g\ L^{-1}$	MALATE $g\ L^{-1}$	POTASSIUM $g\ L^{-1}$	SODIUM $g\ L^{-1}$	[POTASSIUM] / [TARTRATE] Ratio x 10
1. Eden Valley, S.A.	3.15	6.60	3.95	3.13	0.940	0.110	2.38
2. *Kyneton, Vic.	3.15	8.30	3.84	5.20	1.409	0.110	3.67
3. Kyneton, Vic.	3.21	6.50	4.23	4.00	1.283	0.110	3.03
4. Eden Valley, S.A.	3.27	5.60	3.39	3.12	1.120	0.130	3.30
5. *Virginia, S.A.	3.30	8.50	5.80	4.30	1.788	0.100	3.08
6. Roseworthy, S.A.	3.32	8.40	4.72	5.34	1.505	0.109	3.19
7. Barossa Valley, S.A.	3.41	6.60	5.17	2.40	1.075	0.065	2.08
8. Hunter Valley, N.S.W.	3.45	6.50	6.59	2.50	1.934	0.090	2.93
9. Roseworthy, S.A.	3.49	5.53	4.44	2.96	1.370	0.073	3.08
10. Barossa Valley, S.A.	3.52	6.50	4.82	3.01	1.225	0.080	2.54
11. Hunter Valley, N.S.W.	3.54	6.60	6.90	3.10	2.485	0.125	3.60
12. Barossa Valley, S.A.	3.68	5.20	4.15	2.40	1.450	0.090	3.49
13. Roseworthy, S.A.	3.73	4.31	4.10	3.33	1.600	0.094	3.90
14. Roseworthy, S.A.	3.76	4.20	4.12	2.33	1.565	0.088	3.80
15. Roseworthy, S.A.	3.77	3.95	4.14	2.03	1.530	0.092	3.70
16. Virginia, S.A.	3.93	4.43	4.36	3.17	2.248	0.050	5.15
17. Adelaide, S.A.	3.96	6.20	4.22	3.99	2.092	0.090	4.95
18. Virginia, S.A.	4.06	4.40	4.81	3.09	2.383	0.050	4.95
19. Roseworthy, S.A.	4.22	3.50	7.27	2.00	3.900	0.080	5.36

* 'Cabernet Sauvignon', all others are 'Shiraz'

Table 6.3. Linear regression parameters of acidity relationships in grape juice samples.

	Titratable protons vs. expected protons (Fig 6.1)	Sum of monovalent metal cations and titratable protons vs. expected protons (Fig 6.1)	pH vs extent of exchange (Fig 6.2)	Ratio of titratable protons to expected protons vs. potassium concentration (Fig 6.3)
Correlation coefficient (R)	0.64	0.96	0.89	-0.82
Coefficient of determination (R^2) (precision)	0.41	0.92	0.79	0.68
Slope	0.73	1.14	2.15	-6.255
Intercept	-0.003	0.0004	2.65	0.98

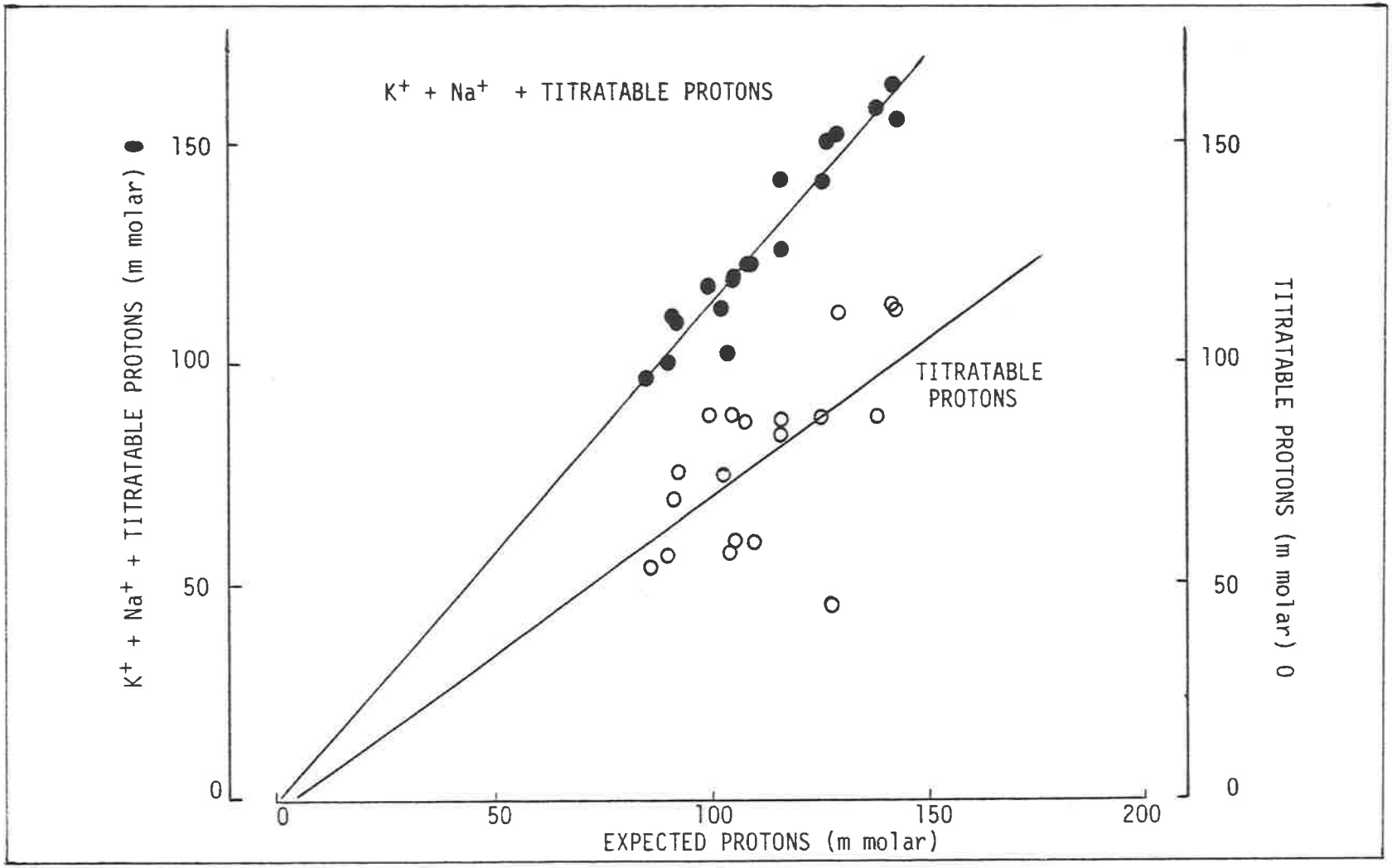

Fig. 6.1. The relationship between titratable protons and expected protons (O-O) (Y = 0.73 X -0.003, R^2 = 0.41), and the relationship between the sum of potassium, sodium and titratable protons and the expected protons of grape juice samples (●-●) (Y = 1.14 X +0.0004, R^2 = 0.92). Sample points represent the 17 'Shiraz' and 2 'Cabernet Sauvignon' grape juices shown in Table 6.2.

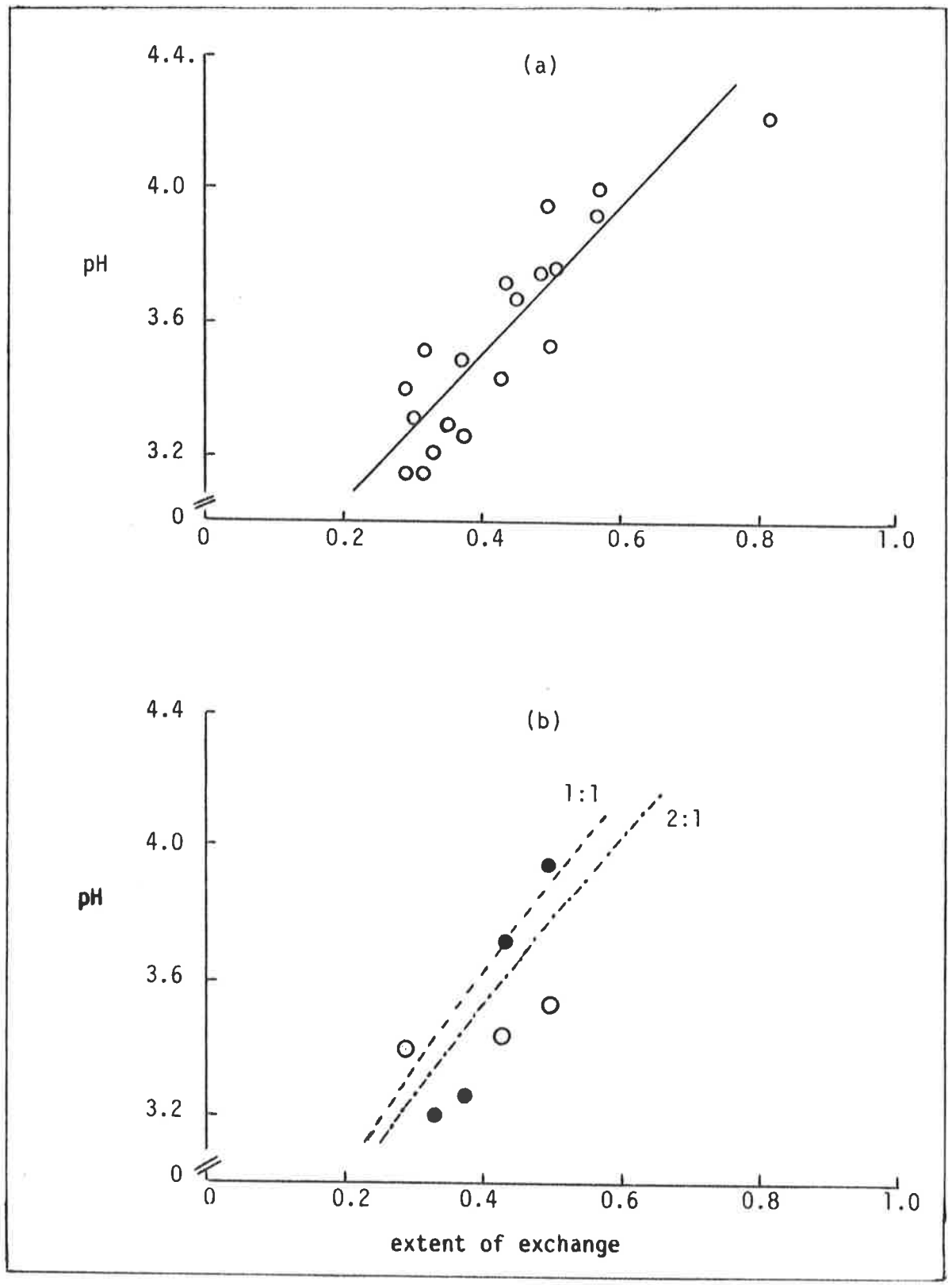

Fig. 6.2. (Top) The relationship between pH and the extent of exchange in grape juices. Sample points represent the 17 'Shiraz' and 2 'Cabernet Sauvignon' grape juices shown in Table 6.2. ($Y = 2.15 X + 2.65$, $R^2 = 0.79$). (Bottom) Dotted lines show the theoretical extent of exchange curves for tartrate to malate ratios of 1:1 and 2:1 (Boulton 1980). Full circles (●) and open circles (○) represent samples from Table 6.2. of known tartrate to malate ratios of 1:1 and 2:1 respectively, indicating that these samples did not fit the theoretical relationship.

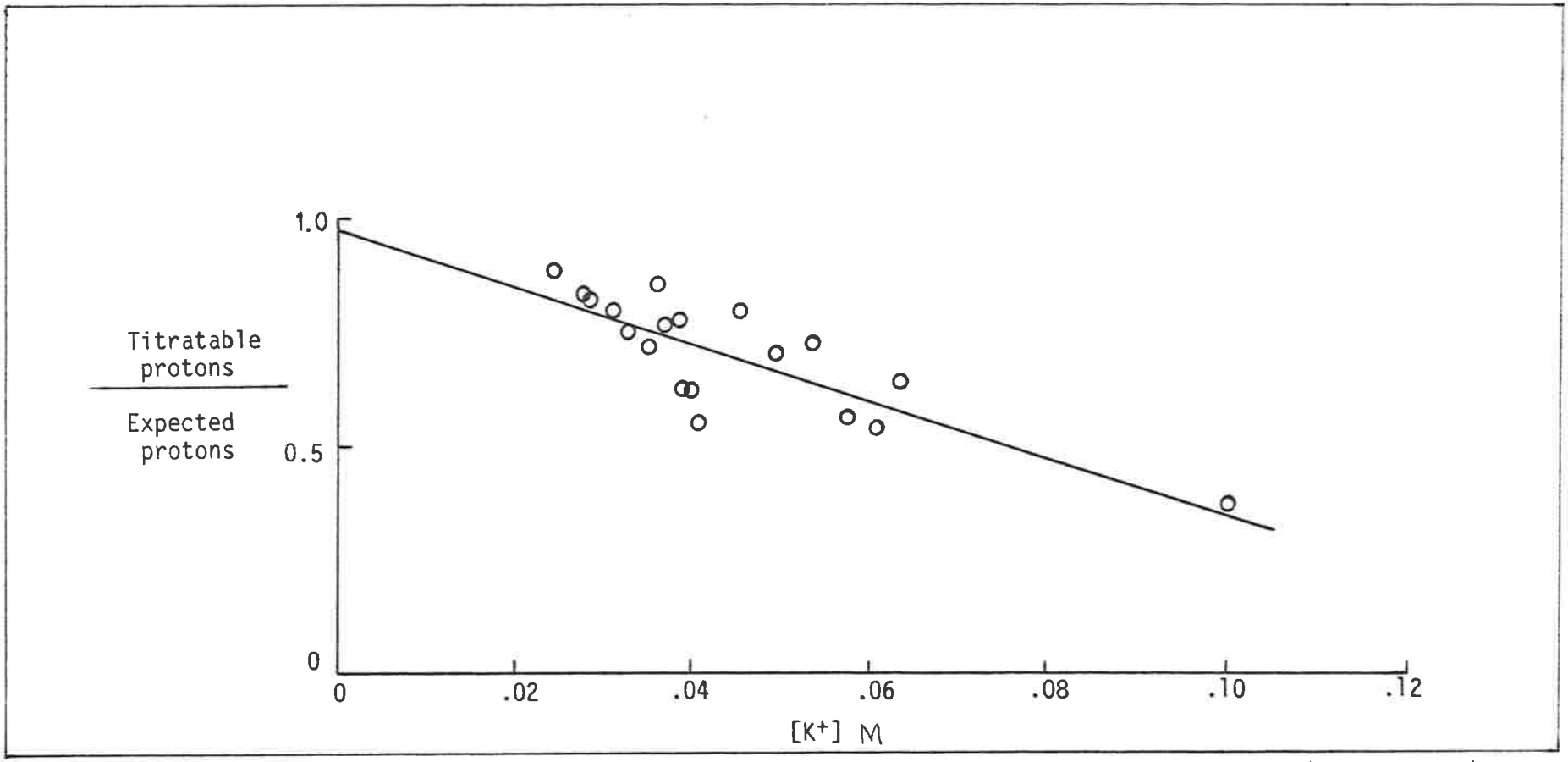

Fig. 6.3. The relationship between the proportion of titratable protons to expected protons and potassium concentration in grape juice samples ($Y = -6.3\ X + 0.98$, $R^2 = 0.68$). Sample points represent the 17 'Shiraz' and 2 'Cabernet Sauvignon' grape juices shown in Table 6.2.

ii: the sum of potassium, sodium and titratable protons (millimolar) versus the expected protons (Fig 6.1).

iii: pH versus the extent of exchange (ratio of potassium concentration, millimolar, to expected protons, millimolar) (Fig 6.2).

iv: $\left[\frac{\text{Titratable protons}}{\text{expected protons}}\right]$ versus potassium concentration (millimolar) (Fig 6.3)

The statistical results from the above regressions are presented in Table 6.3.

6.3. DISCUSSION

The titratable acidity of the juice is obtained by neutralising a known volume of juice with a strong base (0.1M NaOH) to a predetermined end point. This measurement gives the total available amounts of hydrogen ions in the solution. Only species that contain available hydrogen ions participate in the neutralisation reaction. Salt forms of acids e.g. HT^- and $T^=$ have less available hydrogen ions than equivalent amounts of the undissociated (free) acid. Titratable acidity should not be incorrectly referred to as total acidity, as this latter expression represents the sum of all the individual acid forms present, free or as salts, determined by specific chemical methods. This measurement gives the total available amounts of organic acid anions in the solution. In grape juices the protons expected from the organic acid concentration is numerically equal to the sum of the titratable protons, the potassium and sodium concentrations (Boulton 1980b,d,e). Tartrate and malate are the principal organic acids in grape juice hence

$$2[\text{Tartrate}] + 2[\text{Malate}] = [H^+]_T + [K^+] + [Na^+] \quad \text{... equation 6.1.}$$

where $[H^+]_T$ represents the titratable protons, and all terms are expressed as molar concentrations. The total acidity is always greater than the titratable acidity.

In the sample of juices examined here, 73% (the slope of the regression line in Fig 6.1) of the expected protons were recovered by the titratable acidity. This compares to a value of 68% reported by Boulton (1980d,e). The missing protons can be stoichiometrically accounted for by substituting the monovalent cations, potassium and sodium, into the relationship (Boulton 1980b,d,e); when this was done (Fig 6.1) the regression parameters (Table 6.3) showed improved correlation coefficient (R), precision (R^2) and recovery (slope x 100) when compared to those corresponding to the relationship using titratable protons alone. The recovery (114%) was higher than that (98%) reported for the samples investigated by Boulton (1980d,e). This could be due either to uncertainties in the measurements or to the fact that the sum of protons expected from the tartrate and malate concentrations underestimates the total available protons in these grape juice samples. Other acids that would contribute in a minor way to the titratable acidity are other carboxylic acids (e.g. citrate), dihydrogen phosphate ion, amino acids and phenolic compounds. Thus the relationship between the expected protons and the sum of potassium sodium and titratable protons would be improved if the expected protons from these additional components were added to those arising from the tartrate and malate species.

It has been proposed that during berry ripening potassium ions enter berry cells in exchange for protons derived from the organic acid pool (Boulton 1980c). In chapter 2 it was shown that malate existed entirely as the free acid (H_2M) at all stages of berry ripeness; thus it appears that the proposed exchange is particular to protons originating from only tartrate species. Absolute levels of tartrate are essentially constant during berry ripening which means that, progressively, the free acid (H_2T) changes to the bitartrate ion (HT^-) and then at higher levels of potassium exchange the tartrate ion ($T^=$) is formed. When the berry is crushed, only a portion of the total tartrate present in the intact pulp of the berry is dissolved into the juice, while malate is completely soluble (as indicated by recovery indices, Table 6.1). In order to maintain charge balance in the juice equivalent amounts of cations must accompany any anions when they dissolve. Potassium and sodium are the most likely counter ions for HT^- and $T^=$ species. One

and two moles of monovalent cations would accompany each mole of HT^- and $T^=$ that dissolves in the juice. Grape juice or must samples would then represent solutions to which varying amounts of H_2T, HT^-, $T^=$, H_2M, K^+ and Na^+ had been dissolved.

The proposal that malate is dissolved entirely as the free acid does not negate the relationship shown in equation 6.1. A theoretical example is given below:

Consider that when the berry is crushed, 1 mole of tartrate and 1 mole of malate is dissolved in the juice, and that the proportions of tartrate that were added as H_2T, HT^- and $T^=$ are x, y and z respectively where x+y+z = 1. The following balances would result:

	H_2T	HT^-	$T^=$	H_2M	$K^+ + Na^+$
analytical concentration (molar) of each species dissolved in the juice	x	y	z	1	y+2z
molar concentration of titratable protons contributed by each species	2x	y	0	2	-

1 mole each of tartrate and malate would be expected to contribute 2 moles of protons each, making the protons expected from the organic acid concentration equal in total to 4. The sum of the titratable protons (2x + y + 2) and the monovalent cations (y + 2z) also equals 4, thus confirming the relationship.

Accumulation of potassium in the pulp during berry ripening would result in increased amounts of first HT^- and then $T^=$ forms of tartrate in the intact berry pulp and subsequently in the juice on crushing. Since these forms contain less titratable protons than the equivalent amount of H_2T, the titratable acidity is effectively decreased. The lower the potassium concentration the higher is the proportion of titratable protons to the expected protons (Fig 6.3), e.g. at 0.03M K^+ 90% of the expected protons were recovered, while at the other extreme (0.10M K^+) only 37% of the expected protons were found.

Whereas the titratable acidity measures the hydrogen ion concentration quantitatively whether the proton is free in solution (H^+) or bound to anions (e.g. H_2T or HT^-), the pH is related to the concentration of only the free hydrogen ions (H^+) in solution.

The pH of grape juices are higher than values that would be expected from the organic acid content. This is because some of the tartrate dissolving in the juice is in the HT^- and $T^=$ forms. Since malate does not contribute any salt forms, juices should essentially represent a range of tartrate buffers modified to varying degrees by the addition of different amounts of malic acid. The tartrate buffer consists of a mixture of the species H_2T, HT^- and $T^=$. As previously discussed, only portion of the tartrate species dissolve into the juice on crushing. It is proposed that the relative amounts of H_2T, HT^- and $T^=$ species plus the amount of H_2M that actually dissolve in the juice on crushing determines the pH value of the must. Higher pH values are associated with higher levels of HT^- and $T^=$ dissolved in the juice, which corresponds also to higher potassium concentration. The ratio of the different species in the juice at equilibrium will be determined by the final pH; this ratio may or may not be the same as that when the species were dissolved or that when they were contained in the intact berry. Malic acid, even though present only as H_2M in the intact berry, when dissolved in the juice will now be distributed between H_2M, HM^- and $M^=$ species, the relative amounts depending on the pH of the juice. Similarly the ratios of tartrate species that actually dissolve are also modified. The significance of the above phenomenon is that equilibrium relationships in the juice cannot be directly related back to those existing in the intact berry.

Once dissolved the ratio of the forms of the individual acids in grape juice is related to the juice pH according to the Henderson-Hasselbach equation

$$pH = pK_a + \log \frac{[salt]}{[acid]}$$

The tartrate equilibrium system in grape juice is as follows: at juice pH values below 3.56 the predominant equilibrium reaction is between the

H_2T and HT^- species ($H_2T \rightleftarrows H^+ + HT^-$), and above this value the equilibrium between HT^- and $T^=$ ($HT^- \rightleftarrows H^+ + T^=$) is more significant (Fig 1.3). However since the first dissociation of H_2T is not completed before the dissociation of HT^- begins, the relationship between the pH of the mixture and the various forms of tartrate involves both the H_2T/HT^- and the $HT^-/T^=$ equilibria and can be represented by the relationship

$$pH = \frac{1}{2}(pK_{a1} + pK_{a2}) + \frac{1}{2}\log\frac{[T^=]}{[H_2T]}$$

where pK_{a1} and pK_{a2} refer to the first ($H_2T \rightleftarrows H^+ + HT^-$) and second ($HT^- \rightleftarrows H^+ + T^=$) dissociations respectively. A similar relationship could be written for malate species, using the appropriate pK_{a1} and pK_{a2} values.

Boulton (1980b,d) obtained good agreement between predicted pH values calculated by removing protons equivalent to the monovalent cations from the organic acid pool and actual pH values. Again the proposal that malate is added entirely as the free acid does not conflict with this theory, since the same final pH is achieved whether the initial organic acid pool contains both tartrate and malate or if the protons are removed first from tartrate alone (e.g. by titration with KOH) and then malic acid added.

The pH of grape juices had been explained in terms of the extent of exchange expressed as

$$\frac{[K^+] + [Na^+]}{[H^+]_T + [K^+] + [Na^+]} \qquad \text{(Boulton 1980b,d)}$$

There was a good correlation between pH and the extent of exchange calculated for the juice samples examined here (Fig 6.2.a, Table 6.3). Low extent of exchange can be due either to low concentrations of potassium and sodium or to high organic acid content (Boulton 1980d,e). An exception to this is when potassium concentration is excessively high and even though tartrate levels are large the pH will remain high, as in sample 19 of Table 6.2. This is because most of the tartrate in the

juice has initially dissolved as the $T^{=}$ form, and larger amounts are retained in the aqueous medium due to the greater solubility of K_2T. However the influence of this larger concentration of tartrate on lowering the pH or increasing the titratable acidity is negligible since it has been added as the $T^{=}$ form.

When the predicted pH curves for various ratios of tartrate to malate (from Boulton 1980b) were superimposed on the data from Table 6.2, samples with known tartrate to malate ratios showed large scatter and deviation from these theoretical lines (Fig 6.2.b.). The proposal that malic acid is added totally as the free acid while tartrate addition involved a mixture of free acid and salt forms, may negate the separation of the data into tartrate to malate ratios. Perhaps the ratio of potassium to tartrate is more important in setting the extent of exchange. Comparison of tartrate to malate ratios and their significance to grape maturity are only appropriate when samples have the same total acidity and monovalent cation ($k^+ + Ca^{++}$) concentration. When these measures are the same, juices having higher tartrate levels will show lower pH. Normally, though, juice samples will differ in concentrations of all the chief acidity components - tartrate, malate, potassium and sodium.

Table 6.4. shows a range of juices were the titratable acidity is similar, the total acidity varies less than 2 fold, but the hydrogen ion concentration in these juices varies by over 6 fold, emphasising that there is no simple or predictable relationship between pH and titratable acidity nor between pH and total acidity. This is because grape juices are mixtures of different acids and acid salts.

Table 6.4: The pH, titratable acidity and total acidity of a range of grape juice samples.

pH	Titratable acidity	Total acidity
3.15	6.60	7.08
3.21	6.50	8.23
3.41	6.60	7.57
3.45	6.50	9.09
3.52	6.50	7.83
3.54	6.60	10.00
3.96	6.20	8.21

Some general conclusions can be made from the range of juice samples shown in Table 6.2:

- Low titratable acidity and high pH (Table 6.2. sample 19) is probably due to high concentrations of $T^{=}$ ions, reflected by both high potassium to tartrate ratio and high potassium, coupled with low malic acid levels. The use of ion exchange to adjust the pH is more suitable than tartaric acid addition, since this achieves a greater decrease in pH for the same increase in the titratable acidity. These juices are often obtained from grapes grown in hot viticultural areas in Australia.
- Acceptable titratable acidity but still high pH (Table 6.2, sample 17) is probably due to high concentrations of $T^{=}$, reflected by both high potassium to tartrate ratio and high potassium, but coupled with higher malic acid levels. These higher amounts of malic acid contribute titratable protons, but are not effective in reducing pH greatly. These juices require care since although the pH needs to be lowered the increase in titratable acidity should only be minimal. Although the eventual titratable acidity in the wine is lowered by potassium bitartrate precipitation, the acidity due to malic acid in the juice is retained in the wine. Thus excessive increase in juice titratable acidity should be avoided as this may have a direct bearing on the final wine acidity and acid taste. The problem can be countered somewhat by the careful use of ion exchange. These typesof juices are often associated with conditions that cause shading in vine canopies and the problem might best be solved by viticultural techniques, e.g. open vine canopies lead to both decreased potassium and malate levels in the berry pulp (Chapter 3) thus lowering both the juice pH and the titratable acidity.
- Acceptable titratable acidity and low pH (Table 6.2, sample 1) is probably due to a higher ratio of H_2T to HT^{-}, reflected by lower potassium to tartrate ratio and low potassium, coupled with moderate levels of malic acid.
- High titratable acidity and low pH (Table 6.2, sample 2) is probably due to moderate HT^{-} levels, reflected by moderate potassium to

tartrate ratio and moderate potassium, coupled with high malic acid levels. These high levels of malic acid will result in large change in pH during the malo-lactic conversion. Because of this, wine pH should be lowered prior to this occurring. On completion of the malo-lactic the pH rise is coupled with a decrease in titratable acidity, partly compensating for the acidity increase due to the previous adjustment.

These examples represent extreme cases, and other pH values in the mid-range may be due to a variety of combinations of the above components.

A large proportion of the tartrate in the pulp of grape berries exists either as HT^- or $T^=$ (Section 2.2.c). The fact that K_2T is more soluble than CaT would mean that when the $T^=$ concentration is high precipitation of calcium salts are more likely to occur. This is in line with Ruffner's (1982a) finding that the crystals found in grape berry cells are exclusively the calcium salts and not due to potassium, as commonly thought.

Viticultural and climatic factors influence potassium and malate levels in ripening grape berries considerably while only minimally affecting tartrate levels. Variation in potassium concentration alters acidity equilibrium more than does a similar variation in malic aicd (Boulton 1980e). Because of the indirect influence of potassium on both titratable acidity and pH in grape juices, especially the latter, viticultural practices should be aimed at limiting the uptake of this cation during berry ripening, to yield juice samples with low or moderate potassium to tartrate ratios. The balance between pH and titratable acidity in the juice should then be appropriate for quality dry red table wine production.

CHAPTER 7

INTEGRATIVE DISCUSSION.

CHAPTER 7

INTEGRATIVE DISCUSSION

This thesis investigates some factors influencing the organic acid, monovalent cation, total anthocyanin and total phenol content of the pulp and skin of black grapes during the ripening period of berry growth, and the significance of the amounts of these compounds in ripe grapes in relation to juice and wine quality. The literature pertaining to the above aspects is reviewed in Chapter 1.

In Chapter 2, differences between the composition of pulp and skin are examined. It was found that an average 19%, 31%, 38% and 25% of the tartrate, malate, potassium and sodium, respectively of ripe berries was contained in the berry skin. The coloured pigments, the anthocyanins, are located entirely in the skin of the varieties ('Shiraz' and 'Cabernet Sauvignon') examined here, as in most other red wine grape varieties.

During berry ripening amounts of malate in the pulp declined, sometimes to 1/5 of the value at veraison, while in the skin malate levels increased throughout the same period. Comparison of potassium and sodium concentrations in the pulp and skin confirmed that potassium is the major monovalent cation of the grape berry. Levels of potassium increased significantly in both pulp and skin, and during the period from veraison to ripeness the potassium content of whole berries increased as much as 3 to 4 fold. Absolute levels of tartrate remained relatively constant during berry ripening; however, differential extraction of berry pulp with 80% ethanol showed that the free acid form (H_2T) of tartrate was progressively converted to salt forms (HT^-, $T^=$). Malate on the other hand existed entirely as the free acid (H_2M) at all stages of berry ripening.

In the field, the variability in the above measures can be large. Because of this it was thought important to consider some of the controlling factors influencing levels of these components in the berry. The effects of canopy structure and vine water status on the

organic acid, cation, total anthocyanin and total phenol content in pulp and skin of ripening 'Shiraz' grapes were investigated and are reported in Chapter 3. Ripe fruit from shaded canopy environments had higher potassium, malate and pH values in the berry pulp and decreased levels of total anthocyanin in the skin. Shaded conditions induced high potassium in berries at veraison and this correlated positively with potassium levels in ripe berries, indicating that conditions of vine growth prior to veraison are important in setting the pattern of uptake of this ion during the final stages of berry ripening.

The interrelationship between improved vine water status and berry composition appears dependent on the extent to which the applied treatment modifies the canopy structure. If canopy shading is attenuated adversely, then berry composition is affected correspondingly.

Considering that the individual acids of the grape change in different ways during the development of the berry, a knowledge of where each acid is located within berry cells is important in understanding the difference between tartrate and malate metabolism during the ripening phase. Both are likely to be compartmented in cell vacuoles, but due to changes in permeability of cellular membranes may be transported to more metabolically active sites within the cell as ripening progresses. The study of efflux of a solute out of tissue when the tissue is immersed in a defined washing medium provides information about properties of cell membranes in relation to that solute and also compartmentation of that solute. This type of study was carried out using pulp and skin tissue of 'Shiraz' grapes at three stages of berry ripeness (beginning, mid and ripe) and is discussed in Chapter 4.

Efflux studies showed that as ripening progressed, the speed with which tartrate, malate and potassium leached out of pulp tissue increased, indicating an increased membrane permeability with berry ripening. In ripe berries malate leached out more readily than tartrate or potassium, i.e. less was contained in the tissue at the end of the 45 minute period during which it was immersed in the washing medium. These differences would occur if the cellular membranes display selective permeability towards malate compared to tartrate or potassium. If all

compounds are contained in the same vacuoles, then each individual acid would exist in varying proportions of free acid and salt forms depending on the pH of the vacuolar solution. However in Chapter 2 it was shown that malate existed entirely as the free acid form (H_2M) at all stages of berry ripening. A second interpretation of the efflux studies is then, that malate is contained in cells separate to those storing tartrate and potassium. This would then explain how it is possible for malate to exist entirely as the free acid in vacuolar solutions. If the permeability of the tonoplast of these malate type cells changes with berry ripening, then the endogenous pattern of malate respiration is in accord with this speculation. Berry temperature would be an important factor regulating this process in ripening grape berries.

Efflux studies of skin tissue provided further evidence of the different properties of pulp and skin and suggested increased compartmentation of the compounds tartrate, malate and potassium in skin cells of ripening grape berries. This conclusion is consistent with in vivo patterns since in Chapter 2 it was shown that all these compounds are accumulated in skin cells during the ripening phase of berry growth.

The degree to which compounds contained in the skin of black grapes influence the character and quality of red wine is an important oenological variable. The effect that the skin can have on red wine composition is explored in Chapter 5. Even though as much as 40% of the berry's potassium may be located in the skin, it is not fully extracted during vinification. Higher amounts of extractable potassium were associated with natural low pH and low potassium levels in the juice. A further important observation was that the rise in pH associated with fermentation of black grapes was greatest when natural juice pH was low. This rise is due to cumulative effects of cation extraction from the skins, decreased organic acid ionization and metabolism of malic acid to weaker acids, and pH values of even low pH musts can change considerably throughout vinification. Microfermentations of berry pulp with either skin attached or removed indicated that an increase of 0.1 - 0.2 pH units of the pH change could be attributed to the effect of skin contact. Since pressing increased cation extraction from the skin, press fractions require critical evaluation and an adjustment of pH. At

pH values below 3.65 any precipitation of potassium bitartrate acts to decrease pH of the medium, which can compensate partly for the increases incurred during the fermentation; hence it is recommended that pH values should not exceed this critical level at any stage of the vinification.

The total anthocyanin and total phenol concentrations in wines are always less than that possible from the grape material. This is because only partial extraction from the skin occurs and large losses of phenolic material result from precipitation during wine processing. Analysing the skin before and after fermentation provides a means of separating the effects of extraction and precipitation in setting final levels of total anthocyanins and total phenols in the wine. Extended skin contact resulted in higher levels of potassium, pH and total phenols in the final wine, all of which may lead to unbalanced wine composition. If high levels of available total anthocyanins can be achieved in the raw product, then extended skin contact during vinification may not be necessary to achieve satisfactory red wine colour and quality. The viticultural trials described in Chapter 3 indicated that open canopies, providing greater cluster exposure, are involved in mechanisms enhancing total anthocyanin levels in the skin of black grapes.

An understanding of the individual components that contribute to the expressions of pH and titratable acidity in grape juice is important in assessing viticultural practices affecting levels of those components, and also for determining the degree of amelioration prior to fermentation. The interpretation of acidity measures, in a range of 'Shiraz' and 'Cabernet Sauvignon' grape juices obtained from different viticultural areas in Australia, is discussed in Chapter 6.

Grape juice consists mainly of the pulp contents of berries. However, when the berry is crushed, on average only 55% and 66% of the pulp's tartrate and potassium respectively is dissolved into the juice. Malate was fully recovered. In Chapter 2 it was shown that the form of tartrate progressively changes to salt forms with berry ripening, but malate is present only as the free acid. Grape juice or must samples would then represent solutions to which varying amounts of H_2T, HT^-,

$T^{=}$, H_2 M, K^{+} and Na^{+} had been dissolved. It is the relative amounts of these forms that actually dissolve in the juice at crushing that sets the pH and titratable acidity value in the juice. Higher pH values are associated with higher levels of HT^{-} and $T^{=}$, which corresponds also to higher potassium concentration in the juice. The forms HT^{-} and $T^{=}$, contain less titratable protons than equivalent amounts of the free acid, hence the expected titratable acidity is reduced. The higher the potassium concentration in the juice the greater is the reduction in the expected titratable acidity. The proposed relationship (Boulton 1980b,d,e) that the protons expected from the concentration of the organic acids is equal to the sum of the titratable protons and the potassium and sodium concentrations was confirmed, but the regression equation was slightly different to that reported by Boulton.

It was shown that high juice pH was normally associated with high potassium levels in the pulp. This conclusion reinforces the positive correlation found between potassium and pH values in berry pulp (Chapter 3). Because of the indirect influence of potassium on both the titratable acidity and the pH in grape juices, viticultural practices should aim at limiting the uptake of this cation during berry ripening. The evidence to date (Chapter 3) suggests that open grape vine canopies are an appropriate means of achieving this aim.

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