

## Effect of Alcoholic Fermentation in the Content of Phenolic Compounds in Cider Processing

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### ABSTRACT

*The objective of this work was to study the effect of alcoholic fermentation on the content of phenol compounds of five cider apple varieties. The initial content in the apple juice samples, as determined by HPLC, varied from 188.4 to 2776.17 mg.L<sup>-1</sup>. In three of them (DC, PJ, GU), the total phenol compounds remained unaffected by fermentation. However, in two (DM, KE), the final values were lower (55 and 313 mg.L<sup>-1</sup>). In these apple cider, the values of caffeic acid increased from 6.6 mg.L<sup>-1</sup> to 41.8 mg.L<sup>-1</sup>. The catechin content increased during the process, approximately 13 mg.L<sup>-1</sup> independent of the variety. The other phenols class did not present any modifications due to the alcoholic fermentation, maintaining the phenolic compounds of original clarified apple juice in the cider.*

**Keywords:** cider, fermentation alcoholic, polyphenols

### INTRODUCTION

Polyphenols play important roles in the cider quality as they are related to the color, bitterness and astringency, whose balance defines the overall mouthfeel of the beverage (Guyot et al., 1998; Alonso-Salces et al., 2001; Lea and Drilleau, 2003; Alonso-Salces, et al., 2004). They may be involved in the fermentative processes, providing the cider aroma, and as inhibitors of the microorganisms development, controlling the fermentation rates and avoiding some faults that can develop in cider from the action of lactic acid bacteria such as acidification, mannitol taint, “framboisé”, bitterness (Alonso-Salces et al.,

2004). Furthermore, the phenolic compounds participate in the formation of sediments during the cider storage, due to their colloidal interaction with the proteins through the van der Waals forces (Siebert et al., 1996; Kawamoto and Nakatsubo, 1997). They can also inhibit the pre-fermentative clarification enzymes (Cowan, 1999). The polyphenols are receiving increasing attention due to their natural antioxidation and health protective properties (Vanzani et al., 2005; Tsao et al., 2005). The polyphenolic composition of a cider depends on the mixture of the apple varieties and the cidermaking procedures. The five main polyphenol classes in the apples are as follows: [1] **flavan-3-ols**, which includes the monomeric

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(catechin) and polymeric forms (procyanidins), the latter constituted by (-)-epicatechin units; [2] **hydroxycinnamic acids**, 5-caffeoylquinic acid and 4-p-coumaroylquinic acid show the highest contents; [3] **dihydrochalcones**, mainly the phloretin glucoside and xyloglucoside, generally known as specific for the apples; [4] **flavonols** and [5] **anthocyanins** present in the apple peel. The antioxidant activity of these five polyphenols in the apple decreases from cyaniding-3-galactoside > procyanidins > quercetin glycosides > chlorogenic acid > phloridzin (Tsao et al., 2005).

The cidermaking steps mainly responsible for the extraction and content of the phenols in the final product are maceration, pressing, enzymatic clarification of the must prior to fermentation, centrifugation, filtration, and fining. During the maceration and pressing time, intensive oxidation of the polyphenols takes place, due to the activity of the polyphenoloxidase (PPO) and the subsequent coupled oxidation reactions with other polyphenols. In addition, a large proportion of the procyanidins from the fruits remain in the pomace after the pressing step because of their adsorption onto the cell-wall matrix (Renard et al., 2001; Guyot et al., 2003). These lead to musts with lower phenolics content (Siebert et al., 1996). It has been proved that must oxidation was higher when it was in contact with the apple pulp. The enzymatic clarification, centrifugation, filtration and fining of the French ciders lead to the partial elimination of procyanidins due to their ability to precipitate proteins and to interact with cell wall polysaccharides (Alonso-Salce et al., 2004).

Yeast membranes possess the ability to retain some compounds present in the wine (Ribereau-Gayon et al., 1998). The cell wall of *Saccharomyces cerevisiae* is made of the mannoproteins bound to the oligopolysaccharides, which remain exposed on the outside of the cell. These mannoproteins are also bound to the glucanose and chitin (Young, 1987; Walker, 1998). The different polarities and the hydrophilic or hydrophobic nature of these wall polymers define the capacity of the yeast to retain or to adsorb different wine molecules such as the volatile compounds, fatty acids or pigments (Morata et al., 2003). The porosity of the wall also influences the adsorption (Boivin et al., 1998). An increased surface provided by interstitial spaces favors the adsorption.

The surface of yeast cell in the wine fermentation is higher than  $10 \text{ m}^2 \cdot \text{L}^{-1}$  of the must and some

phenolic acids, flavan-3-ol derivatives procyanidins B2 and B4 (Bonilla et al., 2001), phenolic volatiles (Chassagne et al., 2005), colored products formed from phenolics oxidation or condensation reactions can be adsorbed by yeast during the fermentation (red and white wines) (Razmkhab et al., 2002; Mazauric and Salmon, 2005). Another pathway for phenols, with change in the color of the wine involves the direct condensation of the phenols with the acetaldehyde produced by yeasts in fermented drinks (Lopez-Toledano et al., 2004).

The influence of the apple must fermentation in phenol content is still poorly understood. In this work the effect of alcoholic fermentation of five cider apples cultivars in the final phenolics compounds content was studied.

## MATERIAL AND METHODS

### Strain

*Saccharomyces uvarum* - S6U/Lalvin commercialized as dry yeast was used in this work.

### Raw material

Five apple cider varieties were used as fermentation medium. The fruits were harvested and transported to the processing unit stocked at room temperature (20°C) just to the maximum maturation degree, procedures used by local producers to obtain the maximum levels of fermentable sugars. The industrial apple samples (300 kg) were obtained from different producers of Bretagne in France (crop 2000), with respective classification: Douce Coët Ligné [sweet]; Petit Jaune [acid]; Dous Moen [bitter-sweet]; Guillevic [acid] e Kermerrien [bitter] (Boré and Fleckinger, 1997).

### Must preparation

The fruits were selected, milled (Processing Metvisa, Type MPA) and pressed in two steps: 5 min at 40 Bar and 10 min at 200 Bar (Pressoirs Colin, Montreuil). Pectic substances were removed by decanting after the treatment with pectinase (Pectinase ref. C80) for 10h at 20°C ( $8 \text{ mL} \cdot \text{hL}^{-1}$ ).

The must was then microfiltered by tangential filtration (module Millipore/Durapore) at  $0.45 \mu\text{m}$ . Eight liters of each sample were placed in the package bag in box and stocked at -25°C. This was defrosted at room temperature homogenized for 30

minutes in a container of 200 L (Alfa Laval-Type RFT, with agitation). The must was micro-filtered again. Next, they were transferred to sterilized 15 liters glass fermentors (120°C/15min). The dissolved oxygen was partially eliminated by injecting inert gas (nitrogen) with agitation during 60 minutes what warrants the level of 0.5 mg.L<sup>-1</sup> (Orbisphere Laboratoires, modelo 3650). Oxygen was injected in the must (around 50 mg.L<sup>-1</sup>), for a final concentration of 2.5 mg.L<sup>-1</sup>. After this, the fermenters were placed in a room with controlled temperature (10-11°C) and connected with the inert atmosphere system (nitrogen gas) with sterilized filter (PTFE, 0.22 µm) to avoid further contaminations and to release the CO<sub>2</sub> formed during the fermentation.

### **Yeast growth**

Yeast growth was expressed as the cells.mL<sup>-1</sup> using Coulter® Multisizer II System and as colony forming units (FCU) after 24 h of the incubation in YMA.

### **Density**

Degasified (3 min in ultra-son under vacuum) and 2-mL of sample were inserted in digital densimeter (Density Meter, DMA 58) at 20°C (precision was 0.00001 Kg.m<sup>-3</sup>).

### **Alcohol degree**

Degasified samples (10mL) were distilled and the distilled were analyzed in a densimeter (Density Meter, DMA 58) at 20°C, with a function converting in ethanol percentile, respecting the dilution of 1:2.

### **Freeze dried samples**

Microfiltered (Teflon filter 0.45 µm) samples of the apple juice and cider aliquots (2 x 500 µL) were freeze dried in 5 mL vials and stored in desiccators until further analysis.

### **Phenolic compounds**

Freeze dried samples were directly submitted to thiolysis reaction in methanol according to Guyot et al. (2001) and the products were analyzed by reverse phase HPLC. HPLC peaks were identified on the chromatograms according to their retention times and their UV-visible spectra by comparison with standards. Caffeoylquinic acid, (+)-catechin, (-)-epicatechin and phloridizin were commercially available (Sigma-Aldrich). *p*-Coumaroylquinic

acid was purified from a commercial cider (Sanoner et al., 1999). (-)-Epicatechin benzilthioether and procyanidin B2 were kindly furnished by J.M. Souquet (UMR, SPO, INRA, Montpellier, France). Phloretin xyloglucoside identification was performed in a previous work (Sanoner et al., 1999), and its quantification was expressed in phloridizin equivalents. For each compound, the quantification was performed by reporting the measured integration area in the calibration equation of the corresponding standard. Integrations were performed at 280 nm for the catechin monomers, procyanidin B2, thioether adduct, and dihydrochalcone, at 320 nm for hydroxycinnamic compounds and at 350 nm for flavonols.

## **RESULTS AND DISCUSSION**

### **Characteristics of the apple must and alcoholic fermentation**

The characterization of the phenols and the evolution during the fermentation was analyzed in five apples cider, in order to verify a possible absorption of the polyphenols in the cell wall yeast (S6U). The analyses were carried in musts before inoculation (0 day of fermentation); to the end of the growth phase (9-10 days of fermentation) and when the cider had reached 1 015 kg.m<sup>-3</sup> of density (stop fermentation).

In Table 1 the physico-chemical features of the industrial apple musts after despectinization and microfiltration operations are showed.

The Douce Coët Ligne (DC) and Petit Jaune (PJ) varieties show very close total sugars concentrations of 102.67 and 101.65 g.L<sup>-1</sup>, respectively). At a superior level, a quite similar situation was observed for Guillevic (GU) and Kermerrien (KE) varieties, with 130.71 and 127.52 g.L<sup>-1</sup>, respectively. Dous Moen (DM) variety showed an intermediate value 115.32 g.L<sup>-1</sup>. The fluctuation in the total sugar concentration will reflect both in the fermentation time and in the alcoholic potential, which can vary from of 5.9 to 7.6°GL considering a process to dryness.

The nitrogen analysis (Table 1) showed a great dispersion among the samples, with concentrations varying from 32.05 and 36.69 mg.L<sup>-1</sup> for DM and DC respectively, (low nitrogen content), from 65.54 and 76.56 mg.L<sup>-1</sup> for KE and PJ, respectively, (normal varieties) and 375.52 mg.L<sup>-1</sup> for variety GU (excess of the nutrient, due to be a

new orchard, first harvest, with high fertilizations). The phenols in the apple juice varied from 188.4 to 2,776.17 mg.L<sup>-1</sup> (Table 1), which suggested a large genetic variation among the genotypes, a fact already mentioned by some authors (Amiot et al., 1992; Sanoner et al., 1999). Moreover, factors

such as the maturity of the fruit (Burda et al., 1990; Awad et al., 2001), time of the harvest (Vander Luis et al., 2001), exposition to day light (Awad et al., 2000a), storage conditions of the fruits (Awad et al., 2000b) also contribute to establish the proper final phenol content.

**Table 1** - Physical chemical and microbiological features of five apple cider cultivars: apple must, final growth phase (9 days) and apple wine with stopped fermentation in  $\rho_{20^{\circ}\text{C}}$  1 015 kg.m<sup>-3</sup>.

Varieties	Time, d	Fermentation features					Yeast population, cfu.mL <sup>-1</sup>
		Density, kg.m <sup>-3</sup>	Sugar, g.L <sup>-1</sup>	Ethanol, °GL	Nitrogen, mg.L <sup>-1</sup>	Total phenol, mg.L <sup>-1</sup>	
Douce Coet Ligne DC	0	1 043.28	101.65	0.00	36.69	748.3	4.23x10 <sup>5</sup>
	9	1 037.96	89.40	0.68	15.76	757.2	4.27x10 <sup>6</sup>
	48	1 015.24	34.88	3.72	15.73	734.8	5.07x10 <sup>6</sup>
Petit Jaune PJ	0	1 048.48	102.67	0.00	74.56	188.4	6.68x10 <sup>5</sup>
	9	1 040.89	89.02	0.92	39.93	191.3	6.84x10 <sup>6</sup>
	48	1 015.21	32.01	3.90	32.28	186.9	7.08x10 <sup>6</sup>
Dous Moen DM	0	1 049.86	115.32	0.00	32.05	1387.6	7.61x10 <sup>5</sup>
	9	1 045.92	107.51	0.58	18.34	1343.7	4.35x10 <sup>6</sup>
	75	1 017.39	40.16	4.55	17.17	1332.5	4.27x10 <sup>6</sup>
Guillevic GU	0	1 061.52	130.71	0.00	375.52	212.3	6.76x10 <sup>5</sup>
	9	1 053.50	114.47	0.83	297.66	232.5	6.08x10 <sup>6</sup>
	60	1 015.62	35.95	6.16	310.29	230.7	7.36x10 <sup>6</sup>
Kermerrien KE	0	1 056.11	127.52	0.00	65.54	2776.7	6.84x10 <sup>5</sup>
	9	1 049.62	110.16	0.76	30.88	2503.5	5.71x10 <sup>6</sup>
	51	1 015.17	35.16	5.48	28.88	2463.4	7.24x10 <sup>6</sup>

**Table 2** - Effect in phenolics acid content (mg/L) during apple cider fermentation: apple must, final growth phase (9 days) and apple wine with stopped fermentation in  $\rho_{20^{\circ}\text{C}}$  1 015 kg.m<sup>-3</sup>.

Varieties	Time, d	Hydroxycinnamic acids					
		Clorogenic acid			Other hidroxicinnamic acids		
		5-CQA	p-CoQA	Total	Caffeic acid	OHC	Total
Douce Coet Ligne DC	0	363	47	410	nd.	62.3	62.3
	9	376	49	425	nd.	59.2	59.2
	48	375	47	422	1.5	63.3	64.8
Petit Jaune PJ	0	131	18	149	1.8	12.8	14.6
	9	126	18	144	1.5	17.1	18.6
	48	125	18	143	1.8	15.9	17.7
Dous Moen DM	0	492	101	593	3.2	64.3	67.5
	9	475	99	574	1.8	76.9	80.8
	75	505	105	610	2.6	63.4	68.0
Guillevic GU	0	87	62	149	3.7	37.8	41.5
	9	93	65	158	3.6	43.1	46.7
	60	91	65	156	4.4	46.7	51.1
Kermerrien KE	0	824	79	903	6.6	91.8	98.4
	9	780	75	855	15.5	85.8	101.3
	51	749	77	826	41.8	86.3	128.1

Note: (nd) not detected; 5-CQA (5-caffeoylquinic acid); p-CoQA (4-p-coumaroylquinic acid); OHC (others hidroxicinnamic acids).

The phenol compounds are involved with the color, flavor and the bitterness of the musts (Lea and Drilleau, 2003) and it can directly influence the consumption of the dissolved oxygen through enzymatic oxidation process (Nicolas et al., 1994). The level of 200 mg.L<sup>-1</sup> is actually used to classify apple varieties as bitter (Lea and Drilleau, 2003), the DM and KE had 1387.6 and 2776.7 mg.L<sup>-1</sup>, respectively.

The initial yeast population varied from 4.23x10<sup>5</sup> to 7.61x10<sup>5</sup> for the DC and DM, respectively. The maximum yeast population was attained in function of the nitrogen content and did not exceed 7.36x10<sup>6</sup> cfu.mL<sup>-1</sup> in GU cider due to the low content of the dissolved oxygen and also because the apple juices was micro filtered, a process that eliminated the phytosterols which, as oxygen, are essentials for ergosterol synthesis for plasmatic membrane structure (Nogueira, 2003b).

The total phenol content in the apple cider varieties DC, PJ, GU remained the same until the fermentation was stopped. However in the DM and KE, a reduction of the total phenol content (from 55 to 313 mg.L<sup>-1</sup>, respectively) was observed during the fermentation.

#### Effect of the alcoholic fermentation in polyphenols content.

The hydroxycinnamic acid class (Table 2) comprehended from 36% up to 90% of the total

polyphenols (KE and GU, respectively) in these five cider apple juice varieties. According to Guyot et al. (2003) this class shows high yield (65%) in the apple juice processing. The 5-caffeoylquinic acid (5-CQA), the main phenol substrate of the polyphenoloxidase in the presence of oxygen, was found in the largest concentration in all apple varieties (from 45% up to 82% of the total hydroxycinnamic acids) and did not show any changes during the fermentation of the DC, PJ, DM and GU. However, in the case of the KE it fell from 824 mg.L<sup>-1</sup> to 749 mg.L<sup>-1</sup> and, as there was an increase of the caffeic acid from 6.6 to 41.8 mg.L<sup>-1</sup>, it was possible that a deesterification reaction took place by means of the hydrolases of *Saccharomyces* S6U, but such observation was only for this variety fermentation. The 4-p-coumaroylquinic acid (p-CoQA) did not show any changes in the concentration during the process. According to Ramos et al. (1999), in the grape wine fermentation at 14°C, this compound showed a higher concentration at the end.

The flavan-3-ols class (Table 3) corresponded to 25, 49 and 62% of the total polyphenols (DC, DM and KE, respectively) but in the PJ and GU, its presence was not detected. In the other ciders, a sensible reduction and increase of epicatechin and catechins compounds was observed.

**Table 3** - Effect in flavan-3-ols content (mg.L<sup>-1</sup>) during apple cider fermentation: apple must, final growth phase (9 days) and apple wine with stopped fermentation in  $\rho_{20^{\circ}\text{C}}$  1 015 kg.m<sup>-3</sup>.

Varieties	Time, d	Flavan-3-ols						
		Flavan-3-ol monomeric			Flavan-3-ol polimeric			
		(-)Epicat.	(+)-Catec.	Total	B2	Others	Total	GP
Douce Coët Ligné DC	0	26.2	nd.	26.2	Nd.	160.7	160.7	3.1
	9	19.9	8.6	28.5	Nd.	149.3	149.3	2.9
	48	19.2	20.4	39.6	Nd.	152.4	152.4	2.7
Petit Jaune PJ	0	nd.	nd.	0.0	Nd.	nd.	nd.	nd.
	9	nd.	4.4	4.4	Nd.	nd.	nd.	nd.
	48	nd.	15.6	15.6	Nd.	nd.	nd.	nd.
Dous Moen DM	0	75.6	24.7	100.3	45.0	541.0	586.0	3.6
	9	57.3	24.2	81.5	34.0	531.0	565.1	3.1
	75	53.4	37.4	90.8	25.0	517.7	542.7	2.8
Guillevic GU	0	nd.	nd.	0.0	Nd.	nd.	nd.	nd.
	9	nd.	5.9	5.9	Nd.	nd.	nd.	nd.
	60	nd.	13.6	13.6	Nd.	Nd.	nd.	nd.
Kermerrien KE	0	146.9	nd.	146.9	143.0	1421.1	1564.1	3.8
	9	95.3	nd.	95.3	95.0	1293.8	1388.8	3.5
	51	86.8	22.5	109.3	89.0	1279.6	1368.6	3.2

Note: (nd.) not detected; (B2) procyanidin B2; (GP) polymerization degree.

**Table 4** - Effect in dihydrochalcones and flavonols content ( $\text{mg.L}^{-1}$ ) during apple cider fermentation: apple must, final growth phase (9 days) and apple wine with stopped fermentation in  $\rho_{20^{\circ}\text{C}}$  1 015  $\text{kg.m}^{-3}$ .

Varieties	Time, d	Dihydrochalcones			Flavonol	
		Phloretin	Phloridzin	Phloretin Xyloglucoside	Total	Quercetin
Douce Coët Ligné DC	0	nd.	37.1	52.5	89.8	3.3
	9	nd.	33.3	50.1	91.8	3.4
	48	6.2	41.7	46.3	94.2	3.5
Petit Jaune PJ	0	nd.	14.5	9.2	23.1	1.7
	9	nd.	13.9	8.9	22.9	1.4
	48	nd.	14.0	9.1	23.1	1.5
Dous Moen DM	0	nd.	18.6	22.1	38.3	2.5
	9	nd.	16.2	21.2	40.0	2.3
	75	nd.	18.8	18.8	18.8	2.2
Guillevic GU	0	nd.	9.0	12.1	21.8	nd.
	9	nd.	9.7	11.3	21.9	nd.
	60	nd.	10.6	10.0	20.6	nd.
Kermerrien KE	0	nd.	38.7	27.1	63.1	1.2
	9	nd.	36.0	24.6	61.8	1.3
	51	6.7	37.2	23.2	67.4	1.5

Note: (nd.) not detected.

In apple juice processing the extraction of procyanidins was significantly lower, close to 32% due the association of the procyanidins with the solids part of the fruits, particularly cell-wall materials (Renard et al., 2001; Guyot et al., 2003;). This compound of high antioxidant activity (Tsao et al., 2005) was present in the DM and KE and showed to lows down the concentration from 143 to 89  $\text{mg.L}^{-1}$  in the KE. The lower values were also observed in the polymerization degree and according to Bonilla et al. (2001) this was due to the fact that procyanidins could associate with the yeast cell wall. The dihydrochalcone class (Table 4) comprehended from 10 to 12% of the total polyphenols for GU, PJ and DC and from 2.3 to 2.7% for KE and DM, respectively. According to Guyot et al. (2003), the dihydrochalcone showed high yield extraction (80%) in the apple processing.

In a previous work, Guyot et al., (1998) showed that the polyphenols of the cortex accounted for 65% of the total polyphenols of the fruits and that the cortex tissues were shown to comprehend up to ~84% of the fruit fresh matter. The low flavonols content was an indication of the poor extractability of the polyphenols from the peel in the actual conditions of juice preparation (Table 4).

Dihydrochalcone and flavonol classes did not vary during the fermentation. Phloridzin is a phenolic compound found only in apples and it is a

parameter to state the authenticity of apple products (Spanos and Wrolstad, 1992). The concentration of these compounds depends on the variety and the highest content has been found in the DC with 89.8  $\text{mg.L}^{-1}$  (Sanoner et al., 1999), and the lower values were for the PJ and GU with 23.1 and 21.8  $\text{mg.L}^{-1}$ , respectively.

The DC maintained the quercetin content during the fermentation, which was advantageous on the product (Guyot et al., 2003; Tsao et al., 2005), as such phenol compound present in the fruit peel do have high antioxidant activities. In the GU variety no quercetin was detected.

## CONCLUSION

The apple must present a great dispersion in phenols content, with KE and DM showing the largest values and PJ and GU, the lowest. In apple juice with high phenols content (KE) there were observed lower levels after alcoholic fermentation. The phenols class that showed lost was of the polymeric flavan-3-ol, then high concentration, possibly because of interactions with the yeast cell wall. The other phenols class and different apple ciders (DC, PJ, DM e GU) did not show any modifications with the alcoholic fermentation, remaining the phenols compounds of original clarified apple juice in the cider juice.

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## RESUMO

O objetivo deste trabalho foi estudar o efeito da fermentação alcoólica no teor de compostos fenólicos de cinco mostos de maçãs industriais. Os compostos fenólicos foram analisados por HPLC. Os mostos apresentaram fenóis totais entre 188,4 a 2776,17 mg.L<sup>-1</sup>. Os teores de fenóis durante a fermentação permaneceram os mesmos para as variedades DC, PJ e GU, entretanto, em DM e KE foi observada uma diminuição dos teores de fenóis (55 e 313 mg.L<sup>-1</sup>, respectivamente).

Em KE o teor do ácido caféico aumentou de 6,6 mg.L<sup>-1</sup> para 41,8 mg.L<sup>-1</sup>. O teor de catequinas aumentou cerca de 13 mg.L<sup>-1</sup> durante o processo, independente da variedade. As outras classes de fenóis não apresentaram modificações com a fermentação alcoólica, permanecendo na sidra os compostos fenólicos do suco de maçã clarificado.

## REFERENCE

- Alonso-Salces. R. M.; Barranco. A.; Abad. B.; Berrueta. L. A.; Gallo. B.; Vicente. F. (2004), polyphenolic profiles of basque cider apple cultivars and their technological properties. *Journal of Agricultural and Food Chemistry*, **52**, 2938-2952.
- Alonso-Salces. R. M.; Korta. E.; Barranco. A.; Berrueta. L. A.; Gallo. B.; Vicente. F. (2001), Determination of polyphenolic profiles of Basque cider apple varieties using accelerated solvent extraction. *Journal of Agricultural and Food Chemistry*, **49**, 3761-3767.
- Amiot, M. J.; Aubert, S.; Nicolas, J.; Goupy, P.; Aparicio, P. (1992), Phenolic composition and browning susceptibility of various apple and pear cultivars at maturity. *C.r. Jieip 9R*, **16**, 48-51.
- Awad, M. A.; De Jager, A.; Van Westing, L. M. (2000a), Flavonoide and clorogenic acid levels in apple fruit characterisation of variation, *Sci. Hortic.*, **83**, 249-263.
- Awad, M. A.; De Jager, A. (2000b), Flavonoid and chlorogenic acid concentrations in skin of "Jonagold" and "Elstar" apples during and after regular and ultra-low oxygen storage, *Postharvest Biol. Technol.*, **20**, 15-24.
- Awad, M. A.; De Jager, A.; Vanderplas, L. H. W.; Vanderkrol, A. R. (2001), Flavonoid and chlorogenic acid changes in skin of "Elstar" and "Jonagold" apples during development and ripening. *Sci. Hortic.*, **90**, 69-83.
- Boivin. S.; Alexandre. H.; Feuillat. M.; Charpentier. C. (1998), Relative cell wall porosity assay using monomeric and polymeric cations in *Saccharomyces cerevisiae* cultivated in grape juice. *Biotechnology Techniques*, **12**, 471-475.
- Bonilla. F.; Mayen. M.; Merida. J.; Medina. B. (2001), Yeasts used as fining treatment to correct browning in white wines. *J. Agric. Food Chem.*, **49**, 1928-1933.
- Boré, J. M., Flecking, J. (1997), *Pommiers à cidre. Variétés de France*. Paris: INRA, , p. 756.
- Burda, S.; Oleszek, W.; Lee, C. Y. (1999), Phenolic compounds and their changes in apples during maturation and cold storage. *Journal of Agricultural and Food Chemistry*, **38**, 945-948.
- Cowan, M. M. (1999), Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, **12**, 564-582.
- Chassagne. D.; Guilloux-Benatiera. M.; Alexandria. H.; Voilleyb. A. (2005), Sorption of wine volatile phenols by yeast lees. *Food Chemistry*, **91**, 1, 39-44.
- Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J.-F. (2003), Variability of the polyphenolic composition of cider apple (*Malus domestica*) fruits and juices. *Journal of Agricultural and Food Chemistry*, **51**, 6240-6247.
- Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J.-F. (2001), Direct thiolysis on crude apple materials for high-performance liquid chromatography characterization and quantification of polyphenols in cider apple tissues and juices. In: PACKER, L. *Methods in Enzymology - Flavonoids and other polyphenols*, Academic Press, **35**, 57-70.
- Guyot. S.; Marnet. N.; Laraba. D.; Sanoner. P.; Drilleau. J. F. (1998), Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a french cider apple variety (*Malus domestica* var. Kermerrien). *Journal of Agricultural and Food Chemistry*, **46**, 1698-1705.
- Kawamoto. H.; Nakatsubo. F. (1997), Effects of enviromental factors in two-stage tannin-protein coprecipitation. *Phytochemistry*, **46**, 379-483.
- Lea, A.; Drilleau, J.-F. (2003), Cider-making. In: *Fermented Beverage Production*, ed. LEA. A.. London, pp. 59-87.
- Lopez-Toledano. A.; Villano-Valencia. D.; Mayen. M.; Merida. J.; Medina. M. (2004), Interaction of yeast with the product resulting from of condensation reaction between (+)-catechins and acetaldehyde. *J. Agric. Food Chem.*, **52**, 2376-2381.
- Mazauric, J.-P.; Salmon, J.-M. (2005), Interactions between Yeast Lees and Wine Polyphenols during Simulation of Wine Aging: I. Analysis of Remnant

- Polyphenolic Compounds in the Resulting Wines. *J. Agric. Food Chem.*, **53**, 5647-5653.
- Morata, A.; Colomo, B.; Suárez, J. A.; Gómez-Cordove, S. M. C.; Bartolomé, B.; Suberviola, J. (2003) Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *Journal of Agricultural and Food Chemistry*, **51**, 4084-4088.
- Nicolas, J. J.; Richard-Forget, F. C.; Goupy, P. M.; Amiot, M. J.; Aubert, S. Y. (1994) Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition*, **34**, 109-157.
- Nogueira, A.; Lequere, J.M.; Budin, R. (2003a), Oxygène et stabilité. *Pomme à Cidre*, **5**, 16-17.
- Nogueira, A. (2003b), Tecnologia de processamento sidrícola. Efeitos do oxigênio e do nitrogênio na fermentação lenta da sidra. PhD Thesis, Universidade Federal do Paraná. Curitiba.
- Ramos, R.; Faia, M. A.; Andrade, P. B.; Seabra, R. M.; Pereira, C.; Ferreira, M. A. (1999), A preliminary study of non-coloured phenolics in wines of varietal white grapes (codega, gouveio and malvasia fina): Effects of grape variety, grape maturation and technology of winemaking. *Food Chemistry*, **67**, 39-44.
- Razmkhab, S.; Lopez-Toledano, A.; Ortega, J. M.; Mayen, M.; Merida, J.; Medina, B. (2002), Adsorption of phenolic compounds and browning products in white wines by yeast and their cell walls. *J. Agric. Food Chem.*, **50**, 7432-7437.
- Renard, C. M. G. C.; Baron, A.; Guyot, S.; Drilleau, J. F. (2001), Interactions between apple cell walls and native apple polyphenols: quantification and some consequences. *International Journal of Biological Macromolecules*, **29**, 115-125.
- Ribereau-Gayon, P. D.; Doneche, B.; Lonvaud, A. (1998), *Traité D'Oenologie. Microbiologie du Vin Vinifications*, Dunod, Paris, p. 617.
- Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J. F. (1999), Polyphenols profiles of french cider apple varieties (*Malus domestica*). *Journal of Agricultural and Food Chemistry*, **47**, 4847-4853.
- Siebert, K. J.; Carrasco, A.; Lynn, P. Y. (1996), Formation of Protein-Polyphenol Haze in Beverages. *Journal of Agricultural and Food Chemistry*, **44**, 1997-2005.
- Spanos, G. A.; Wrolstad, R. E. (1992), Phenolics of apple, pear, and white grape juices and their changes with processing and storage—a review. *Journal of Agricultural and Food Chemistry*, **40**, 1478-1487.
- Tsao, R.; Yang, R.; Xie, S.; Sockovie, E.; Khanizadeh, S. (2005), Which polyphenolic compounds contribute to the total antioxidant activities of apple? *Journal of Agricultural and Food Chemistry*, **53**, 4989-4995.
- Vander Der Luis, A. A.; Dekker, M.; Skrede, G.; Jongen, W. M. F. (2002), Bioactivity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *J. Agric. Food Chem.*, **50**, 7211-7219.
- Vanzani, P.; Rossetto, M.; Rigo, A.; Vrhovsek, U.; Mattivi, F.; D'Amato, E.; Scarpa, M. (2005), Major phytochemicals in apple cultivars: Contribution peroxyl radical trapping efficiency. *J. Agric. Food Chem.*, **53**, 3377-3382.
- Walker, G. M. (1998), *Yeast physiology and biotechnology*. John Wiley and Sons, Scotland, p. 350.
- Young, T. W. (1987) The biochemistry and physiology of yeast growth. In *Brewing microbiology*, PRIEST, F. G.; Campbell, I., pp. 15-46.

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