# ANTHOCYANIN EXTRACTABILITY ASSESSMENT OF GRAPE SKINS BY TEXTURE ANALYSIS

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## Abstract

**Aims**: The aim of this work was to evaluate the kinetics of anthocyanin extraction in berries of cv Brachetto and Nebbiolo, having the same level of total soluble solids, but with different skin hardness.

**Methods and results**: A puncture test was carried out on berries calibrated according to their density, estimated by flotation in different salt solutions. For each variety, two groups of berries of different skin hardness were selected: soft (< medium value) and hard (> medium value). Spectrophotometric and HPLC methods were used to evaluate the kinetics of anthocyanin extraction in a hydroalcoholic buffer solution. In each of the examined cultivars, the grapes with a higher break skin force produced extracts with a higher content in total anthocyanin. The anthocyanin profile of extracts of Brachetto hard skin is characterized by a higher content of acetyl-glucosides.

**Conclusion**: Texture analysis, which is fast and inexpensive, turned out to be an excellent analytical technique to verify skin hardness measurements. The break skin force proved to be a mechanical parameter able to estimate the extractability of anthocyanins with adequate reliability.

**Significance and impact of study**: The possibility of having an easy way to determine maturity index able to estimate the extractability of phenol compounds with sufficient reliability, can be a valuable tool for the enological community.

**Key words**: texture analysis, anthocyanin extractability, *Vitis vinifera*, Nebbiolo, Brachetto

## Résumé

**Objectif** : L'objectif de ce travail est d'évaluer les cinétiques d'extraction des anthocyanes des baies des cépages Brachetto et Nebbiolo, avec une teneur semblable en solides solubles totaux, mais avec différentes duretés de pellicules.

**Méthodes et résultats** : Le test a été mené sur des baies calibrées selon leur densité estimée par flottation dans des différentes solutions salines. Pour chaque cépage, deux groupes de baies avec différentes duretés de pellicule ont été sélectionnés : tendres (valeur inférieure à la moyenne) et dures (valeur supérieure à la moyenne). La spectrophotométrie et la chromatographie en phase liquide à haute performance (HPLC) ont été utilisés pour évaluer les cinétiques d'extraction des anthocyanes dans une solution hydro-alcoolique. Pour les deux cépages étudiés, les raisins avec une force plus importance pour transpercer la pellicule ont donné des extraits plus riches en anthocyanes totaux. Le profil anthocyanique des pellicules les plus dures de Brachetto a été caractérisé par une teneur supérieure en acétyl-glucosides.

**Conclusion** : L'analyse de la texture s'est démontrée une technique très rapide et peu coûteuse pour évaluer la consistance de la pellicule. La force nécessaire à transpercer la pellicule est un paramètre mécanique capable d'évaluer l'extractibilité des anthocyanes avec fiabilité.

**Signification et impact de l'étude** : Disposer d'un moyen facile pour déterminer l'indice de maturité pouvant estimer l'extractibilité des composés phénoliques semble intéressant pour les opérateurs du secteur vitivinicole.

**Mots-clés** : analyse de la structure, extractibilité des anthocyanes, *Vitis vinifera*, Nebbiolo, Brachetto

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## INTRODUCTION

Knowledge of the grape anthocyanin characteristics, content and extractability, may permit the rationalization of maceration and winemaking processes and allow winemakers to best exploit the grape potential reached in the vineyard (SAINT-CRIQ *et al.*, 1998a; GONZALEZ-NEVES *et al.*, 2004).

Many studies have been conducted aimed at defining the best method to evaluate polyphenolic compounds in grapes and the ease with which they are released (GLORIES and AUGUSTINE, 1993; AMRANI-JOUTEI and GLORIES, 1994; VENENCIE *et al.*, 1997; SAINT-CRIQ *et al.*, 1998b; CAYALA *et al.*, 2002; MATTIVI *et al.*, 2002; CELOTTI *et al.*, 2007).

The structural and chemical properties of the cell-walls may determine the mechanical resistance of berry skin to releasing the anthocyanins (BARNAVON *et al.*, 2000; ORTEGA-REGULES *et al.*, 2006; ORTEGA-REGULES *et al.*, 2008).

Texture analysis is a current analytical technique used for measurement of the physical properties of plant tissue (BOURNE, 2002; ROUDOT, 2006). Previous studies have indicated the potential of grape texture measurement to estimate grape ripeness (ABBAL *et al.*, 1992; ROBIN *et al.*, 1997; GROTTE *et al.,* 2001).

Nevertheless, these scientific contributions on grape texture mechanical properties did not investigate their usefulness for estimating extractability.

The aim of this work was therefore to determine the kinetics of anthocyanin extraction in berries, with the same level of total soluble solids, but with different hardness.

## MATERIALS AND ANALYTICAL AND EXPERIMENTAL METHODS

### Grape samples

*Vitis vinifera* var. Brachetto (B) and Nebbiolo (N) grape samples were harvested in 2006 from vineyards located in Piedmont (North West Italy). Berries were calibrated according to their density (i.e., total soluble solids). Density was estimated by flotation of the berries in different salt solutions (from 130 to 190 $gL^{-1}$ NaCl) so that the difference in total soluble solids of two consecutive batches of berries was about 17 $gL^{-1}$ (i.e., 1 vol % in potential alcohol) (FOURNAND *et al.*, 2006).

One stage of maturity was studied: 230 ±8 $g/L^{-1}$ sugar. This sugar content is that at which the grapes are usually harvested in accordance with their respective Italian Wines Disciplinary of Production.

### Texture analysis of berry skin

A puncture test was carried out on the side of all the berries (~300) present in the class defined by flotation. The measurements were made using a Universal Testing Machine TAxT2i Texture Analyzer (Stable Micro System, Godalming, Surrey, UK) equipped with a HDP/90 platform, needle probe P/2N and a 5 kg load cell. Tests were performed at 1 $mms^{-1}$ and the berry skin hardness were expressed as break skin force (Fsk), evaluated in newton (N) (ROLLE *et al.*, 2007; LETAIEF *et al.*, 2008a). All the data acquisition was carried out at 400 Hz and using the Texture Expert Exceed software. For each variety, two groups of berries with different skin hardness were selected: S, soft (< medium value) and H, hard (> medium value).

### Anthocyanin extraction in hydroalcoholic solution

Sixty berries (20 x 3 replicate) belonging to each of the four groups formed (BS, BH, NS, NH) were used for studying the extractability of anthocyanins.

The berry skins, removed manually from the pulp and dried with absorbent paper, were quickly immersed in 75 mL of hydro-alcoholic buffer (pH 3.20), containing 200 $mgL^{-1}$ of $K_2S_2O_5$ to limit oxidation of phenolic compounds (FOURNAND *et al.*, 2006) and 3 % (Brachetto) or 12 % (Nebbiolo) of ethanol to simulate the respective extraction conditions during industrial production. The red aromatic Brachetto grapes are used for the production of sweet sparkling wines (6 - 7.5 % vol. ethanol) and the product obtained must not exceed, before second fermentation (prise de mousse), 3.5 % vol. of ethanol.

The kinetics of extraction were monitored at regular intervals: 10, 20, 30 minutes and 1, 2, 4, 8, 12, 24 hours. At the end of the extraction, the residual solid berry skins were rinsed with a hydroalcoholic solution and quickly immersed in 75 mL of a buffer solution containing 12 % ethanol, 600 mg/L of sodium metabisulfite, 50 mg/L $NaN_3$, 5 g of tartaric acid and adjusted to pH 3.20 with 1N NaOH. After homogenization with an Ultraturrax T25 (IKA Labortechnik, Staufen, Germany), the extract was centrifuged (3000 rpm; 5 min; 20 °C). The supernatant was then used for the analysis of non-extracted anthocyanin to estimate the extractability yield (%).

### Spectrophotometric and HPLC analysis

Spectrophotometric and HPLC methods were used to evaluate the total anthocyanin index (TAI) of hydroalcoholic solutions and berry skins before and after hydroalcoholic extraction and their relative anthocyanin profile (DI STEFANO and CRAVERO, 1991; ROLLE and GUIDONI, 2007). The identification of the free forms of anthocyanins in the berry skin extract was performed by comparison with external standards (Extrasynthèse, Genay, France). The acylated forms of anthocyanins were identified by comparing the retention time of each chromatographic peak with available data in the literature (DI STEFANO *et al.*, 1995). The percentages of individual anthocyanins were determined by comparing the area of the individual peak with the total peak area.

**Statistical analysis**

Statistical analysis was performed using STATISTICA for Windows Release 7.1 (StatSoft Inc., Tulsa, OK, USA).

## RESULTS

The total amounts of anthocyanin and their relative profiles in Brachetto and Nebbiolo grapes at harvest are reported in table I. The data observed was in good agreement with that reported in the literature (DI STEFANO and CORINO, 1984; GUIDONI *et al.*, 1991; GUIDONI *et al.*, 2008).

Break skin force values determined by puncture test are shown in table II. Therefore, groups S (soft) and H (hard) were composed by berries with Fsk lower and higher than the medium value: 0.428 N (Brachetto) and 0.353 N (Nebbiolo). In particular, four groups of berries were formed in experimental design: BS = 0.247-0.425, BH 0.430-0.696, NS = 0.169-0.350 N, NH 0.355-0.691 N.

Figures 1 and 2 show extractability of total free anthocyanins from the different groups of berries identified in a model hydroalcoholic solution of Brachetto and Nebbiolo grapes. Hard skins, under our experimental conditions, presented a extractive capacity of 71.4 % ± 3.02 (Brachetto) and of 88.1 % ± 1.10 (Nebbiolo) compared to the 58.6 % ± 0.32 and 77.0 % ± 3.36 respectively, of soft skins. In both cultivars, the toughest skins showed a higher than average capacity to release anthocyanins: + 12.8 % Brachetto, + 11.1 % Nebbiolo. The different extraction percentage of anthocyanins between Brachetto and Nebbiolo grapes is probably influenced by ethanol concentration in the water-alcohol solution (CANALS *et al.*, 2005).

The results of analysis of variance, carried out on TAI ($mgL^{-1}$; %) of extracts obtained from skins of differing hardness after different extraction times, showed

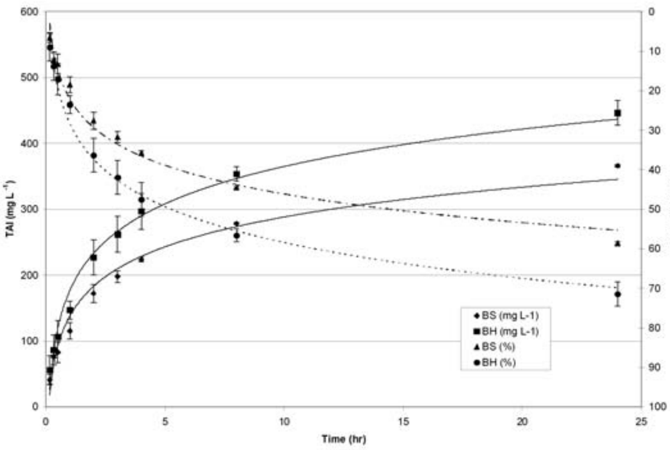


**Figure 1 - Extractability ($mgL^{-1}$; %) of total free anthocyanins from the two different groups of Brachetto (B) berries by a model hydroalcoholic solution**

S= soft skin 0.247-0.425N; H = hard skin 0.430-0.696 N).

**Table 1 - TAI and anthocyanin profile of Brachetto and Nebbiolo grapes in 2006 vintage**

| | Brachetto | | Nebbiolo | |
|---|---|---|---|---|
| | Avg | SD | Avg | SD |
| TAI (mg malvdin-3-gluc. Kg grape$^{-1}$) | 566 | 32 | 413 | 17 |
| Delphinidin-3-glucoside (%) | 7.32 | 0.98 | 6.61 | 0.40 |
| Cyanidin-3-glucoside (%) | 5.02 | 0.31 | 6.60 | 0.82 |
| Petunidin-3-glucoside (%) | 7.10 | 0.67 | 6.37 | 0.24 |
| Peonidin-3-glucoside (%) | 25.65 | 2.12 | 30.98 | 1.14 |
| Malvidin-3-glucoside (%) | 50.85 | 2.35 | 35.66 | 1.88 |
| Delphinidin-3-acetyl-glucosides (%) | nd | - | 0.40 | 0.37 |
| Cyanidin-3-acetyl-glucosides (%) | nd | - | 0.38 | 0.11 |
| Petunidin-3-acetyl-glucosides (%) | nd | - | 0.17 | 0.09 |
| Peonidin-3-acetyl-glucosides (%) | 0.18 | 0.05 | 1.91 | 0.12 |
| Malvidin-3-acetyl-glucosides (%) | 0.26 | 0.07 | 2.33 | 0.18 |
| Delphinidin-3- *p*-coumarylglucoside (%) | 0.12 | 0.04 | 0.05 | 0.06 |
| Cyanidin-3- *p*-coumarylglucoside (%) | 0.19 | 0.03 | 0.76 | 0.26 |
| Petunidin-3- *p*-coumarylglucoside (%) | 0.15 | 0.01 | 0.36 | 0.06 |
| Peonidin-3- *p*-coumarylglucoside (%) | 1.59 | 0.17 | 3.99 | 0.13 |
| Malvidin-3- *p*-coumarylglucoside (%) | 1.57 | 0.02 | 3.30 | 0.15 |
| Peonidin-3- *p*-caffeoylglucoside (%) | nd | - | 0.13 | 0.12 |
| Malvidin-3- *p*-caffeoylglucoside (%) | nd | - | nd | - |

nd = not detected

Means of three replicates. (Avg = average; SD = standard deviation).

significant differences for both varieties only from 8 hours of maceration.

In Brachetto grapes, some anthocyanin species presented different behaviours during extraction (Figure 3 a,b,c,d). In the extracts obtained both from the hard, as well as the soft skins, the dissolution of peonidin-3-glucoside and cianidin-3-glucoside was fastest in the early stages of maceration confirming the results obtained in previous studies on Nebbiolo grapes (GERBI *et al*., 2002).

The results of analysis of variance carried out on the anthocyanin profile of extracts obtained from the skins of Brachetto grapes of different hardness at different extraction times, showed significant differences between the distribution of different anthocyanins only in terms of total quantity (mg/L$^{-1}$), except for peonidin 3-glucosides and cinnamoyl-glucosides. Apparently hard skins have a greater capacity to release these compounds in the extraction process.

The anthocyanin profile of the extracts (%), indeed, after 24 hours of maceration showed no differences except for the acetyl-glucosides content, higher in extracts BH (+ 28 %), but always in total < 1 %.

## DISCUSSION

The elaboration of high quality red wines requires the assessment of grape phenolic maturity by the determination of concentration of phenolic compounds and their extractability during the winemaking process.

The cell maturity index (EA%) is currently one of the best known and most used indexes to assess the extractability of anthocyanins (GLORIES and AUGUSTINE, 1993; ROMERO-CASCALES *et al*., 2005; ORTEGA REGULES *et al*., 2006).

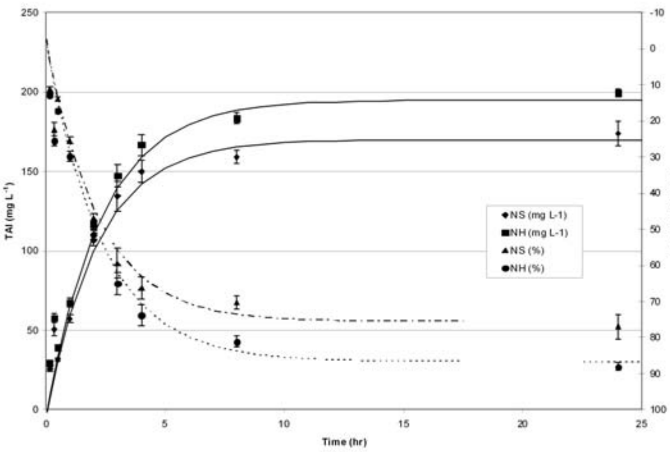


**Figure 2 - Extractability (mgL$^{-1}$; %) of total free anthocyanins from the two different group of Nebbiolo (N) berries by a model hydroalcoholic solution.**
S = soft skin 0.169-0.350N; H = hard skin 0.355-0.691 N).

**Table 2 - Average, minimum and maximum value of break skin force (Fsk) of Brachetto and Nebbiolo grapes expressed in Newton (N), vintage 2006**

| | Avg | Min | Max | SD |
|---|---|---|---|---|
| Brachetto | 0,428 | 0,247 | 0,696 | 0,095 |
| Nebbiolo | 0.353 | 0.169 | 0.691 | 0.082 |

(SD = standard deviation).

On the basis of studies conducted on grapes in Piedmont and in particular on Nebbiolo, we can state that the EA% index measures the ease with which anthocyanins can be extracted in the first phase of skin contact (decreasing values correspond to increased ease of extraction) (CAGNASSO *et al*., 2008). Therefore, this parameter is useful for programming the pre-fermentation process and/or maceration technique, in particular for grape varieties rich in easy degradable anthocyanins. In fact, in grapes rich in peonidin-3-glucoside and cyanidin-3-glucoside, like Brachetto and Nebbiolo, the remarkable loss of di-substituted anthocyanins, easily extractable from the first phases of maceration (GERBI *et al*., 2002), is probably due to the complex processes of combination, oxidation and insolubilisation that characterise anthocyanin-like substances during the course of winemaking (CHEYNIER *et al*., 1997; ATANASOVA *et al*., 2002). Nevertheless, if EA% provided information about how quickly anthocyanins can be extracted during the skin contact phase in industrial winemaking, it can not differentiate the percentage of recovery of anthocyanins of grapes with different value of this parameter (CAGNASSO *et al*., 2008).

Although further studies are probably needed to deepen our knowledge, in this work the break skin force has allowed the assessment of the percentage of recovery (% extraction). Therefore, the Fsk might be seen as a useful index to determine during the ripening of grapes to estimate the cellular permeability. This mechanical parameter, obtained quickly and at low cost, could supplement information obtained from EA%.

In studies conducted on grapes in Galicia has been demonstrated to be a multiple linear regression between cellular maturity index, and break force and thickness of berry skin (RIO SEGADE *et al*., 2008).

The skin hardness at harvest characterize the variety and is an effective tool to discriminate between different vineyards, although the Fsk values are strongly affected by climate trends of the vintage (LE MOIGNE *et al*., 2008; LETAIEF *et al*., 2008b).

In Nebbiolo grapes, from veraison to ripeness, but above all in the first phases, an increase of Fsk is noted,

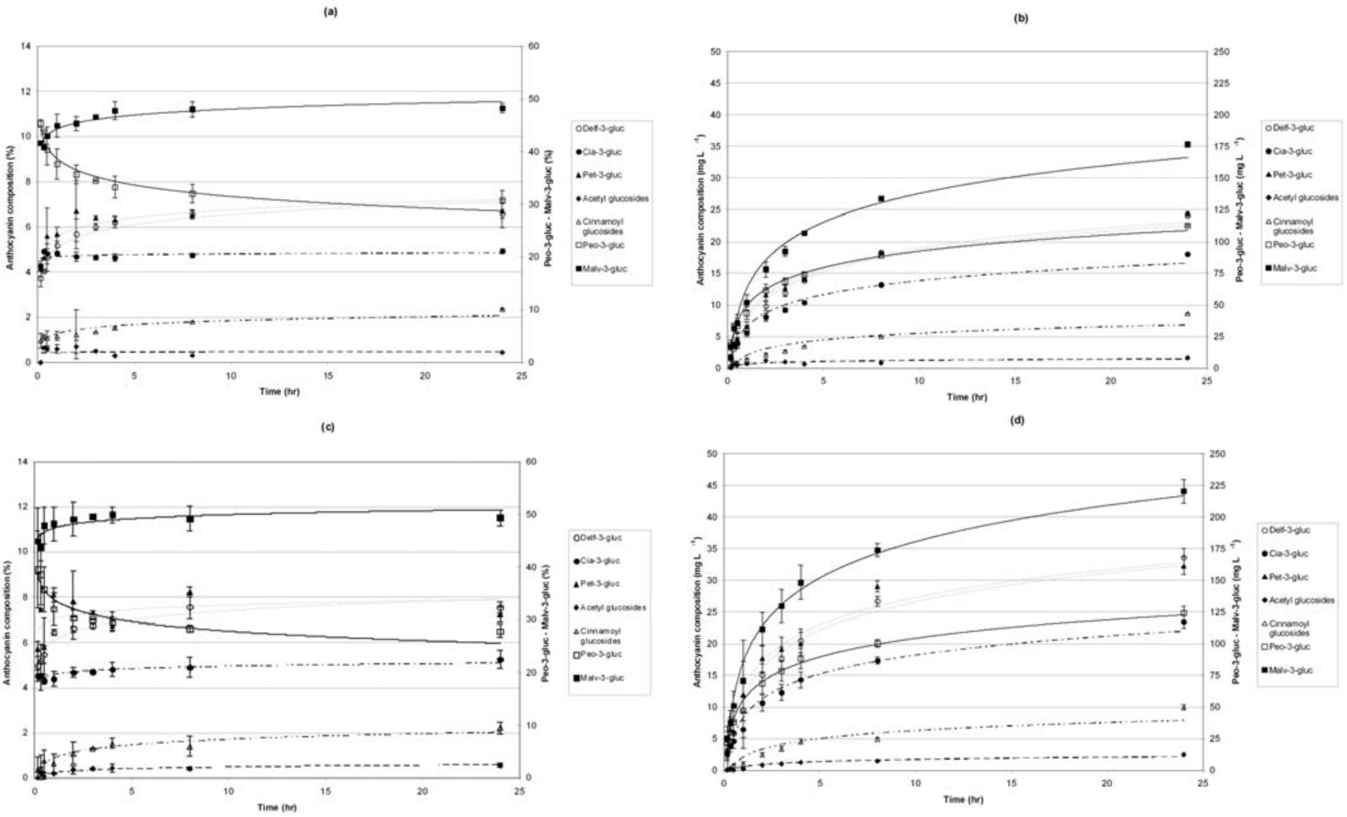


**Figure 3 - Anthocyanin extractability of Brachetto grapes in a model hydroalcoholic solution.**
BS% (a), BS mgL-1 (b), BH% (c), BH mgL-1 (d). Cinnamoyl-glucosides included both p-coumaroyl and caffeoyl anthocyanin forms.

with a steady or slight decrease in the proximity of « technological maturity » and a renewed increase in the phases of over ripeness. The reduced increases in Fsk values close to harvest, if confirmed in several vintages, could, however, place a limit on the choice of this parameter as an indicator of maturity of grapes.

## CONCLUSION

The study shows the influence of skin hardness, determined by texture analysis using a puncture test, on the capacity of anthocyanin extraction from Brachetto and Nebbiolo grapes. In particular, the grapes with a higher break skin force produced, during a maceration in a model hydroalcoholic solution, extracts with a higher content of total anthocyanins.

An easy to determine index of maturity able to estimate the extractability of phenol compounds with sufficient reliability can be a valuable tool for operators in the field.

Further studies are necessary to develop a mathematical model,which, given the average value of Fsk of a vineyard on a given date, will allow the value of the total extractable anthocyanins.to be predicted.

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