Pre-maceration, Saignée and Temperature affect Daily Evolution of Pigment Extraction During Vinification†

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Consumer demand for intensely coloured wines necessitates the systematic testing of pigment extraction in Sangiovese, a cultivar poor in easily extractable anthocyanins. Pre-fermentation (absent, cold soak pre-fermentation at 5 °C, cryomaceration by liquid N_2 addition), temperature (20 or 30 °C), and saignée were compared during vinification (800 kg). Concentrations of anthocyanins, non-anthocyanic flavonoids and SO_2-resistant pigments were recorded daily. A semiparametric Bayesian model permitted the kinetic description and the comparison of sigmoidal- and exponential-like curves. In total anthocyanins, saignée at 30 °C yielded a significant gain, later lost at drawing off; cryomaceration had little effect and cold soak no effect at drawing off. Non-anthocyanic flavonoids increased steadily with saignée and at 30° C. SO_2-resistant pigments were higher, particularly for the higher temperature/saignée combination. Using daily recordings, the model indicates turning points for concentration rise or fall, thus allowing a precise and detailed comparison of the vinification methods.

1 Introduction

A large number of oenological methods and combinations thereof are today employed in winemaking in order to develop new wines or to meet market demand. Recently, consumers are increasingly requesting red wines which combine an intense colour, a powerful tannic structure and a fruity flavour. In Tuscany, Sangiovese, a cultivar poor in easily extractable anthocyanins, usually undergoes a long post-fermentation maceration after vinification. Other methods, such as pumping over, punching down, and temperatures higher than 28-30 °C (controlled or uncontrolled) may however be used for pigment extraction. In response to market demand, experiences with novel winemaking methods not traditionally applied to Sangiovese have been reported[1,2]. Some of these vinification procedures and conditions have been tested primarily on Pinot noir, a cultivar also poor in easily extractable anthocyanins[3,4]. Cold soak pre-fermentation, where the destemmed-crushed berries are kept at low temperatures before fermentation, has been used on Sangiovese and other cultivars[3,5–8]. The contrasting results in the literature are most probably due to variability in the parameters used, such as contact time (1 to 8 days), temperature level (0 to 14 °C), refrigeration method (normal refrigeration or solid CO_2), and the use of inert gas[4]. Cryoextraction, where the berries are kept in contact with liquid nitrogen to lower their temperature and break cell walls and membranes via thermal shock, thus favouring the release of compounds from the skin has given encouraging results with Sangiovese[1]. During fermentation, many processes (extraction from solids, diffusion, solubilization, sorption on solids etc.), occur simultaneously growing or diminishing in importance over time as their physical-chemical-biological properties continuously change. For example, in anthocyanin concentration, a maximum followed by a drop has been observed[9,10], whereas tannin extraction continues to increase[11]. The evolution of such complex and dynamic systems is difficult to describe with simple and canonical kinetic laws, therefore some mathematical generalizations have been studied.

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2 Materials and methods

2.1 Winemaking

Sangiovese grapes (2008 vintage) were manually harvested at maturity in the area of Maremma, South Tuscany, and collected into 20 kg buckets which were allocated to bins (500 L) by a randomization procedure. The bins were transported to the experimental winery and then assigned to one of the 36 cylindrical stainless steel tanks by an extra randomization procedure. The combination of experimental factors resulted in 12 vinification trials, which were run in triplicate. At the beginning of vinification, each tank contained 800 kg of grapes. A programmable control unit (Parsec s.r.l.) regulated the temperatures and pumping-overs of each single tank. The saignée was targeted at 20% vol/weight of the volume of the tank (1000 L). The required final weight (800 kg) was reached by drawing off the free-run juice (200 L) from the bottom valve. After the saignée, $K_2S_2O_5$ (15 g hL^{-1}, corresponding to 70 mg L^{-1} SO_2) was added to each tank, and the temperature

set to 20° or 30°C as required. Finally, the tanks were inoculated with 25 g hL^{-1} of EC1118 yeast strain (Lalvin). During the maceration, the tanks underwent pumping over cycles the number and duration of which were adjusted daily on the basis of the must density (Table 1). The fermentative maceration continued for 14 days in all tanks. The CSPF required a further four days prior to the beginning of fermentation.

The pre-maceration was performed as follows: CTRL; the tanks were filled with destemmed-crushed berries, the saignée applied where necessary and the content brought to 20 or 30 °C as required: CSPF; the tanks were treated as for CTRL, but the temperature was lowered to 5°C by conventional refrigeration, maintained for 48 hours, and then raised in two steps (see note on Table 1) to the planned heat: CRYO; the destemmed-drained berries were treated using experimental equipment (Parsec s.r.l.) consisting of a freezing tunnel containing a stainless steel conveyor belt, on top of which some sprinklers for liquid N_2 were installed. The contact time of N_2 with berries was around 5-10 seconds, as required to obtain a temperature of the mass below 0 °C, as regulated by the speed of the conveyor belt. The resulting temperature proved to be between 7 and -5 °C in the flowing mass. The berries were then crushed and put back with the previously drained must. The saignée was applied where required, and the inoculation was carried out after 18-24 h, when the temperature returned to 20°C.

2.2 Lab measurements

After a pumping over, 100mL were withdrawn each day from the sampling valve of each tank, to which 400 mg of NaF were added to stop fermentation, and centrifuged at 3840 g for 15 minutes. The instrument (an Agilent 8543 UV-Visible DAD spectrophotometer equipped with a 1FS peristaltic pump, a G18011A XY-autosampler and a UV-Visible ChemStation software (Rev B01.01[21])) was zeroed with a water/ethanol/37% HCl solution (29:70:1 vol). The supernatants (80 *mu*L) were diluted with 3 mL of the zeroing solution, and the UV/VIS spectra (230-900 nm) recorded in a 1-cm wide quartz flow cell. Total anthocyanins (TANT) and non-anthocyanic flavonoids (NAF) were expressed in malvidin-3-glucoside (the most representative anthocyanin, in Sangiovese[12] and catechin equivalents (mg L^{-1}), respectively. Briefly, the peak height at 280 nm, corresponding to the absorbance of total flavonoids, was measured from the baseline between the two nearest valleys. TANT was calculated from the absorbance at A520 (peak minus valley). NAF was calculated by subtracting from total flavonoids the contribution of TANT at 280 nm as estimated by A520[13]. Further details of the analyses are also described in[14].

The concentration of polymeric anthocyanins (SO_2 resistant pigments, SO_2-RP), was measured on 3 mL of supernatant to which were added 600 μL of either a diluting solution (water/ethanol/tartaric acid 88/12/5 vol/vol/weight) or a 30% $K_2S_2O_5$ solution. The absorbance at 520 nm in a 1-mm path-length quartz flow cell was recorded both in the diluted and in the decolored samples. The SO_2-RP were expressed as an index, δAU520, which was the difference between the diluted and diluted-decolored samples[15].

2.3 Statistical Analysis

A semiparametric linear mixed model was formulated to estimate the conditional mean of pigments concentration given the days elapsed since the start of maceration and given the treatment. The master equation states that at time x_i the response y_i is a smooth non linear function plus an error term $y_i = h_w(x_i) + \varepsilon_i$ with w the treatment for a given tank at time x_i. As for time, the CSPF procedure resulted in a extended maceration, thus requiring more days to be completed. The time scale was normalized by scaling time in the range 0-100%. The smooth nonlinear function was represented by a truncated basis expansion, with $K = 6$ terms. Following[16], an auxiliary variable $z_{i,l}$ which is equal to one if $z_{i,l} = l$, otherwise is null, was defined and the observation within a tank l at time x_i is defined by the following linear model:

$$\begin{aligned} y_i = \beta_0 + \beta_1 x_i + \beta_2 x_i^2 + \sum_{k=1}^{K} b_k (x_i - \kappa_k)_+^2 + \\ \sum_{l=2}^{L} z_{i,l} (\gamma_{0,l} + \gamma_{1,l} x_i + \gamma_{2,l} x_i^2) + \\ \sum_{l=1}^{L} z_{i,l} \sum_{k=1}^{K} c_{k,l} (x_i - \kappa_k)_+^2 + \varepsilon_i \end{aligned} \quad (1)$$

where $\varepsilon_i \sim N(0, \sigma^2)$, $b_k \sim N(0, \sigma_b^2)$ and $c_{k,l} \sim N(0, \sigma_{c,l}^2)$ and with $N()$ the normal distribution, with $L = 3$ because of 3 tanks for each treatment. Random coefficients (β_s and c_s) are independently and normally distributed with treatment–specific variances; moreover they are also independent from errors ε_i. The expected value of a treatment was estimated by averaging over tanks. A Bayesian model was formulated by specifying the prior distribution of model parameters, and model fitting was performed by Markov Chain Monte Carlo (MCMC) simulation using the JAGS software[17]. Prior distributions of model variances were defined to be weakly informative, for example the initial distribution of the inverse of the error variance is a Gamma distribution with scale 0.001 and shape 0.001. The initial distribution for all parameters but β_0 in the linear predictor were defined to be normal with mean zero and small precision, for example $N(0, 100000)$. All the model parameters were considered to be a priori independent. The β_0 parameter received a prior distribution $N(m, 10000)$, where m is a prior mean judged to be plausible

Table 1 Maceration and fermentation settings: temperature profiles[a] are reported in Fig. 1

Stage	Pre-fermentation	1	2	3	4	5
Must density (kg l^{-1})		1100-1080	1079-1065	1064-1055	1054-1000	999-990
Pumping over duration (times x min.)	1 x 10	1 x 3	1 x 4	1 x 4	1 x 3	1 x 2
Air allowed	No	No	For 48 h from start of stage 2	No		

a For CSPF the temperature control was set to 20°C immediately after the end of pre-fermentation period, while for 30 °C was set to 26°C during the stages 1-2, and then to 30°C for stages 3-5

by the winemaker expert. Bayesian predictive distributions of the expected response $u_w(x)$ at each time x given the treatment w were summarized by the mean and quantiles 2.5% and 97.5%. Time-dependent contrasts were approximated over a grid of normalized days to evaluate the magnitude of changes $u_{w_2}(x_i) - u_{w_1}(x_i)$. Uncertainty of estimated contrasts is indicated as interval around the expected value (Fig. 3 and 4). All computations were performed using the R software [18] and rjags, coda and lattice libraries [19–21].

The approach proposed here has three main advantages over other predictive methods: i) kinetic behaviour is estimated in a very general class of smooth functions: this is a point of major importance since it becomes possible to capture specific features such as concentration decay at conclusion and it is possible to compare samples although of very different kinetic shapes; ii) there is no need to measure other chemical/physical/biological parameters other than those of interest (TANT, NAF, etc.); iii) the differences between treatments can be summarized by contrasts, calculated on a grid of time points, which can be plotted as in Fig. 2, 3 and 4.

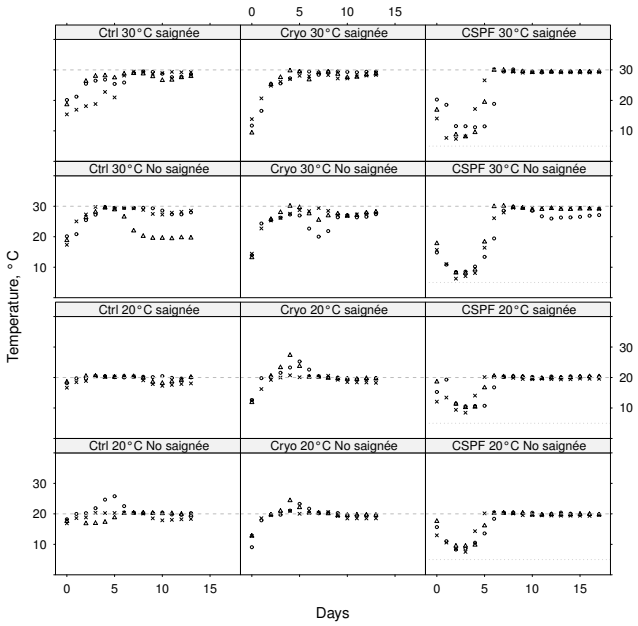

Fig. 1 Temperature recordings during maceration: the dashed lines indicate the intended levels of temperature in the experiment, 20 or 30 °C; for CSPF treatment the dotted line indicates the prefermentation temperature (5°C)

3 Results and discussion

3.1 Temperature

In the second part of the fermentation, three tanks (CTRL, CRYO, and CSPF 30 °C No saignée, Fig. 1) were accidentally set to a lower temperature.

Despite this, no effects were noted either in the extraction kinetics nor in the contrasts and in the analysis of residuals (not reported), which did not show any relevant deviation from the assumed normality of errors. This apparent discrepancy in the effects of temperature can be explained by observing that the misprogramming took place after the plateau for all the monitored pigments had been reached (Fig. 1).

For CSPF tanks, it should be noted that the established pre-fermentation temperature (5 °C, dotted line) was almost reached, while there was no difference in the time needed to reach 20 or 30 °C. On the other hand, an extensive survey of similar experiments in the literature revealed that only the thermostatic setting temperatures had been reported while the actual values never actually had been reported. This leads us to suppose that temperature monitoring is indeed a critical step in cold soak experiments and may be a reason for the possible contrasting results found in literature.

3.2 Modelling and curve shapes

The curves for TANT (Fig. 2, top) in CTRL and CRYO show an apparent first-order behaviour, but after reaching a maximum they begin to decline towards the conclusion rather than showing a horizontal asymptote, as in first order kinetics. A similar decay at conclusion is also observable in CSPF (right column, Fig. 1), but here the curves are decidedly S-shaped: at the beginning they run roughly parallel to the time axis,

indicating no extraction of TANT. The curves start to rise at around 25% of the maceration time (5 out of 19 days), i.e. after the increase of temperature from 5 to 20-30 °C. An exhaustive review of literature regarding phenolic extraction[22] emphasized the role and effects of the most common winemaking methods such as fermentation temperature, SO_2, cold soak, must/grape freezing, saignée etc.. However, detailed studies on the extraction kinetics of phenolic compounds are lacking, particularly as regards Sangiovese, and the reported data generally refers to wines at drawing off or later.[23] in a kinetic study on the effect of maceration methods on Merlot grapes, found anthocyanin extraction after 7 days (600-800 mg L^{-1}) followed by a decrease with ageing.[24] in a kinetic study on Sangiovese musts, found maximal extraction (100-200 mg L^{-1}) after 3-4 days from the onset of fermentation, while[25] found a huge concentration (500-600 mg L^{-1}) after 3 days for Sangiovese. It is not easy to compare the shape of the curves observed here with those reported in the literature. Nevertheless, for TANT, many authors observed a concentration at drawing off in the same range as that found by us (100-200 mg L^{-1}). The initial lag phase is most probably due to the lower temperature here used during pre-maceration.

The curves for NAF (Fig. 2, centre) are S-shaped: in many panels there is also a final decrease, albeit not as rapid as that in TANT. The increase in concentration, when observed, seems negligible in the early stages and the lag phase seems somewhat longer in CSPF. The sigmoidal shapes we found are similar to the patterns reported by[26] for catechin and phenolic compounds. The daily monitoring confirmed and added detail to that which is reported in the literature[27] upon colour development.

The initial low temperature appears to influence the curve shape for TANT, but not for NAF. The sigmoidal shape most probably results from two concurrent phenomena: i) ethanol build up in the solution, (NAF solubility is regulated by the alcohol content of the medium[27]; ii) maceration of skin and seeds, since NAFs are mostly contained in compartments from which extraction is more difficult (i.e. walls of cells in the skins or seeds).

Formation of SO_2-RP takes place principally via cross-reactions between tannins and anthocyanins during the ageing process. However, the varying ways and times in which tannins and anthocyanins enter the solution during fermentation may affect their formation even in this early state of vinification, and it was for this reason that monitoring of daily variations in concentration was felt to be necessary. At the beginning (Fig. 2, bottom) all the treatments have values which do not differ significantly from, or are even significantly below, zero, but which become significantly positive at about 5-10 % for CTRL and CRYO and later for CSPF (11-18 %). Again, the curves apparently belong to a first-order kinetic: CRYO and CTRL show a fast growth rate and a slight final decay,

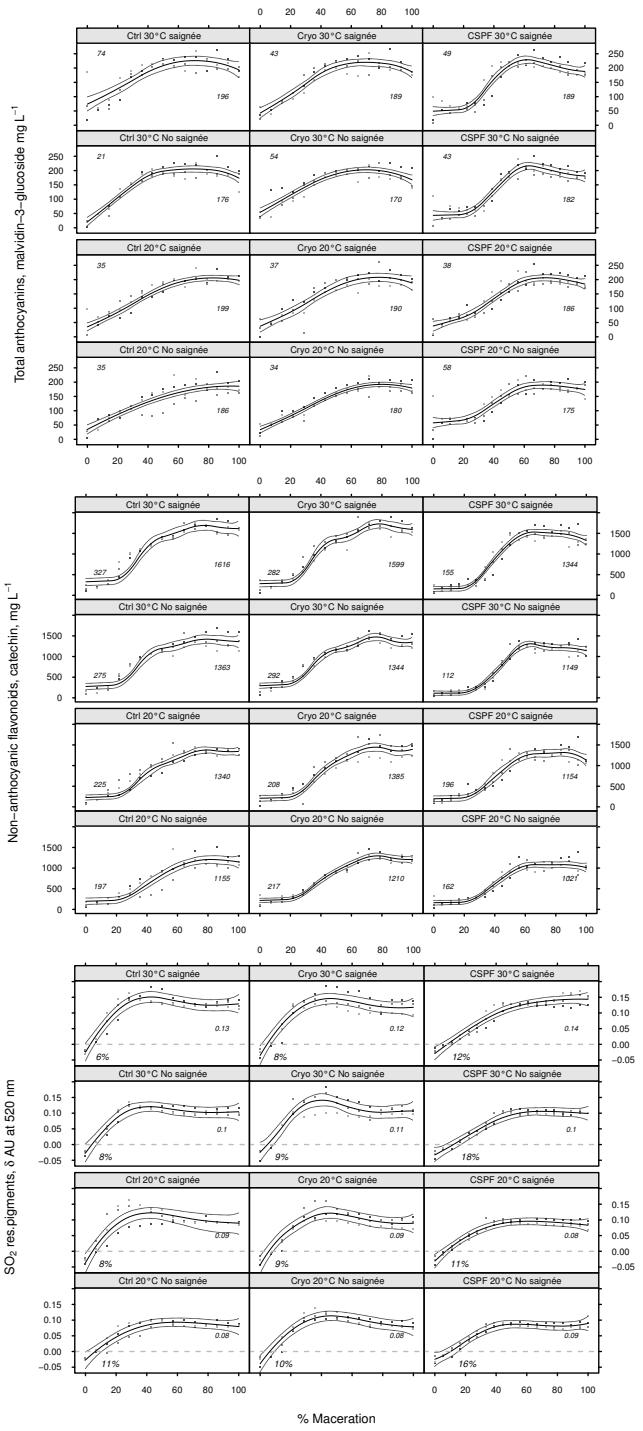

Fig. 2 Development of TANT, FNA and SO_2-RP during maceration. The central solid line is the mean of three tanks, while the two dotted lines are the boundaries of the 95 % credibility interval. Values on the left and on the right are those relative to beginning and end of the vinification, respectively. The % reported in SO_2-RP panels indicates when the value rises significantly above zero (grey dashed line).

features which are not visible in CSPF.

3.3 Time point contrasts

In Fig. 3 and 4, each panel reports the percentage of maceration on the x axis and on the y axis the difference in concentration against CRTL 20°C No-saignée and CSPF 30 °C saignée, respectively. The solid horizontal line at zero indicates no difference, so a procedure compared with itself appears as the left lower panel in Fig. 3. When two treatments are significantly different, i.e. where the pointwise credible intervals of the 95% level (outer dotted lines) do not include zero, a grey area is shown.

The complete set of comparisons consisted of 144 plots, and the results were therefore summarized by comparing each procedure against the one presumed to be the mildest (CTRL 20 °C No saignée, Fig. 3), and against CSPF 30 °C saignée (Fig. 4). This last was selected as the most intense treatment since it showed the maximum value found for TANT, without considering either time or treatment (229 mg L^{-1} at 62 %, mean value of three tanks). The discussion will be divided in two sections: in the first we will consider the pigments, while in the second we will consider the vinification methods.

3.4 Pigments

3.4.0.1 Total Anthocyanins. The transparent areas (top of Fig. 3) indicated that, at drawing off, all the procedures were statistically indistinguishable from the reference. Nevertheless the concentration was significantly higher before the end of vinification (grey areas above zero). The values range from a minimum of +15 mg L^{-1}(CRYO 20 °C No-saignée) to a maximum of +66 mg L^{-1} (CSPF 30 °C saignée). During the early stages of maceration, CSPF tanks contain significantly less TANT than the reference (grey area below the zero line). Later on, the difference in concentration becomes increasingly great until it finally drops to zero, giving a peculiar wiggly shape to the curve.

Both saignée and temperature have a positive effect, but to a different extent. Saignée increases the extraction (1st row against the 2nd, and 3rd against 4th from the bottom), but temperature (1st vs 3rd row and 2nd vs 4th) yields larger differences, with maximal values almost doubled and maintained for longer periods.

All the contrasts in Fig. 4 share a wiggly appearance, due to the sigmoidal shape of the reference's kinetic (CSPF 30°C saignée, lowest right panel on top of Fig. 2). In the first stages, (0-40%) CTRL and CRYO extract much more TANT than CSPF, which is more efficient at mid-maceration (grey area under zero around 40-80% of maceration time). Fig. 4 show the same data as Fig. 3, but observed from a different perspective; it is therefore unsurprising to find no significant

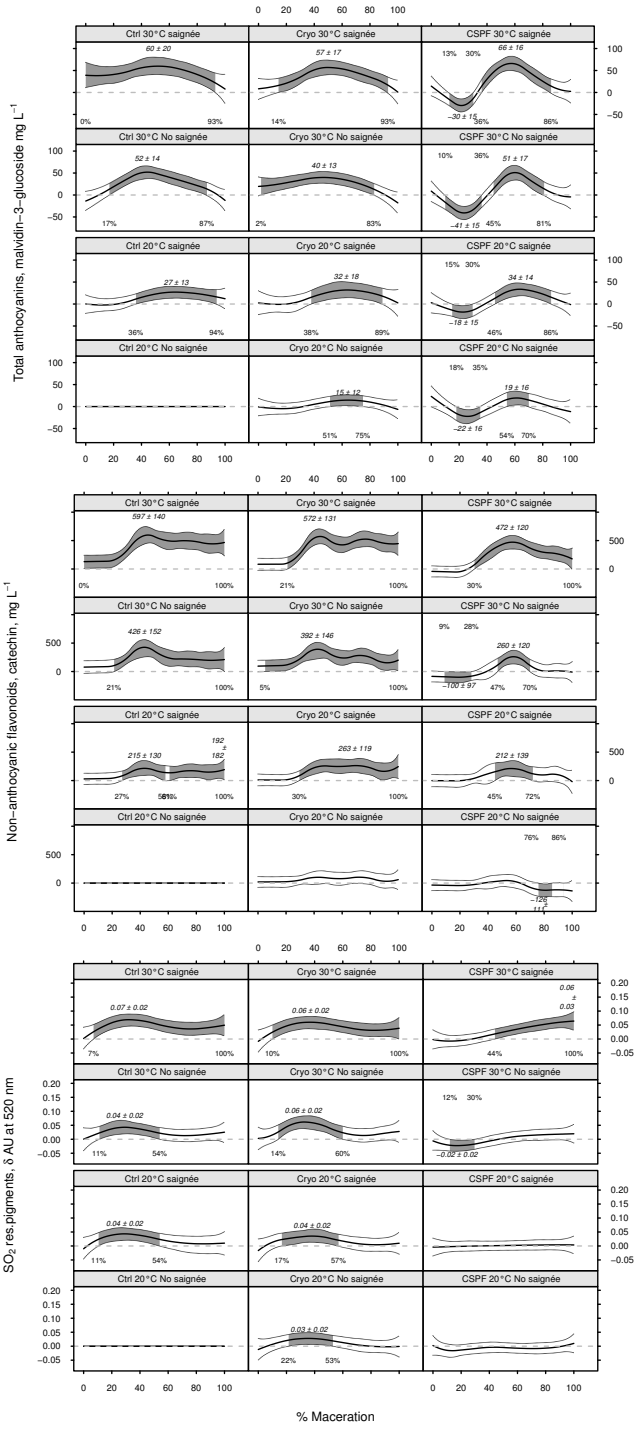

Fig. 3 Contrasts for TANT, FNA and SO_2-RP: the shaded area between the two lines (95 % credible interval) indicates a significant difference between the treatment and CTRL 20°C without saignée. The figures for central values +- credibility interval are shown on the maximum and minimum points of the curves

differences at the end of vinification.

3.4.0.2 Non-Anthocyanic Flavonoids. Most of the panels (Fig. 3, centre) show the grey area above the zero line, indicating that CTRL 20 °C No-saignée (reference) was one of the least efficient, as already seen for TANT. Conversely to the results observed with TANT, most of the procedures lead to a final gain. Saignée and temperature had a significant positive effect, the extent of which was similar in CTRL and CRYO. CTRL and CRYO saigneè at 20 °C yielded about +200 ml L^{-1} (Fig. 3 second line from the bottom), and the increase in temperature yielded about +400 ml L^{-1}(third line from the bottom). Their combined effect appears to be an additive phenomenon without interaction, since the gain is about +600 mg L^{-1} (top line).

In the initial lag phase (before 20-30 % of the maceration time, 3-5 days) few procedures differ significantly from CTRL 20 °C No-saignée (reference). Even though some panels display a grey area close to the zero line in the initial stages, they show only a negligible difference (3^{-1}0 mg L^{-1}). This behaviour is consistent with the well known low solubility of NAF in water. CSPF seems unable to extract more NAF then the reference, most probably due to the low temperature in the initial stages. For this treatment, it is necessary to combine both temperature and saignée to achieve significantly larger concentrations (top right panel) whose additive behaviour, observed for CTRL and CRYO, is not evident.

3.4.0.3 SO_2-Resistant Pigments. Despite the fact that the values are in the range -0.05 + 0.15 units (Fig. 2 bottom), the model is able to detect significant differences even for very small values (Fig. 3 bottom). Three out of four CSPF procedures did not yield significant gains, which were instead observed in all the remaining procedures. Saignée and temperature must be coupled to obtain a stable and final significant gain. CSPF 30 °C saignée gains 0.06 units, a remarkable 75 % increase with respect to the reference (plateau at about 0.08, lower left panel in Fig. 2 bottom).

The bottom part of Fig. 4 shows another perspective of the same data. All the grey areas of CSPF column lie below zero, confirming that both high temperature and saignée are necessary in order to achieve a stable and significant gain. All the CRYO procedures show grey areas below zero toward the end of maceration, while CTRL is not significantly different from the reference, except for CTRL 30 °C saignée which remains significantly above the zero line except for a brief period. It should be noted that the change of reference allows the highlighting of very subtle differences, such as those in the behaviour of CTRL and CRYO 30 °C saignée, which were undetectable in Fig. 3.

Several authors have studied the evolution of individual phenolic compounds during wine ageing[28–31], but the data on the kinetic evolution of the SO_2-RP during wine maceration/fermentation is scattered throughout the literature. In a study on Sangiovese treated with carbonic maceration on an industrial scale[2], found a δAU520 of about 0.12 at the time of drawing off, with an increase in SO_2-RP with respect to a standard vinification of about 0.015 units. While the δAU520 values observed here are of the same magnitude, the differences with respect to the reference are larger (about 0.06 units in Fig. 3 bottom). The formation rate of SO_2-RP is constant up to 40%, (6-7 days, Fig. 2 bottom) and then drops to a value near zero, meaning that the concentration does not vary with time. This suggests that the initial period is important for the formation of a stable wine colour.

The presence in wines of non-polymeric pigments which do not bleach with bisulfite, such as Vitisin A and Vitisin B[32], must be taken into account. A recent paper[12] reports that in Sangiovese such pigments are in the range of 5-10 % of the total pigments, and it is therefore reasonable to assume that such small amounts do not influence the general trends and behaviours here observed for SO_2-RP.

3.5 Vinification methods

3.5.0.4 Cold Soak Pre Fermentation. The initial phase was not monitored by Gordillo et al.[6], on Tempranillo with a CSPF held at 5-8 °C for 8 days. A first order kinetic-like shape in the same phase was noted by Gómez-Míguez et al.[33] on Shiraz, where CSPF was held at 15 °C for 6 days. Both authors recorded clear gains (about +100-200 mg L^{-1}) in TANT concentration with respect to traditional vinification held at 25 °C, both at the beginning and at the end of fermentation.

Reynolds et al.[8] on investigating the effect of CSPF on Shiraz, held at 2 °C for one or ten days, found smaller increases in TANT concentration (50-70 mg L^{-1}) which is the range we also observed (top of Fig. 3 and 4). Other results on CSPF[4,5,34], confirmed a rapid decrease in the early-extraction of anthocyanins and a lower final concentration of these molecules as compared with other extraction procedures, and the heterogeneous nature of the data in the literature therefore makes it difficult to draw conclusions.

Here, the CSPF temperature set at 5°C was almost reached, and the monitored values were low enough to reduce spontaneous fermentation activity to a minimum before the inoculation of the selected yeast. The low temperature is possibly the main cause for the lack of TANT extraction over the first 40% (7 days) for CSPF (top of Fig. 2 and 3). Later on, when the temperature was raised, the sudden increase of TANT was probably a consequence of the cold soak, which may have weakened the cell walls of the berries. This hypothesis is consistent with the results obtained by[25] who reported a decrease in the rates of anthocyanin diffusion in the liquid phase upon decreasing the temperature.[22] reports that "the rationale offered," (for cold soaks) "is that aqueous extraction improves

wine colour". This early aqueous extraction seems not to occur for TANT on Sangiovese at 5 °C. From a review of the literature we therefore hypothesize that the improvements in TANT are only possible when the temperature during the cold soak is kept at 10-15 °C, where faster and more effective biotic and enzymatic reactions are most likely to occur.

The initial lag phase or smaller slope observable in NAF (Fig. 2) can be explained again by the very low temperature used in the cold soak. The sudden rate change occurs for NAF exactly when the temperature is raised to 20-30 °C: therefore further experiments on temperature profiles during cold soak may elucidate the effect on NAF extraction.

At the beginning, when low temperatures slow down the diffusion processes, TANT and NAF concentrations remain low, but they then rapidly increase at mid-maceration (Fig. 2). Most probably, the higher concentration of SO_2-RP observed at the end of maceration may be due to the simultaneous availability in solution of both TANT and NAF. This seems to be confirmed by the higher % values (x axis) at which SO_2-RP rises significantly above zero (Fig. 2).

3.5.0.5 Cryoextraction. No reference to this was found in the literature except [1], which does not report the kinetics. The shape is again first-order like, similarly to that observed for the CTRL. Temperature and saignée being equal, no significant differences were found between CRYO and CTRL (contrasts not reported) either for TANT, NAF or SO_2-RP. The variations between these results and those obtained [1] previously by adding cryogen via a stainless-steel hand-lance with nozzle are probably due to differences in the cryogen delivery equipment and/or to the vintage and ripeness of the grapes.

3.5.0.6 Combination of effects of temperature and saignée. CRYO and CSPF procedures are able to extract more TANT only in combination with saignée and/or high fermentation temperature, but the extraction gain is in any case lost at the end of maceration. Since oxidation and sorption reactions both occur with monomeric anthocyanic forms, it is impossible to predict the role of the two phenomena without further information or data. An effective extraction procedure would couple greater extraction of oxidable/degradable anthocyanic forms with a timely combination with flavonols to form stable polymeric pigments. As is evident from Fig. 3, NAF availability results in a higher production of stable pigments (SO_2-RP) towards the end of maceration. This is true when both higher temperature and saignée are combined (top line of Fig. 3 top, centre and, bottom), and is particularly true for CSPF.

To maximize the gain in SO_2-RP in Sangiovese it is advisable to combine pre-fermentation treatments with higher fermentation temperature and saignée (top line of Fig. 3). Since the gain in SO_2-RP in Sangiovese can be only temporary when temperature and saignée are not coupled, it may be necessary

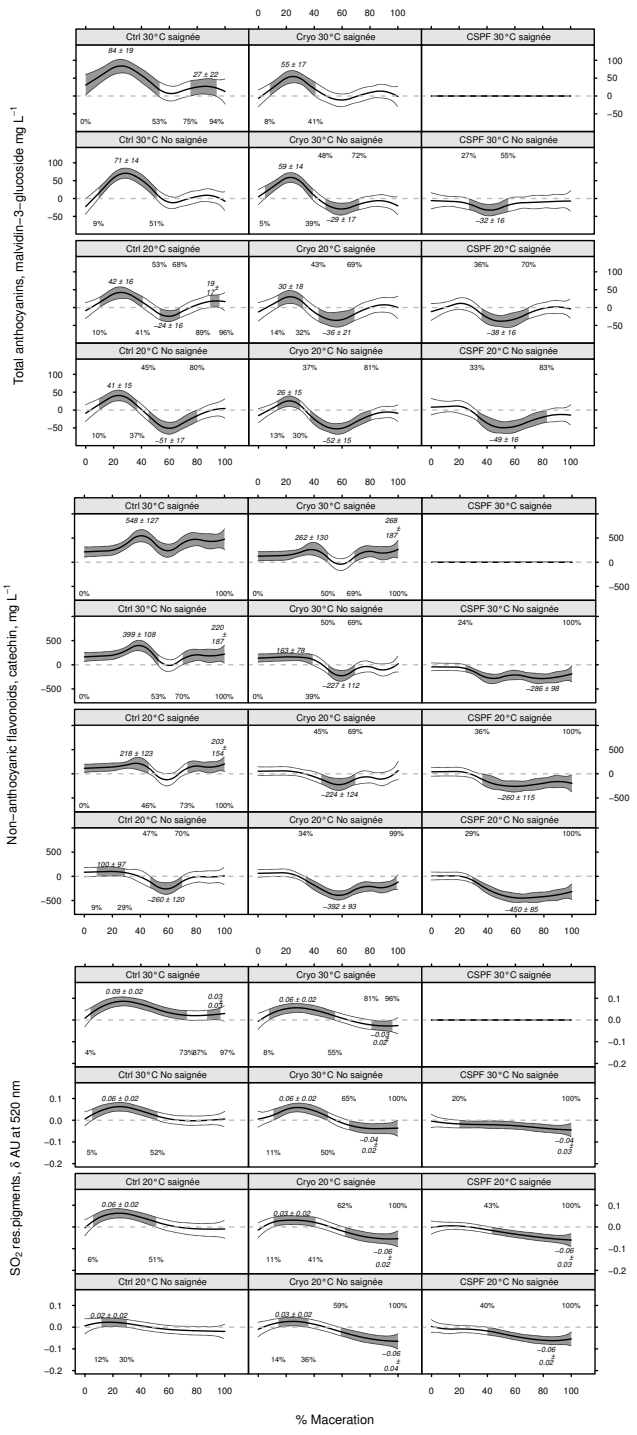

Fig. 4 Contrasts for TANT, FNA and SO_2-RP: the shaded area between the two lines (95 % credible interval) indicates a significant difference between the treatment and CTRL 20°C without saignée. The figures for central values +- credibility interval are shown on the maximum and minimum points of the curves

to use other procedures (e.g. macro-oxygenation, tannin addition) to maintain the achieved gain.

4 Conclusions

The semiparametric Bayesian model here developed was able to capture complex kinetic behaviours well beyond those typically described by "textbook" kinetic equations, and allowed precise and meaningful comparisons even between nearly sigmoidal and nearly first-order behaviours.

In the extraction of total anthocyanins, all procedures proved indistinguishable from the reference at drawing off, and temperature proved much more effective than saignée. In cold soak pre-fermentation, total anthocyanins displayed hitherto unreported sigmoidal behaviour which was likely due to the low temperatures (5°C) used. As vinification proceeded, the increase in temperature yielded larger differences than saignée, displaying almost doubled values which maintained for longer periods.

Conversely, in non-anthocyanic flavonoids most of the procedures yielded a final gain: saignée and temperature had an additive effect for control and cryoextraction, while in cold soak, where the coupling of both methods was necessary for achieving a final yet undramatic gain, the additive effect was not evident. In cold soak, the initial lag phase (before 20-30 % of maceration time) was either not influenced or only slightly influenced by thermal treatment.

For SO_2-resistant pigments both high temperature and saignée were necessary to achieve a significant, stable gain, regardless of pre-fermentation treatment. The rate of their formation was constant up to about 40% of fermentation (6-7 days) and their subsequent concentration remained constant, which suggests that the initial period is important for the formation of a stable wine colour.

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