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Caftaric acid in grapes and conversion to a reaction product during processing

by

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L'acide caftarique des raisins et sa conversion en produit de réaction au cours de la vinification

Résumé. — A l'aide de HPLC il a été mis en évidence que l'acide caftarique (caffeoyl tartrique) subit des pertes rapides lors du foulage des raisins dans le cas où ceux-ci ne sont pas complètement protégés contre l'oxidation, comme par ex. foulage en atmosphère inerte en présence d'un niveau élevé de métabisulfite de potassium et d'acide ascorbique. En l'absence de telles précautions les valeurs de la teneur originelle en acide caftarique, considérées comme étant caractéristiques des variétés de vigne, sont douteuses. Des échantillons totalement protégés de 5 variétés blanches et 3 variétés rouges de *Vitis vinifera*, mûres, se rangent entre 200 mg/l de jus pour Grenache et 50 mg/l pour Carignane. Dans la mesure que l'exposition à l'oxygène et l'activité de phénolase sont évaluables la perte peut être importante. Une majeure partie de cette perte consiste dans un produit de réaction de l'acide caftarique ici pour la première fois décelé (CRP). Ce CRP peut pénétrer dans le vin et sa détermination par HPLC ainsi que celle de l'acide caftarique non transformé montrent, en fonction de la variété de vigne, une grande conversion au cours de la préparation normale du moût. De telles analyses de vins peuvent servir d'indicateurs de l'exposition à l'oxygène au cours de la préparation du moût. La nature du CRP est actuellement étudiée et sa présence peut contribuer à expliquer la faible récupération de l'acide caftarique du moût ou du vin.

Introduction

Caftaric acid (caffeoyl tartaric acid) and its relatives, coutaric and fertaric acids (*p*-coumaroyl and feruloyl tartaric acids), make up a major portion of the phenolic substances of fresh grape juice and wines made from it (ONG and NAGEL 1978, SINGLETON *et al.* 1978). They are major phenolic components, oxidation substrates, and browning precursors in wines made without appreciable pomace contact. It is therefore important to know the true content of these substances in grapes and understand their modification during normal processing.

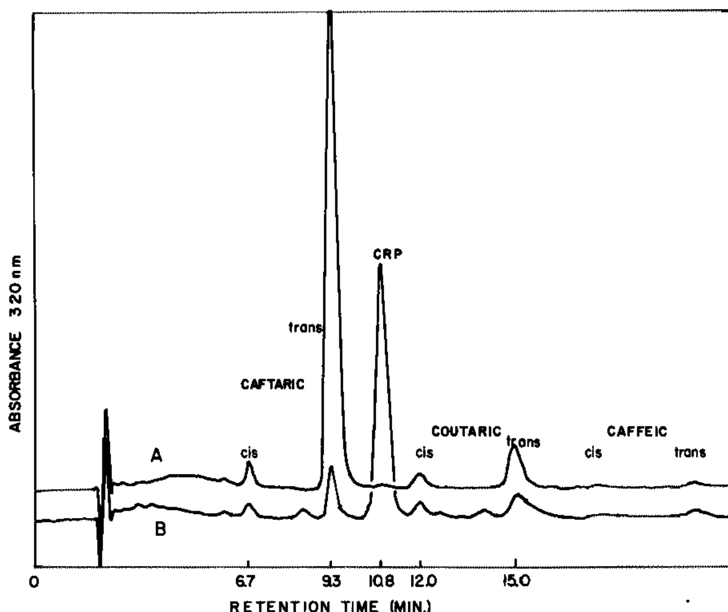
The content of caftaric acid reported in various samples has been quite variable, ranging from tens of mg/l to more than three hundred. Part of the variation is varietal, some can be weather or ripeness related, but part appeared to be technique and sample preparation. Our previous values for *trans*-caftaric acid content by HPLC averaged lower on wines than on the corresponding musts and were generally lower on musts than others had reported in grapes (SINGLETON and TROUSDALE 1983). NAGEL *et al.* (1979) reported that wines retained less than half of the caftaric and coutaric acids of their musts. MYERS and SINGLETON (1979) examining the nonflavonoids of white wine found the situation complex. A considerable portion of the 320 nm absorbance characteristic of hydroxycinnamates was due to highly polar unknown compounds that resembled products from chlorogenic acid under certain conditions of enzymic oxidation. Whether these were artifacts remained uncertain.

Isolation of caftaric acid from California juices and wines prepared under conditions similar to good commercial practice produced much lower recovery than the same isolation procedure applied to an English experimental wine (SINGLETON *et al.* 1978). Extraction with solvents from acidified musts, even with ethanol after saturation with ammonium sulfate (SINGLETON 1961), recovered much less of the compound from the sample than expected from the characteristics of caftaric acid.

To better explain these observations, we wished to determine the original content of caftaric acid in the grape and to investigate the size and nature of the losses incurred in normal processing.

Materials and methods

The grapes were harvested directly from the University vineyards and sampled by carefully snipping with small scissors at the torus without damaging the berry skin. The whole group of clusters being processed to wine was sampled with several hundred



HPLC chromatograms of: A — fully protected fresh Chenin blanc juice, B — the same juice unprotected from air oxidation during crushing.

Chromatogrammes HPLC de: A — jus frais de Chenin blanc totalement protégé, B — même jus non protégé contre l'oxidation pendant le foulage.

berries being collected, a few at random from about every fifth cluster. The berries were gently mixed, subsamples counted, weighed, and processed immediately.

Berry subsamples (100 berries as a rule) were placed in a glove box under an atmosphere of CO_2 flowing in at a high rate and verified as essentially oxygen-free by an oxygen electrode system. Two to four subsamples of berries from each variety were processed in the glove box entirely to the stage of filtered juice ready for HPLC. To the 100 berries were added 1 g of potassium metabisulfite and 1 g of ascorbic acid. Levels of 100 mg of each agent seemed about adequate, but to ensure maximum effect and since excess had no apparent deleterious effect on chromatography, the larger amounts were used. The mixed mass was crushed and pressed in cheesecloth by means of a hand-operated press as quickly, completely and uniformly as possible. The juice was measured and a portion filtered for HPLC through a Millipore 0.45 µm membrane filter. Meanwhile, other berry subsamples were also prepared with and without additions of ascorbic acid and bisulfite but in air. One set was unprotected and the crushed grapes exposed to air for 15 min to 1 h.

HPLC was carried out on each juice sample as soon as possible, although once protected by high SO_2 and ascorbic acid and filtered further change was slow. The HPLC apparatus was a Waters Associates system including a 720 controller, 730 data module, M6000 A pumps, a 710B autoinjector and a Perkin-Elmer LC-55B variable wavelength detector. The effluent was monitored at 320 nm and known dilutions of *trans*-caftaric acid were used to determine the response factor (unit mass per unit peak area) and thus to calculate the amount of caftaric acid or derivatives in each peak. The column was reversed phase, C18 (5 µm packing), 4.6 × 250 mm, and protected with a guard cartridge of the same packing (Brownlee Labs., Inc.). Isocratic development was with 0.05 M ammonium dihydrogen phosphate made to pH 2.6 with phosphoric acid, 16 % methanol at 1.5 ml/min and ambient temperature. Direct injections, 50 µl, were made of the closely filtered sample along with comparable injections, 30 µl, of a standard solution of known caftaric acid.

The commercial-style musts were prepared by passing the grapes through a small Garolla-type crusher-destemmer, making the must to 50 ppm in SO_2, and pressing lightly. The musts were allowed to settle in a 0 °C room overnight and the supernatant juice sampled to compare with the sample taken at pressing. A second portion of the whole must (pomace included) was fermented 5 d and then pressed and sampled as wine.

Results and discussion

There are three obvious possible sources of loss of *trans*-caftaric acid during processing — oxidation, hydrolysis, and *cis*-isomerization. Oxidation will be shown to be rapid and important. Hydrolysis, unless external pectinase or other esterase has been added, is relatively slow and usually minor. Even at equilibrium *cis*-caftaric acid content is small.

Caftaric acid content is maximal and should represent the true content in fresh juice of the grape berry when processing is cool, rapid, with minimal light, and fully protected from oxidation, i.e., inert atmosphere, immediately both high SO_2 and ascorbic acid to maintain reducing conditions. Under such conditions a typical HPLC chromatogram (Fig.: A) shows *trans*-caftaric acid, *trans*-coutaric acid and traces of their *cis*-forms. In time some caffeic acid, both *trans* and *cis*, slowly appears.

The figure (B) shows a typical HPLC chromatogram of the same juice after incomplete protection from enzymic oxidation. Note that the major change is the production

Table 1

Caftaric and coutaric acid content of juice and berries well protected from oxidation

Teneur en acides caftarique et coutarique de jus et baies bien protégés contre l'oxidation

Variety	Vintage	Caftaric acid							Coutaric acid	
		100-berry replicates, mg/l juices						mg/kg [1]) fresh berries	mg/l juice Mean	mg/kg [1]) fresh berries
		A	B	C	D	Mean	SO_2 + Asc. no CO_2			
Carignan	1983	47	51	52	49	50	53	41	11	9
Chardonnay	1983	207	196	182	180	191	197	158	20	16
Chenin blanc	1982	—	—	—	—	74	13	62	—	—
Chenin blanc	1983	102	100	98	98	100	90	82	7	6
French Colombard	1982	—	—	—	—	105	87	88	—	—
French Colombard	1983	76	76	76	77	76	78	64	11	9
Grenache	1983	190	201	202	210	201	193	158	16	13
Ruby Cabernet	1983	63	66	67	70	67	67	55	13	11
Semillon	1982	—	—	—	—	142	119	118	—	—
Semillon	1983	92	92	90	86	90	79	74	3	2
Thompson Seedless	1982	—	—	—	—	112	85	99	—	—
Thompson Seedless	1983 a	62	65	64	64	64	41	55	2	2
Thompson Seedless	1983 b	—	—	—	—	—	71	62	7	6

[1]) Calculated assuming all berry fluid has the same content as the best-protected expressed juice, subtracting an approximate value (10 % for seeded, 5 % for unseeded) for dry, nonsugar solids, and using Brix density to convert from volume to weight.

of a new symmetrical peak at a retention time of 10.8 min representing caftaric reaction product (CRP). Based upon retention time under identical conditions the same CRP is produced in all grape varieties tested. It is called caftaric reaction product because it was possible to produce additional product from added caftaric acid and grape phenolase. Furthermore, the relative amounts of the remaining *trans*-caftaric acid and the CRP are reciprocal, i.e., the less the caftaric acid is protected from oxidation the more CRP appears and *vice versa*. In fact, the sum of the percentage contribution of these two components to the total remaining 320 nm absorbance was nearly constant and caftaric acid and CRP inversely proportional.

The means of triplicate HPLC determinations of *trans*-caftaric and *trans*-coutaric acids on protected, replicate, 100-berry samples are shown in Table 1 for grape varieties harvested in 1983 and a few protected samples from 1982. The ultraviolet absorption of CRP is similar to that of caftaric acid with a maximum at 325 nm in 0.1 N formic acid in 20 % methanol. Therefore, amounts of CRP calculated from the caftaric acid response factor should be meaningful. A separate response factor was used to calculate *trans*-coutaric acid content. The replicate analyses were highly reproducible (coefficient of variability averaged 1.6 %) and the 100-berry samples only slightly more variable. This gives additional confidence that the maximum caftaric acid contents indicated are good estimates of the original berry content. Other factors studied were the reproducibility of injection and of the response factor. It was found that the amount injected and the preparation of the standard could easily produce variation in response factor sufficient to vary the calculated caftaric acid content by a factor of 2. Weighing a relatively large amount (100 mg) of pure caftaric acid to make the standard solution, preparation of dilutions to a similar level as the samples being measured (about 50—100 mg/l), and use of similar amounts per injection (not less than 10 μl per injection) were found preferable and used to develop the 1983 data in Table 1. The 1982 data are perhaps less reliable because the techniques were under development and less replication was used.

There is apparent loss of caftaric acid to other products because the sum of the remaining *trans*-caftaric acid plus CRP is appreciably lower in most cases than the original *trans*-caftaric acid content (Table 2). This loss presumably results from conversion of caftaric to additional products sufficiently changed in spectral properties or too numerous to be detected as significant peaks. (Note extra peaks in the figure, B.) Hydrolysis and *cis*-isomerism could contribute. Nevertheless, the CRP represents in most instances a very sizable fraction of the remaining 320 nm absorbance and a major fraction of the original *trans*-caftaric acid content. In no instance was CRP detected in juices well protected from oxygen (Table 2).

Coutaric acid appeared more readily retained than caftaric acid. Little or none was lost in the semi-protected laboratory juice samples, but in unprotected samples the losses were: 26 % for Chardonnay, 13 % for Chenin blanc, and 53 % for Semillon. The rate of loss of caftaric acid was rapid, being considerable by 10 min and approaching completion by 1 h after exposure to air by unprotected crushing. CRP was higher after about 15 min than after longer oxidation. It seems again clear (Table 2) that the caftaric acid content of grapes and wines represents a component of the easily expressed vacuolar juice and is not contributed appreciably from the firmer tissues, in sharp contrast to anthocyanins and other flavonoids. The slightly higher content of caftaric acid in wine fermented on the pomace than corresponding wine from juice is attributed to less complete oxidation in the former.

Discovery of this CRP entity is considered exciting and details of its characteristics and identity will be the subject of following reports. It appears to help explain a number of observations. Previously we had been able to recover about half or less of the

Table 2

Loss of *trans*-caftaric acid and generation of CRP by storage and processing

Perte en acide *trans*-caftarique et génération de CRP par stockage et traitements

	Char-donnay (Reg. II)	Chenin blanc (Reg. II)	Grenache (Reg. IV)	Ruby Cabernet (Reg. IV)	Semillon (Reg. IV)	Thompson Seedless (Reg. IV)
Juice, fully protected						
A. caftaric mg/l	191	100	201	67	90	64
% loss/wk (refrigerated)	1.5	2.7	—	—	10.6	3.8
CRP mg/l	0	0	0	0	0	0
Intact berry, refrigerated 0 °C in air (caftaric, no CRP, coutaric 0)						
% loss/wk from A	9.5	—	—	—	—	—
Juice, air exposed, SO_2 and ascorbic acid added as crushed						
caftaric mg/l	187	90	193	67	79	41
% loss from A	2	10	4	0	12	38
CRP mg/l	0	0	0	0	0	0
Juice, laboratory, unprotected						
B. caftaric mg/l	95	17	101	0	4	0
% of A	50	17	50	0	4	0
C. CRP mg/l	21	50	56	64	56	38
% of A	11	50	28	96	62	59
% disappeared [A−(B+C)]/A	39	33	22	4	34	5
Juice, winery, immediately pressed, 50 ppm SO_2						
D. caftaric mg/l	124	54	107	0	3	13
% of A	65	54	53	0	3	20
E. CRP mg/l	23	16	44	49	57	32
% of A	12	16	22	73	63	50
% disappeared [A−(D+E)]/A	23	30	25	27	31	30
Juice, racked after overnight cold settling						
caftaric mg/l	115	60	—	—	3	14
% change from D	− 7	+11	—	—	0	+8
CRP mg/l	23	17	—	—	56	31
% change from E	0	+ 6	—	—	−2	−3
Fermented 5 d with pomace						
F. caftaric mg/l	124	46	—	7	0	46
% change from D	0	−15	—	+	−100	+250
G. CRP	12	7	—	3	28	18
% change from E	−48	−56	—	−94	− 51	− 44
% disappearance [A−(F+G)]/A	29	47	—	85	69	0

caftaric acid as a proportion of 320 nm absorbance from Californian wine as from an English wine (SINGLETON *et al.* 1978). This was true in spite of meticulous attention to details of the isolation procedure including use of a portion of the same lot of activated carbon used in England. We now know the Californian wines had less unmodified caftaric acid and thus the difficulty. Owing to shortage of winemaking equipment, the English wine was made by placing grapes, inoculum and SO_2 within a polyethylene bag, flushing the bag with CO_2 and then crushing the grapes by treading the bag. The same technique applied to Californian grapes gave wine with little CRP and more caftaric acid. When a large lot (400 kg) of Napa Gamay grapes was crushed with 900 g each of ascorbic acid and potassium metabisulfite in a strong stream of CO_2, the drained and settled juice retained 42 mg/l of caftaric acid and 68 % of that absorbed on charcoal was recovered in the eluate — a recovery better than the best (54 % overall, 61 % of adsorbed) obtained in England.

The CRP was considerably more resistant to extraction than caftaric acid and this at least partly explains the poor recovery of 320 nm absorbance from solvent extraction of ordinary musts and wines (Table 3). CRP was found in wines up to at least 2 years old. In some instances caftaric acid was also present and in others it was gone.

Table 3

Distribution coefficients (A_{320} in solvent/A_{320} aqueous)

Coefficients de distribution (A_{320} en solvant/A_{320} aqueux)

	EtOH/satd. $(NH_4)_2SO_4$	EtOAc
Coutaric acid	10.3	3.4
Caftaric acid	6.8	1.1
CRP	1.5	0.2
Wine (60 mg/l CRP, no caftaric)	1.0	0.1

The values previously reported in the literature for caftaric acid content cannot be evaluated correctly because the degree of oxygen exposure is unknown. The highest values reported here are higher than some previously reported for ripe grapes of the same varieties, but for other varieties they appear similar. Most of the previous reports indicated efforts to minimize oxidation, but perhaps not as complete as we found necessary. ONG and NAGEL (1978), for example, froze the berries in liquid nitrogen, thawed them in a microwave oven and crushed them with 1,000 ppm SO_2. Caftaric values obtained were high, but some oxidative modification appears possible. Other workers have been apparently less careful in some instances and any values obtained on ordinary winery musts do not represent accurately the original berry content of caftaric acid.

There are no data to indicate that the formation of CRP is deleterious. It must always have been a usual but unrecognized component of wines. Its recognition should, however, lead to useful new understanding as more is learned about it. Furthermore, the relative content of caftaric, CRP, and caffeic acid should serve as indicators of the original handling of the must and vinification. It is gratifying that the amounts of caftaric acid originally present in grapes and the sum of that remaining plus reaction products approach those estimated from gross evaluations of the phenol content by different means (SINGLETON 1982).

Summary

Grapes were crushed in the presence of high levels of the anti-oxidants SO_2 and ascorbic acid, in an atmosphere deprived of oxygen. Hydroxycinnamic derivatives, particularly caftaric acid in the grape juice were found in much higher amounts than when the usual processing methods were employed.

Partial oxidation of caftaric acid is not only very rapid and extensive during unprotected grape crushing and juice separation but gives a major reaction product that behaves as a single entity and survives through vinification. It gives a sharp HPLC peak and behaves reciprocally with caftaric, i.e., when caftaric acid in must remains high relative to the berry content the reaction product is low and *vice versa*. The product appeared identical in all grape varieties tested.

The reaction product is about one-sixth as extractable by immiscible solvents as is the parent caftaric acid. It can constitute a large part of the 320 nm absorbance of a wine and explains the variable results and poor yields in attempts to isolate caftaric acid from ordinary wine or juice.

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