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Influence of Prefermentary Clarification on the Composition of Apple Musts

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The polyphenol contents and colors of cider apple juices were compared before (NCM, not clarified must) and after five clarification treatments: enzymatic depectinization by pectinases followed by (i) sedimentation (depectinized and decanted juice), (ii) tangential microfiltration (microfiltered juice) or (iii) fining using gelatin (gelatin-treated juice); (iv) enzymatic gelification of pectin by pectin methylesterase followed by natural keeving by a cider manufacturer (producer keeved juice), or (v) flotation (floated with nitrogen gas juice). The pressing of the apples led to the highly selective extraction of the flavan-3-ols with the lowest molecular weights: In the apples, the number average degree of polymerization of the flavanols was 14.7, and it dropped to 2.2 in the NCM. Keeving had the highest impact on the reduction of both flavanol content and number average degree of polymerization. The flavanol concentrations were decreased in the permeate by fining (30%) much more than by depectinization. The clarification step led to a further decrease of the number average degree of polymerization. Hydroxycinnamic acids were less affected by the extraction process (with extraction yields >50%) and not affected by clarification. The color evolved with all treatments: L^*, a^*, b^*, and chromaticity distance index measures indicated a reduction of orange-yellow saturation except after sedimentation.

KEYWORDS: Polyphenols; fining; color; cider; pectin

INTRODUCTION

French cider is a slightly alcoholic, fizzy, and sweet drink obtained by slow and partial fermentation of the juice of specific apple varieties (*1*). The final taste depends on the varieties blended, but it can be strongly affected by some specific steps of the process such as clarification. The prefermentary clarification is carried out to eliminate the cloud of raw must. The cloud composition has been studied (*2*). It contains colloidal pectin, cell wall fragments, and pulps. Two techniques are used to remove the cloud. The first method, commonly used to obtain clear apple juices, consists of hydrolyzing the pectic substances by an enzymatic mix containing pectin methylesterase (PME), endo-polygalacturonase, and pectin lyase (*3*). These pectolytic enzymes hydrolyze the soluble pectin and partially solubilize the pectic fraction from suspended particles. The must viscosity decreases, and the particles aggregate slowly. This depectinization is sometimes combined with fining with proteins, most often gelatin. As gelatin is positively charged at the pH of apple juice, the flocculation could be assisted by electrostatic interactions with the negative charges of remaining pectin in the particles. Gelatin, which is a proline-rich protein, also precipitates with the tannins in the juice (*4*). A decrease of all classes of phenolics occurs after gelatin−bentonite treatment in the clarification of Golden delicious juices (*5*). After depectinization and fining, the juices are usually filtered, by either tangential microfiltration or ultrafiltration. Weak sensory differences were reported between the apple juice permeates from ultrafiltration and microfiltration; however, these differences were significant for total solids and soluble solids (*6*). The second method of clarification is based on the gelling properties of the pectin after the action of PME. Specifically used in French cider production, this method called keeving (known as "defecation" in French) is derived from phenomenon that occur spontaneously when cider apple must is left to stand. At low temperatures, around 7 °C, PME slowly demethylates pectin. The newly formed sequences of free galacturonic acid combine with juice cations, mainly calcium, to form a weak gel (*7*). Cloud particles are entrapped in the gel, and the gel network rises to form the so-called "chapeau brun". This is buoyed up by small bubbles of CO_2 from the incipient fermentation, leaving a clear juice layer under the foam. For commercial cider production, this phenomenon is now controlled by the addition of PME from *Aspergillus niger* and calcium ions (*8*). This traditional keeving process is a static one, but in some French factories, a dynamic process is now used. After pressing of the apples, the must is incubated with highly purified PME overnight for pectin demethylation. Then, the must is pressurized with nitrogen in an enclosure. At the same time, a calcium chloride solution

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($CaCl_2$) is injected to ensure gelation (*9*). Flotation and keeving thus share the same mechanisms but differ by their kinetics of gelation and foam rise.

Differences in polyphenol pattern can be expected between the raw must and the clarified juices depending on the process used. Therefore, organoleptic characteristics such as color, astringency, and bitterness could be differentially affected. In apple juice, bitterness and astringency are due to the presence of condensed tannins and are strongly modulated by the degree of polymerization (DPn) of these compounds (*10*, *11*). Their amphiphilic properties and hydroxyl groups confer to polyphenols interaction properties with polysaccharides and proteins, which can both be actively involved in must clarification (*11*, *12*). Thus, fining could favor depletion in tannins, while keeving could favor oxidation and polyphenol−pectin interactions due to the long standing time of the juice in open vats.

This study aimed to collect data on the impact of various treatments of clarification on the composition of apple must, especially on the phenolic compounds. We used two varieties, Dous Oignon (bittersweet apple) and Avrolles (sharp apple), which present flavanols of low and high molecular weights, respectively.

MATERIALS AND METHODS

Reagents. Acetonitrile, high-performance liquid chromatography (HPLC) gradient grade, was purchased from Fisher Bioblock Scientific (Illkirch, France). (+)-Catechin, (−)-epicatechin, phloridzin, 5-caffeoyl quinic acid, and quercetin were obtained from Sigma Aldrich Co. (St. Louis, MO). Procyanidin B2 and *p*-coumaroyl-quinic acid were purified from a commercial cider by liquid−liquid extraction followed by reversed-phase HPLC at semipreparative scale and identified by electrospray ionization−mass spectrometry. Epicatechin benzylthioether was a gift from J.-M. Sauquet (INRA, UMR SPO, Montpellier, France). All others reagents were analytical grade. Ultrapure water was obtained using a Milli-Q water system (Millipore, Bedford, MA).

Plant Materials. For laboratory-scale experiments, 500 kg of apples (*Malus domestica*) was hand picked in October 2004 in the orchard (Gaël, Ille et vilaine, France). Varieties Dous Oignon and Avrolles were used in the proportions 2:1 (w/w). For keeving at industrial-scale treatment by the producer, 7000 kg of apples was mechanically picked in the same orchard and in the same proportion.

Processing of Apple Must and Juices. At the laboratory scale, the apples (500 kg) were dipped in sulfited water (sodium metabisulfite, 1 g/L) to decrease the microorganism load of the fruits and to avoid the onset of fermentation. Then, they were rinsed with running tap water, crushed, and pressed in a belt press (Voran, Austria; remaining time, <5 min). After they were pressed, dry ice (2 kg) was added to the must (400 L) to limit the oxidation of polyphenols. As such, the oxidation level at this small scale was similar to industrial products in a large tank. The producer used a tank press (Vaslin Bucher) to extract the must from 3.5 tons of apples per 2 h cycle.

Seven different products were prepared (**Figure 1**). Not clarified must (NCM): The raw must (30 L) was used as a control. Depectinized and decanted juice (DCJ): Rapidase C80L (115000 viscosimetric unit/mL, DSM Food, Seclin, France) was added to the must (15 mL/150 L); it was left to stand for 18 h at 10 °C, during which time the particles decanted. The clear supernatant was separated from the mud by racking. Microfiltered juice (MFJ) was obtained by microfiltration on a polyvinylidine difluoride membrane, 0.45 μm (Millipore Pellicon, Millipore Corp.), of an aliquot (35 L) of the depectinized and DCJ described above. Gelatin fined juice (GTJ): An aliquot (50 L) of the raw must was depectinized by Rapidase C80L added at 0.1 mL/L for 7 h at 10 °C. Fining was carried out by the addition of 0.35 g/L of gelatin (Rousselot 75 bloom; Angoulème, France), followed by an overnight incubation for decantation and separation of the clear supernatant by racking. Floated juice (FNJ): An aliquot of the raw must (80 L) was treated by PME (defecation enzyme P10; 100 UI/mL; Standa industrie, Caen, France, 0.5 mL/L, incubation for 18 h at 10 °C) for enzymatic demethylation of its pectins. This was followed by pressurization to 6 bar with N_2. The must was then passed through a nozzle with a narrowing inlet (4 to 1.5 mm) and a widening outlet (1.5−12 mm) to ensure sufficient flow speed and depressurization with the addition of calcium ions. The flow rates of the must and the $CaCl_2$ solution (4.35 M) were 108 L/h and 250 mL/h, respectively. The calcium dichloride final concentration in the must was 10 mM.

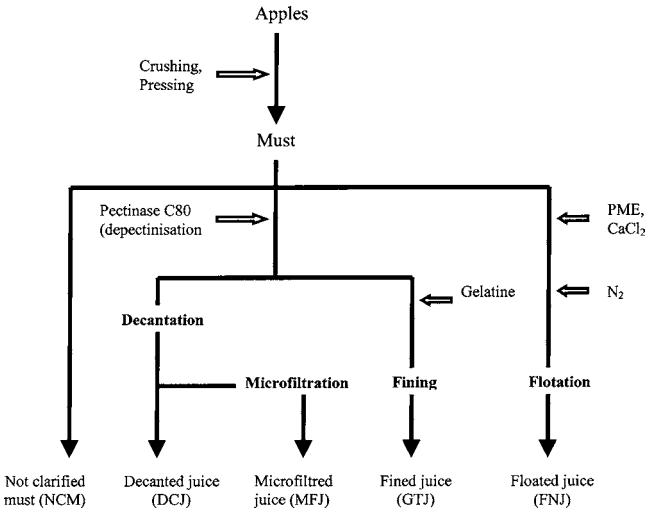

Figure 1. Preparation scheme of the five different juices with different clarification treatments in the laboratory (PME).

All of these clarification treatments were carried out in duplicate. Keeving was carried out at the production site by the cider producer (Cidrerie de Brocéliande, Gael), as it relies on the wild yeasts for fermentation initiation. PME (500 mL defecation enzyme P10) and $CaCl_2$ (2200 g) were added to 5000 L of juice. Samples of raw must (PRM, producer raw must) and clarified juice (PKJ, producer keeved juice) (25 mL) were collected before and after keeving.

Polyphenol Analysis. Polyphenols were quantified by reversed phase (RP)-HPLC after thiolysis according to ref *13*. Three aliquots of apple fruits were prepared as described in ref *13*, freeze-dried, and ball-milled prior to thioacidolysis (50 mg of powder). Aliquots of apple must (0.5 mL) were sampled in tubes spiked with NaF (final concentration 1 g/L) to avoid oxidation. The samples were freeze-dried prior to thioacidolysis. To measure the free monomeric flavanols, aliquots of freeze-dried samples not thiolyzed were extracted by sonication for 15 min in acidic methanol (1% acetic acid, 1.2 mL). After filtration (PTFE, 0.45 μm), the liquid phase was analyzed by RP-HPLC.

A Waters HPLC apparatus (Milford, MA) was used as follows: system 717 plus autosampler equipped with a cooling module set at 4 °C, a 600 E multisolvent system, a 996 photodiode array detector, and a Millenium 2010 Manager system. The column was a Purospher RP18 end-capped, 5 μm (Merck, Darmstadt, Germany). The solvent system was a gradient of solvent A (aqueous acetic acid, 25 mL/L) and solvent B (acetonitrile): initial, 3% B; 0−5 min, 9% B linear; 5−15 min, 16% B linear; 15−45, 50% B linear, followed by washing (90% B) and reconditioning of the column. The solvent flow was 1 mL/min. HPLC peaks were identified on chromatograms according to their retention times and their UV−visible spectra by comparaison with available standard compounds. Quantification is performed by reporting the measured integration area in the calibration equation of the corresponding standard. Phloretin and phloretin xyloglucoside were calculated as phloridzin equivalents. Total flavonols and total polyphenols were the sums of the related quantified compounds. The DPn of flavan-3-ols was calculated as the molar ratio of all of the flavan-3-ol units (thioether adducts plus terminal units) to (−)-epicatechin and (+)-catechin, corresponding to terminal units.

Determination of the Polyphenol Extraction Yield (Y%). Y% corresponded to the ratio of polyphenol concentrations in the raw juice (NCM) to their concentrations in the apples. To calculate this concentration, we assimilated an apple to a sponge (with as solid material: the cell wall material, skin, and pips) filled with a liquid

(juice), and the concentrations in this liquid (in g/L) were calculated by correcting the amounts (in g/kg fresh weight) as described below.

To estimate the volume of juice per kilogram of apples (V_j), we considered the liquid compartment as homogeneous. Moreover, we assumed that the theoretic water percentage into the juice of the apple was equal to the percentage of the extracted juice. We used the dry matter contents of apples and juices to determine the apple and raw juice contents. This last hypothesis allowed us to express the total mass of juice M_j:

$$M_j \times E_j\% = M_{ap} \times E_{ap}\% \quad (1)$$

with E_j and E_p, respectively, corresponding to the water content into the juice and apples. M_{ap} represented the total mass of the apple containing the juice. For 1 kg of apples, eq 1 corresponds to:

$$M_j = \frac{E_{ap}\%}{E_j\%} \quad (2)$$

The total volume of juice into 1 kg of apples (V_j) could be calculated with the density of the juice (d):

$$V_j = \frac{M_j}{d} \quad (3)$$

The eqs 2 and 3 allowed us to express the polyphenol contents of apples $[PP]_{ap}$ (g/kg) by [tot] (g/L), the theoretical polyphenol contents in juice into the apples.

$$[tot] = \frac{[PP]_{ap}}{V_j} = \frac{\left([PP]_{do} \times \frac{2}{3}\right) + \left([PP]_{av} \times \frac{1}{3}\right)}{V_j} \quad (4)$$

with $[PP]_{av}$ and $[PP]_{do}$, respectively, corresponding to the polyphenol contents of Avrolles and Dous Oignon.

The polyphenol extraction yield (Y%) corresponded to the ratio between the polyphenols of juice determinate with the juice $[PP]_j$ and [tot].

$$Y\% = \frac{[PP]_j \times 100}{[tot]} \quad (5)$$

Total Yeast Count. The total yeast count of the raw must was determined on yeast maltose agar medium using Spiral System sower (AES Laboratoires; Cincinnatti, OH). It spread 52 μL of juice on a conventional Petri plate and was appropriate for populations in the range of 500−500000 CFU/mL. Determinations were carried out in duplicate.

Nitrogen Quantification. Nitrogen was mineralized according to a modified Hach's method (*14*). Sulfuric acid (5 mL; 16 N) was added to 5 mL of centrifuged juice (10000*g*; 15 min). During the experiment, the temperature was maintained at 440 °C. Five minutes later, 10 mL of hydrogen peroxide (17 M) was added over 5 min. The reaction mixture was cooled at room temperature before neutralization by potassium hydroxide (32%) with methyl red (200 μL, 200 mg/L) as a pH indicator. Ultrapure water was added to the digest up to 250 mL. Nitrogen was consequently quantified using the modified Berthelot method (*15*) as described by ref *16*.

Color Determination. The L^*, a^*, and b^* coordinates (Commission Internationale de l'Eclairage, 1976) of the juices were determined after centrifugation (10000*g*, 15 min) using a Minolta CM-3600d (Japan) spectrophotometer, against pure water. The color difference, as chromaticity distance (DE) = $(\Delta a^2 + \Delta b^2 + \Delta L^2)^{0.5}$, was calculated (*17*) with NCM as a reference for the juices FNJ, GTJ, DCJ, and MFJ and PRM as the reference for PKJ.

Pectin Quantification. Pectin was precipitated by the addition of four volumes of 96% ethanol. The precipitate was washed four times with 70% ethanol and then dissolved in water. D-galacturonic acid was quantified by the metahydroxydiphenyl method (*18*) adapted for microplates. The sample (50 μL) was treated by sulfuric acid (16 N, 300 μL; 85 °C; 6 min). After it was cooled in a water bath, 15 μL of metahydroxydiphenyl solution (200 mg/L in 0.125 M NaOH) was added. After thorough mixing and 15 min of incubation at room temperature, the absorbance was read at 540 nm in a microplate spectrophotometer (Spectramax Plus; Molecular Devices Corp., Sunnyvale, CA).

Table 1. Nitrogen, Galacturonic Acid, and Total Biomass Determination in Apple Juices Obtained after Different Clarification Treatments[a]

clarification processes	nitrogen (mg/L)	D-galacturonic acid (mg/L)	total biomass (CFU/mL)
	laboratory		
NCM	66	15	10000
GTJ	69	ND	200
DCJ	59	2	4300
MFJ	54	ND	ND
FNJ	60	6	2690
	producer		
PRM	108	140	
PKJ	111	3	
CI(±)	*3*	*5*	

[a] CFU, colony forming unit; italic numbers, CIs expressed in units of each measure ($\alpha = 0.05$, degrees of freedom = 6); and ND, not detected.

Statistical Analysis. Confidence intervals (CI) were calculated for each series of replicated measurement using the sum of individual variances weighted by the reciprocal of individual degrees of freedom according to the Box and Hunter formula (*19*). Bonferonni's *t* test and a two-way analysis of variance on the polyphenols concentrations data were performed using Statgraphics plus Software v. 5 (Manugistics Inc., Rockville, MD) software. The first factor was the clarification type, and the second factor was the duplication of each treatment.

RESULTS AND DISCUSSION

Total Yeasts, Nitrogen, and Pectin. Standard musts obtained in our laboratory commonly have microbial loads inferior to 10^5 CFU/mL. The must obtained after washing with sulfited water and subsequent rinsing contained less than 10^4 CFU/mL, demonstrating the efficiency of this procedure to diminish the yeast load (**Table 1**). At this low level, microorganisms did not have any noticeable influence on the must composition during the clarification processes.

Nitrogen is one nutrient that regulates the yeast growth during fermentation (*8*, *20*). Enzymatic depectinization significantly reduced the nitrogen content. Tangential microfiltration (MFJ) was more efficient in this reduction than decantation (DCJ) or flotation (FNJ). However, the addition of gelatin in depectinized must (GTJ) increased the residual nitrogen in the clear juice up to the initial value (NCM). A small amount of added gelatin presumably did not react with tannins and stayed in solution. Surprisingly, keeving did not change the soluble nitrogen content of juices (PRM/PKJ). A reduction of 50−60% has been reported (*8*, *21*). The differences of nitrogen levels for all laboratory clarifications were significant, but they were very limited by comparison with the variations needed to induce changes in fermentation kinetics.

The pectin content in the industrial juice was nine times higher than in those from our laboratory due to the differences in the conditions of extraction. The diffusion of soluble pectin from the mash to the juice is slow (*22*); according to these authors, a significant amount of soluble pectin was released only after a 30 min maceration. All clarification methods removed the pectin from juice as it was expected.

Polyphenols. *Apples.* The polyphenol composition of the two cultivars was very different (**Table 2**). The total concentration was 54% higher in Dous Oignon than in Avrolles. The main differences concerned hydroxycinnamic acids and flavanols.

Table 2. Polyphenols Contents of Apples and Clarification Treatments[a]

	flavan-3-ols					dihydrochalcones			hydroxycinnamic acids		flavonols	
	(+)-catechin	(−)-epicatechin	B2	total flavanols	DPn	PLT	PLZ	XPL	5-CQA	pCoQA	QCE	total
						apples						
Avrolles	ND	ND	ND	2238	38	4.4	69	80	23	107	4.3	2576
CI(0.05)				*450*		*0.4*	*4.6*	*6.4*	*27*	*21*	*0.3*	*430*
Dous Oignon	129	365	306	2245	2.7	21	221	60	1096	27	8.2	3976
CI(0.05)	*5.7*	*6.0*	*6.0*	*230*		*6.4*	*56*	*8.5*	*19*	*2.8*	*7.8*	*280*
						laboratory musts						
NCM (Y%)	25 b (26)	63 c (23)	22 c (10)	307 a (12)	2.2 a	3.7 a (22)	66 a (35)	38 a (52)	411 b (50)	39 b (66)	ND	912 (22)
GTJ	29 a	71 b	34 b	210 c	1.5 e	3.7 a	62 a	40 a	453 a	46 a	ND	875
DCJ	26 b	66 c	29 b	263 b	2.0 b	3.3 a	64 a	37 a	415 b	43 a	ND	879
MFJ	29 a	71 b	34 b	274 b	1.8 d	4.3 a	66 a	40 a	446 a	44 a	ND	940
FNJ	30 a	76 a	38 a	297 a	1.9 c	4.3 a	66 a	40 a	455 a	46 a	ND	975
						producer musts						
PRM	15.0	22.8	ND	123	3.2	ND	30.5	18	175	44	ND	470
PKJ	15.4	22.9	ND	84	2.2	2.5	28.2	18	180	43	ND	423
CI(0.05)	*2.2*	*5.0*	*0.6*	*9.6*	*0.01*	*1.0*	*2.3*	*1.3*	*16*	*2.6*	*ND*	*25*

[a] Polyphenols concentrations (mg/kg for apple; mg/L for juice). Italic numbers, CIs ($\alpha = 0.05$, degrees of freedom = 2 for apple and $\alpha = 0.05$, degrees of freedom = 15, $n = 20$ for juice). Different letters a–e in the same column denote significantly different values. B2, procyanidin B2; XPL, phloretin xyloglucoside; PLZ, phloridzin; PLT, phloretin; pCoQA, *p*-coumaroylquinic acid; QCE, quercetin; and ND, not detected.

5-Caffeoylquinic acid (5-CQA) was much more abundant in Dous Oignon than in Avrolles apples. The two cultivars had the same total flavanols content, but Avrolles had neither monomers [(+)-catechin and (−)-epicatechin] nor dimer B2. All flavanols in Avrolles were highly polymerized; the DPn reached 38. It was only 2.7 in Dous Oignon apples. The Avrolles composition was in agreement with previously published data (*23*).

Raw Juices (NCM and PRM). The total polyphenol concentration in NCM was 1 g/L; that is, it was a juice with a low polyphenol content (*24*). Flavan-3-ols represented 30% of total polyphenols. The transfer from apples toward must could be represented as the ratio of concentration in the raw juice to the concentrations in the "theoretical juice" and symbolized by *Y*%. The procyanidins with the highest DPn and the dimer B2 were less extracted (*Y*% = 10%) than monomers (*Y*% > 20%). The DPn of flavanols in the must was in consequence lower than the expected value from the composition of the native apples due to the ability of the pomace to retain the most polymerized procyanidins (*25*−*27*). Hydroxycinnamic acids were the main polyphenols in the raw juices (45%). These compounds were not retained on the pomace. Moreover, 5-CQA, which is a substrate of polyphenoloxydase, was partially lost by oxidation, whereas pCoQA was not. Their extraction yields were 50 and 70%, respectively. Pressing inverted the proportions of flavanols to hydroxycinnamic acids. Dihydrochalcones were also relatively well-extracted (*Y*% > 20%). The PRM had a very low amount of native polyphenols, only half as much as NCM due to a higher oxidation level.

Clarified Juices. Clarification processes mainly affected both the content and the size of procyanidins. Almost a third of the flavanols were lost after fining (GTJ). Enzymatic depectinization followed by sedimentation (DCJ) and microfiltration (MFJ) reduced the flavanols to a lesser extent (loss of 14 and 10%, respectively). Keeving (PKJ) also removed 32% of flavanols. Flotation did not modify the flavanols content. This may be related to the kinetics of gel formation. Keeving is a slow process over several days, which favors interactions of polyphenols with pectin and cell wall material, explaining that the procyanidins could be retained in the "chapeau brun". Gel formation during flotation is almost instantaneous (<5 min). The rate of the adsorption of procyanidin on the cell wall material has been reported to be slow and needs at least 10 min (*25*). The DPn of procyanidins was significantly different depending on the clarifications treatments. It was less reduced in DCJ (2.0), whereas the tangential microfiltration and the fining dropped the DPn to 1.8 and 1.5, respectively. This was due to the specific adsorption of higher procyanidin on the retentate or on the gelatin lies (*28*, *29*). Flotation was an intermediary treatment, which conserved the relatively high concentration and DPn (1.9) of procyanidins.

The amounts of catechins, dimer B2, and hydroxycinnamic acids were significantly lower in NCM and DCJ than in other laboratory juices. This might be related to oxidation, as these juices kept suspended particles, which bind PPO (*28*), for a longer time. Thus, these oxidized the 5-CQA more extensively. The CQA quinone oxidized, in turn, the other polyphenols, resulting in a further loss of native phenolic compounds. This chain of reaction led to the formation of the colored molecules of apple juices and ciders (*29*). In the juices from producer, catechin and hydroxycinnamic acids were low and constant. The duration of the different steps of the process (milling, pressing, and juice transfer) was longer than in laboratory processing (several hours vs minutes), so that the oxidation was completed in the raw juice (PRM). Bitterness and astringency depend on the flavanol contents, their DPn, and the residual pectin (*30*). The manufacturer could use the adequate clarification techniques to adjust juices to a desired flavanols concentration.

Color. The parameter a^* ranged between −3 and 19, and the b^* was positive in all juices: The hue varied in the yellow or orange-yellow space (**Table 3**). The saturation in this hue varied in a wide range ($40 < b^* < 84$). The luminance (L^*) increased after clarification except in DCJ. The variations of color were consistent with the level of oxidation. Accumulation of oxidized products led to gain in yellow, orange-yellow saturation (NCM and DCJ). Removing these pigments by gelatin or keeving treatments reduced the saturation (GTJ and PKJ). The treatments, which limited the oxidation (MFJ and FNJ), led to an intermediate color. Ultrafiltration has been reported to preserve the initial color of the juice in comparison to different fining treatment (*5*). For instance, the absorbance of the depectinized ultrafiltered must was higher than the only ultra-

Table 3. Color Measures L^*, a^*, and b^* of Juices Obtained by Different Clarification Treatments[a]

clarification processes	a^*	b^*	L^*	DE*
		laboratory juices		
NCM	5.3	70	87	0
GTJ	−2.7	40	94	30
DCJ	11.2	76	82	8
MFJ	2.4	61	89	10
FNJ	2.7	60	89	10
		producer juices		
PRM	19	84	72	0
PKJ	−3.2	49	92	38
CI(±)	*1.4*	*5*	*1*	*2*

[a] L^*, a^*, b^*, and DE* = averages of two repetitions; and italic numbers, CIs ($\alpha = 0.05$, degrees of freedom = 6).

filtered one (*31*). The chromatic difference DE, which integrates the variation of the hue, the saturation, and the luminance, was affected by the process. This indicated that the clarification method was indeed a point on which the producers could act to modulate the organoleptic qualities of juices. The DE to a reference juice could be a parameter to evaluate the commercial quality. It should be more accurate than the current value of absorbance at 420 nm. Both of these criteria were related to the brown pigments content depending on the process used.

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