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Effect of Pulsed Electric Fields Treatment and Mash Size on Extraction and Composition of Apple Juices

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This study explored the effect of pulsed electric field (PEF) treatment (E = 450 V/cm; t_t = 10 ms; E < 3 kJ/kg) and apple mash size on juice yield, polyphenolic compounds, sugars, and malic acid. Juice yield increased significantly after PEF treatment of large mash (Y = 71.4%) and remained higher than the juice yield obtained for a control small mash (45.6%). The acid sweet balance was not altered by PEF. A correlation was established between the decrease of light absorbance (control: 1.43; treated: 1.10) and the decline of native polyphenols yield due to PEF treatment (control: 9.6%; treated: 5.9% for small mash). An enhanced oxidation of phenolic compounds in cells due to electroporation of the inner cell membrane and the adsorption of the oxidized products on the mash may explain both the lower light absorbance and the lower native polyphenol concentration.

KEYWORDS: Processing; energy consumption; *Malus domestica*; polyphenols; sugars; malic acid

INTRODUCTION

The consumer's demand for high-quality natural juice has increased. Nowadays, juices with high phenolic content are preferred because of their assumed healthy and beneficial effects (*1*). Now the objective of the industrial manufacturers is to commercialize minimally processed food with the characteristics of color, flavor, and nutritional value as close as possible to the fresh product.

Pressing is a crucial unit operation in apple juice extraction (*2*). In order to improve the extraction of juice, mash may be treated with thermal (*3*), electrothermal (*4*), enzymatic (*5*), or pulsed electric field (PEF) treatment techniques. PEF is a new technology that was introduced as a new alternative for improving extraction.

PEF is a nonthermal technology which preserves the organoleptic and nutritional quality of food. PEF processing was shown to be useful by maintaining a redness and ascorbic acid content higher than those of thermally processed tomato and orange−carrot juice, respectively (*6*−*8*). Pulsed electric field pretreatment of maize and rapeseed increased the phytosterol and polyphenol contents in oil (*9*, *10*). The extraction in water at 70 °C of polyphenolic compounds from grape peels treated by PEF was 2-fold higher than the control (*11*). At higher electric field (10−60 kV/cm), PEF can reduce the microbial load (*12*−*15*) and enzymatic activity (polyphenol oxidase, peroxydase, ...) (*16*) of apple and cranberry juices.

The application of pulsed electric field has shown many benefits for extraction of juices (*15*, *17*−*20*) and valuable molecules (*21*−*23*). For instance, clear apple juice was obtained at moderate temperature without any enzyme addition (*19*, *24*). Juice expression was facilitated when moderate field intensity (0.5−1 kV/cm) and short duration treatment (10^{-4} to 10^{-2} s) were applied (*25*−*27*). The juice yield increased when the untreated mash size decreased while the PEF treated mash improved the yield (*19*).

Many studies described the effect of high intensity PEF treatment to pasteurize juices on their chemical composition (*6*,*7*,*12*,*13*). Recently, some authors highlighted quality change due to high intensity PEF treatment prior to extraction. The anthocyanin content of the wine significantly increased when the PEF treatment was applied to the grape in the range 5−10 kV/cm (*28*). The quality attributes of apple juice did not significantly differ from controls after the PEF treatment of the mash (1−5 kV/cm) (*29*).

A few papers described the biochemical quality of apple juices after PEF treatment of mash and pressing (*18*, *19*). The only existing data focuses on the general qualitative characteristics of apple juice, such as turbidity, color, solid contents, etc. But there is a lack of information in the literature about the effect of the low intensity pulsed electric field (E < 0.5 kV/cm) treatment of mash on the apple juice biochemical composition. The aim of this study was to assess the effect of the size of apple mash on the juice yield and its polyphenol, sugars, and malic acid contents after a pulsed electric field treatment of low intensity (<0.5 kV/cm).

MATERIALS AND METHODS

Reagents. Acetonitrile, high-performance liquid chromatography (HPLC) gradient grade, was purchased from Fisher Bioblock Scientific (Illkirch, France). (+)-Catechin, (−)-epicatechin, phloridzin, and 5-caffeoyl quinic acid were obtained from Sigma Aldrich Co. (St. Louis, MO). Quercitrin was purchased from Extrasynthese (Genay, France). Procyanidin

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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 other reagents were analytical grade. Ultrapure water was obtained using a Milli-Q water system (Millipore, Bedford, MA).

Apples. Golden delicious apples from France were purchased in a local supermarket and stored at 4 °C until use. In order to reduce the effects related to the material heterogeneity, the samples came from the same lot of fruits that had approximately the same size and the same degree of ripeness. The moisture content of apples determined by desiccation at 104 °C for 24 h was within 84−87%.

Preparation of Apple Slices. For each experiment, two apples were cut into eight vertical segments, and each one was crosscut into two wedge-shaped pieces. Each piece was distributed on the different experimental units (control, PEF treatment, or apple analysis) using an allotment method to reduce the effect of the compositional variation between the different parts of an apple (*30*). Apple pieces which were not processed to juice were quickly weighed and then sprayed with a sodium fluoride solution (1 g/L) to avoid oxidation of phenolic compounds by polyphenol-oxidase. This lot was stored at −25 °C until analyzing the native polyphenols.

Juice Processing and PEF-Treatment. Juice expression experiments were carried out in duplicate using a mash of two different sizes prepared using the food cutting equipment CL 50 (Robot-Coupe S.N.C., France) with different graters which gave plane-parallel shaped pieces with a constant section. The average length was estimated from 50 pieces of mash. The small-sized mash (S1) was 2 mm × 2 mm × 30 mm (average volume = 120 mm^3), and the large-sized one (S2) was 7 mm × 3 mm × 30 mm (average volume = 630 mm^3).

Mechanical expression of juice was performed in a laboratory pressing cell. Apple mash (40 g) was introduced in the polypropylene cylindrical chamber of the pressing cell (20 mm thick, 56 mm in diameter). Then both sides were tightly closed with steel covers. At the first side of the chamber, a mobile electrode was attached to a rubber diaphragm. The rubber is impermeable and elastic, so it is able to deform by mechanical action. Expression of material is made by sending compressed gas (from a nitrogen bottle). At the second side, a stationary wire gauze electrode (stainless steel 316 L) with square holes of 0.2 mm × 0.2 mm was installed between a filter cloth (0141 AP, Sefar Fyltis, Lyon, France) and the bed of mash. Constant pressure (p = 3 bar) was applied to the mash for one hour. The PEF-treatment was applied during expression using a high voltage pulse generator, 1500 V−20 A (Service Electronique UTC, Compiegne, France), which provided monopolar pulses of near-rectangular shape. A detailed description of the compression chamber and PEF-generator was presented earlier (*31*). The PEF was applied to different samples after 15 s of expression. The 15 s initial expression removed the air from the space between slices and made the PEF treatment more efficient (*31*). Preliminary experiments have determined the exact distance between electrodes depending on the mash type after 15 s of pressing. All the experiments were carried out using 10 pulses delivered continuously at the electric field strengths E = 450 V/cm (U = 540 and 670 V for small and large mash, respectively). Total treatment time (t_t) was 10 ms, which gave a disintegration index (Z) of 0.75. Electric treatment parameters were fixed based on previous work in our research group (*32*, *33*).

Q (J/kg), the energy consumption of a PEF treatment, was calculated using eq 1.

$$Q = \frac{U \times I \times t_t \times 1000}{m_i} \quad (1)$$

where U (V) was the potential difference, I (A) was the current, t_t (s) was the treatment time, and m_i (g) was the initial mass of the apple mash sample before pressing.

Juice Yield. The weight of expressed juice was recorded during 3600 s at a 1 s step using the electronic scale PT 610 (Sartorius AG, Germany). The juice yield (Y) was calculated using the eq 2.

$$Y_{(t)} = \frac{m_{(t)}}{m_i} \times 100 \quad (2)$$

where $m_{(t)}$ (g) is the mass of the juice and t (s) is the pressing time.

The kinetics of juice expression were modeled according to an empirical approach (eq 3). This model was already applied to sugar beet slices under similar conditions of PEF treatment and pressing (*34*).

$$Y = \frac{t}{\frac{1}{v_0} + at} \quad (3)$$

v_0 (g/s) is the initial mass velocity of expressed juice. The value of $Y(t)$ at $t \rightarrow \infty$ approaches the constant value of $Y_\infty = 1/a$. This value characterizes a maximum yield of expressed juice at given conditions.

Extraction and Analysis of Polyphenols. Polyphenolic compounds were analyzed according to the methods described previously (*35*). Apple slices which were stored at −25 °C were freeze-dried (Lyovac GT 2, Leybold-Heraeus, Germany) and ball-crushed in closed vials to avoid hydration. Apple juice (0.5 mL) was mixed with sodium fluoride (200 μg) to avoid oxidation and then was freeze-dried. The samples were kept in a desiccator and in darkness between three and five days until analysis.

Aliquots (40 mg) of freeze-dried apple powders were extracted with acidic methanol (1% acetic acid; 1.2 mL), and the raw extract was analyzed by HPLC. Another aliquot was directly submitted to the thioacidolysis reaction in methanol. Juice samples were analyzed both by direct injection and after freeze-drying and thioacidolysis.

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 comparison with available standard compounds. Quantification is performed by reporting the measured integration area in the calibration equation of the corresponding standard. Phloretin xyloglucoside was 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.

Polyphenols yield (PY) was given as in eq 4.

$$PY = \frac{[\text{polyphenols}]_{\text{juice}} \times m}{[\text{polyphenols}]_{\text{apple}} \times m_i} \times 100 \quad (4)$$

[polyphenols] was the concentration of polyphenols in the recovered juice (mg/L) and in the apple (mg/kg). m and m_i are the masses of juice and mash, respectively.

Sugars and Malic Acid Contents. About 2 mL of recovered juice was kept at −25 °C until analysis. Glucose, fructose, and sucrose as well as L-malic acid were quantified by respective enzymatic−spectrophotometric methods using UV/vis spectrophotometer (Libra S32, Biochrom, France) test kits according to the recommendation of the manufacturer (R-Biopharm, Darmstadt, Germany).

Miscellaneous Measures. The total soluble solid content (° Brix) was measured using a refractometer AR 200 (Leica Microsystems Inc., Buffalo, New York). The pH-value and conductivity of apple juice were measured using a Consort K912 pH/conductimeter (Consort nv, Turnhout, Belgium). The light absorption of undiluted juices was measured using a CO75 colorimeter (WPA, Pocklington, U.K.) with an orange filter at 590 nm. This wavelength was chosen in order to reduce absorbance fluctuation due to turbidity of the juices.

Statistical Analysis. The analysis of variance (ANOVA) at $P < 0.05$ and the Least Significant Difference test were used to compare the mean values of juice yield observed at 3600 s; polyphenols, sugars, and malic acid contents; and miscellaneous measures.

A standard two factors design was used to estimate the effects of the mash size (S) as averaged particles volume and the field intensity of electric treatment (E) and the interaction between these quantitative factors at two levels. A general linear model including an analysis of variance was applied

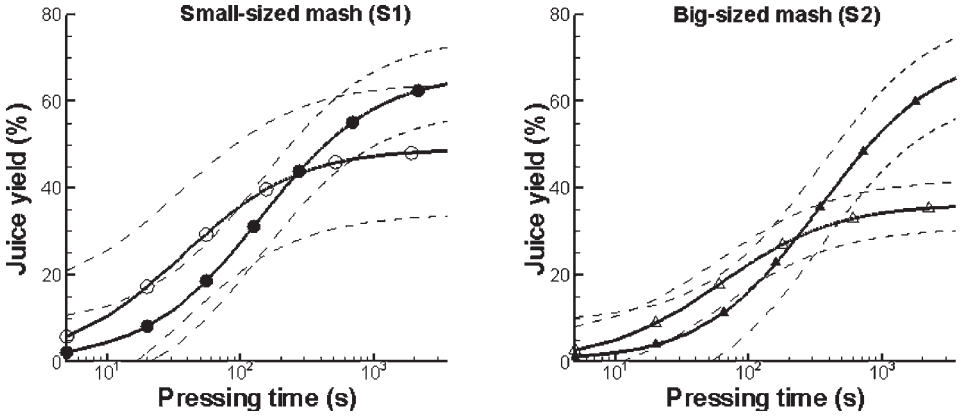

Figure 1. Juice yield (%) vs pressing time (s) from golden delicious apples. PEF-treatment was applied 15 s after mechanical expression that lasts 3600 s. Open and whole symbols represent control and treated extraction kinetics. Dashed lines are the 95% confidence interval for predicted values according to the mathematical model (eq 3).

Table 1. Apple Juice Extraction and Analytical Characteristics[a]

mash size	apple juice from small mash (S1)		apple juice from large mash (S2)	
E (V/cm)	0	450	0	450
v_0 (g/s)	2.84 ± 1.4 [a]	0.97 ± 1.4[a]	1.34 ± 1.4 [a]	0.71 ± 1.4[a]
$Y_{(3600)}$ (%)	52.3 ± 12.0 [ab]	68.5 ± 12.0 [bc]	38.5 ± 12.0 [a]	71.4 ± 12.0 [c]
Y_∞ (%)	50.5 ± 12.2 [ab]	67.7 ± 12.2 [bc]	37.5 ± 12.2 [a]	67.9 ± 12.2 [c]
conductivity (μS/cm)	1860 ± 111 [a]	1846 ± 111 [a]	1865 ± 111 [a]	1944 ± 111 [a]
energy consumption (J/kg)		2620 ± 220 [a]		2030 ± 220 [b]
total sugar (g/L)	133 ± 22.8 [a]	129 ± 22.8 [a]	121 ± 22.8 [a]	141 ± 22.8 [a]
fructose (g/L)	89 ± 13.1 [a]	85 ± 13.1 [a]	85 ± 13.1 [a]	95 ± 13.1 [a]
glucose (g/L)	25 ± 2.6 [a]	24 ± 2.6 [a]	24 ± 2.6 [a]	27 ± 2.6 [a]
sucrose (g/L)	18 ± 8.4 [a]	20 ± 8.4 [a]	12 ± 8.4 [a]	20 ± 8.4 [a]
Brix	14.0 ± 2.5 [a]	12.8 ± 2.5 [a]	13.9 ± 2.5 [a]	13.6 ± 2.5 [a]
pH	3.84 ± 0.11 [a]	3.22 ± 0.08 [b]	2.97 ± 0.08 [c]	3.15 ± 0.08 [b]
malic acid (g/L)	4.9 ± 1.7 [ab]	5.4 ± 1.7 [ab]	5.9 ± 1.7 [a]	5.4 ± 1.7 [b]
light absorption (590 nm)	1.43 ± 0.17 [a]	1.10 ± 0.17 [ab]	1.21 ± 0.17 [ab]	0.87 ± 0.17 [b]

[a] v_0 is the initial mass velocity of expressed juice; $Y_{(3600)}$ is the observed juice yield after 1 h of expression; Y_∞ is the estimated maximum juices yield for an infinite time; ±95% confidence limits; different letters for a parameter indicate significant differences at $\alpha = 0.05$.

to the orthogonal polynomial model defined by eq 5 to test the significance rate of the factors (*36*).

$$X = a_0 + a_1 S + a_2 E + a_{12} S \times E \quad (5)$$

in which a_0, a_1, a_2, and a_{12} are the constant and linear and interaction coefficients of the model, respectively.

Finally, a general linear model was applied to significant factors in order to identify their corresponding coefficients. This analysis was performed for juice yield and each polyphenol family.

All experiments were repeated twice. Statistical analysis of the data was conducted with Statgraphics plus 5.1 software (Statistical Graphics Corp. Rockville, MD).

RESULTS AND DISCUSSION

Juice Yield. The evolution of juice yield with time was adequately described by eq 3 for the different sizes of mash (**Figure 1**). The characteristic values of the juice extractions are given in **Table 1**. The observed yield (Y_{3600}) reached the maximum theoretical yield (Y_∞) for both control and PEF treated mashes after one hour of pressing. For controls, the increase of mash size leads to a decrease of the juice yield from 52.3% to 38.5%. The application of PEF at 450 V/cm and $n = 10$ square pulses of 1000 μs (energy input = 2.6 and 2.0 kJ/kg of small and large size mash, respectively) increased the juice yield, which reached 68.5% and 71.4% for S1 and S2, respectively ($P < 0.05$). These results about PEF concerning the juice yield were consistent with those of the literature (*18*, *19*, *37*, *38*). There is a significant interaction ($P = 0.0028$) between the size of the mash and the electric treatment (**Table 3**). The effect of PEF treatment was more pronounced for larger mash size (**Figure 2**). The electropermeabilization of cell plasmalemma led to the turgidity loss due to the leak of intracellular liquid. PEF efficiency increases with the number of intact cells in the mash.

A previous work (*29*) showed that apple mash treatment with exponential decay pulses at 5000 V/cm and $n = 30$ pulses of 400 μs (energy input = 27.0 kJ/kg) led to a maximum juice yield of 71.3%. In this study, square pulses with less than 3 kJ/kg of input energy were used to reach a similar yield (71.4%). The exponential decay pulse is made up of a long tail with a low intensity of electric field. The square pulse maintains a peak voltage for a longer time than the exponential decay pulse (*39*). It was reported that square wave pulses are more efficient than the exponential decay pulse for the microorganism inactivation (*40*). Moreover, monopolar pulses show noticeably higher disintegration efficiency for larger pulse duration (*31*). Another paper mentioned that the optimum particle size for electrotreatment was 3−6 mm (*41*).

Quality of the Apple Juice. Various analytical parameters were evaluated to compare the juice after one hour of pressing (**Table 1**). The average sugar concentration (131 g/L) was unchanged whatever the treatment of mash. No significant differences were found either in the levels of fructose, glucose, and sucrose or in the total soluble solid. This confirms the results already obtained: Treatment with PEF does not alter the composition of solutes analyzed in the juice (*29*). Because the extraction efficiency of juice was improved, the quantities of extracted sugars increased when the

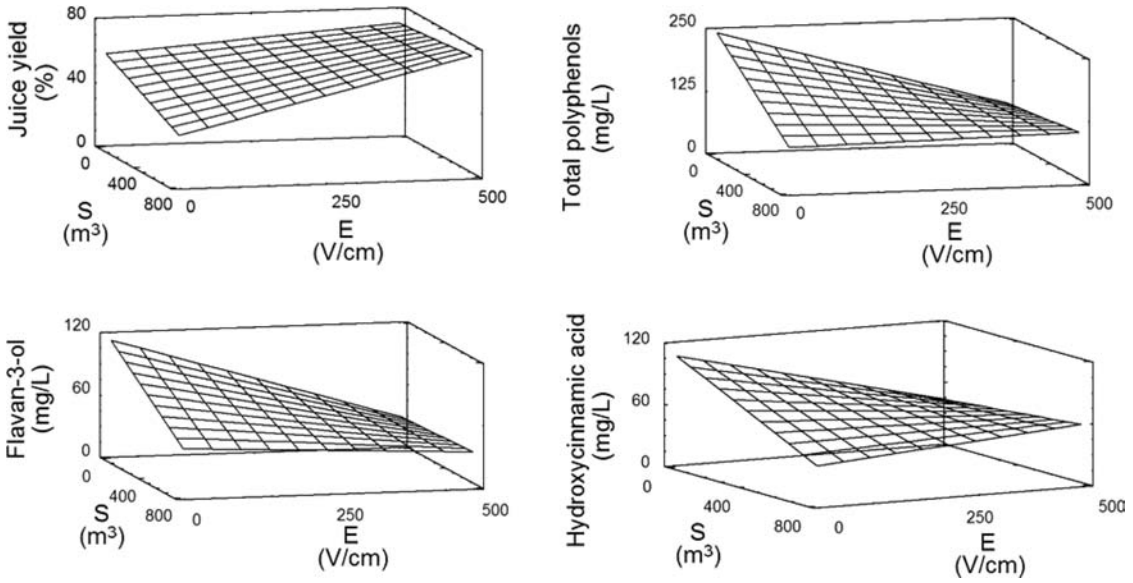

Figure 2. Effect of electric treatment (E; V/cm) and apple mash (S; mm^3) changes on the response surface of juice yield (%) and the total polyphenols, hydroxycinnamic acids, and flavan-3-ols concentrations (mg/L) of golden delicious apple juice.

Table 2. Effects of PEF and Mash Size on the Concentration and Extraction Yield of Phenolic Compounds in Apple and Raw Juices[a]

mash size		apple	apple juice from small mash (S1)				apple juice from large mash (S2)			
			0		450		0		450	
E		mean[i]	mean[ii]	PY	mean[ii]	PY	mean[ii]	PY	mean[ii]	PY
hydroxycinnamic acid	5CQA	142 ± *35.3*	78.4 ± *7.5*^{a}	27.1	38.8 ± *8.3*^{b}	17.7	43.5 ± *7.5*^{b}	11.0	44.8 ± *11.8*^{b}	21.2
	PCQ	7.5 ± *2.9*	19.1 ± *2.0*^{a}	126	13.9 ± *2.2*^{b}	120.1	10.6 ± *1.9*^{c}	51.0	11.3 ± *3.1*^{bc}	102.1
	total	150 ± *37.2*	97.5 ± *8.9*^{a}	32.1	52.7 ± *10.0*^{b}	22.8	54.1 ± *8.9*^{b}	13.0	56.1 ± *14.1*^{b}	25.2
flavan-3-ol	CAT	18.0 ± *2.7*	3.9 ± *0.7*^{a}	10.6	2.4 ± *0.8*^{b}	8.7	3.2 ± *0.7*^{ab}	6.4	4.1 ± *1.2*^{a}	15.1
	ECAT	22.7 ± *7.3*	15.7 ± *2.1*^{a}	34.0	6.7 ± *2.3*^{b}	19.1	10.4 ± *2.1*^{c}	16.5	9.8^b ± *3.3*^{c}	29.0
	B2	119 ± *27.5*	8.4 ± *1.3*^{a}	3.5	2.5 ± *1.7*^{b}	1.4	4.3 ± *1.3*^{b}	1.3	3.8 ± *2.0*^{b}	2.2
	PCD	810 ± *213*	83.9 ± *19.7*^{a}	5.1	29.3 ± *17.1*^{b}	2.3	46.5 ± *17.1*^{b}	2.1	22.7 ± *24.2*^{b}	1.9
	aDP	3.2 ± *0.45*	2.1 ± *0.8*		2.9 ± *0.8*		2.5 ± *0.7*		1.8 ± *1.0*	
	total	851 ± *222*	103.5 ± *20.0*^{a}	6.0	38.5 ± *17.3*^{b}	2.9	60.2 ± *17.3*^{b}	2.5	36.6 ± *24.5*^{b}	2.9
dihydrochalcon	PLZ	35.8 ± *16.8*	4.4 ± *0.6*^{a}	6.0	2.9 ± *0.7*^{b}	5.3	2.1 ± *0.6*^{b}	2.1	2.3 ± *1.0*^{b}	4.3
	XPLT	23.1 ± *2.7*	6.3 ± *0.8*^{a}	13.5	4 ± *0.9*^{a}	11.1	3.9 ± *0.8*^{b}	6.1	4.2 ± *1.3*^{b}	12.2
	total	58.8 ± *19.0*	10.7 ± *1.1*^{a}	8.9	6.9 ± *1.2*^{b}	7.5	6 ± *1.1*^{b}	3.7	6.5 ± *1.7*^{b}	7.4
flavonol	Q-Rha	38.5 ± *20.3*	3.5 ± *0.4*^{a}	4.4	2.1 ± *0.5*^{b}	3.6	2.4 ± *0.4*^{b}	2.3	2.4 ± *0.6*^{b}	4.3
total polyphenols		1098 ± *224*	215.2 ± *31.1*^{a}	9.6	100.2 ± *26.9*^{b}	5.9	122.7 ± *26.9*^{b}	4.0	101.7 ± *38.1*^{b}	6.2

[a] 5CQA, 5-caffeoylquinic acid; PCQ, *p*-coumaroylquinic acid; CAT, (+)-catechin; ECAT, (−)-epicatechin; B2, procyanidin B2; PCD, procyanidins; aDP, averaged degree of polymerization of flavan-3-ols; PLZ, phloridzin; XPLT, phloretin xyloglucoside; Q-Rha, quercitrin. Each compound was quantified in reference to pure standard. PY, extraction yield of polyphenolic compounds as described in eq 44. Subscripts a, b, c: different letters in one column indicate significant differences (least square difference, $P < 0.05$). CI, ±95% confidence limits; E, electric field intensity (V/cm). Mash size: S1 = 120 mm^3, S2 = 630 mm^3. (i) mg/kg of fresh apple; (ii) mg/L of apple juice.

mashes were pretreated by PEF. This should facilitate the processing of residual pomace before drying.

The pH value varied between 2.97 ± 0.08 and 3.84 ± 0.11. The difference between control and treated mash is random and may be related to the variability between samples. This is confirmed by the variation in the malic acid content, whose changes are not really significant (5.4 g/L). The absorption of light by the juices was not significantly affected by the size of the mashes. Instead, it decreased when the mashes were treated by PEF. This could be explained by the higher oxidation levels of polyphenols when mash is treated by PEF.

Polyphenolic Composition of Apple. Table 2 presents the phenolic composition of apple. Four families of phenolic compounds were present in golden delicious apple. Flavan-3-ol was the outweighed family (77.5% of total phenolics) with a low averaged degree of polymerization (aDP = 3.2). This result was in accordance with previous work (*42*). The second was the family of hydroxycinnamic acid (13.6%), which was mainly represented by 5-caffeoylquinic acid (5CQA). Dihydrochalcon (5.3%) and flavonol (3.5%) were minor families.

Effect of Mash Size and PEF Treatment on the Polyphenolic Composition of Juices. Table 2 also presents the concentration as well as the extraction yield (PY) of each phenolic compound. For both control and treated juices, the extraction yield of phenolic compounds was calculated from eq 4. The native phenolic compounds (total polyphenols) in the juices are extracted by pressing, but with low efficiency (PY < 10%).

Increasing the size of the mash without PEF caused a decrease in the extraction yield (PY) of different classes of polyphenols. For the control juices, 9.6% of the total apple polyphenols were extracted from the small mash (S1) and only 4.0% of polyphenols were extracted from the large mash (S2). The reason of this difference could be the increase of the damaged cells number by cutting when the mash size decreased. For the PEF treated juices, only 5.9% of the total polyphenols were extracted from the small

Table 3. Significance Test for Linear and Interaction Factors and Regression Coefficient of the Statistical Model for Juice Yield and Polyphenol Concentration

factors		juice yield	hydrocinamic acid	flavan-3-ol	aDP	dihydrochalcon	flavonol	total polyphenols
mash size (S)	*F* ratio	11.66	15.06	12.02	1.88	15.67	2.3	16.69
	Prob	0.0058	0.0026	0.0085	0.2122	0.0022	0.1641	0.0035
electric treatment (E)	*F* ratio	99.18	17.13	40.02	0.01	6.76	7.52	35.45
	Prob	0.0000	0.0016	0.0002	0.9251	0.0247	0.0228	0.0003
interaction	*F* ratio	14.63	20.5	10.47	2.79	11.2	7.9	17.95
	prob	0.0028	0.0009	0.012	0.1391	0.0065	0.0203	0.0029
A0	value	6.1×10^{1}	1.1×10^{2}	1.1×10^{2}		1.2×10^{1}	3.7	2.4×10^{2}
	standard error	2.4	5.2	1.1×10^{1}		6.3×10^{-1}	2.2×10^{-1}	1.7×10^{1}
a1	value	-3.5×10^{-2}	-8.5×10^{-2}	-8.3×10^{-2}		-9.2×10^{-3}	-2.0×10^{-3}	-1.9×10^{-1}
	standard error	5.3×10^{-3}	1.1×10^{-2}	2.3×10^{-2}		1.4×10^{-3}	5.1×10^{-4}	3.6×10^{-2}
a2	value	2.1×10^{-2}	-1.2×10^{-1}	-1.7×10^{-1}		-1.1×10^{-2}	-3.7×10^{-3}	-3.2×10^{-1}
	standard error	8.1×10^{-3}	1.7×10^{-2}	3.3×10^{-2}		2.1×10^{-3}	8.0×10^{-4}	5.1×10^{-2}
a12	value	8.3×10^{-5}	2.0×10^{-4}	1.8×10^{-4}		1.9×10^{-5}	5.9×10^{-6}	4.3×10^{-4}
	standard error	2.0×10^{-5}	4.3×10^{-5}	7.7×10^{-5}		5.3×10^{-6}	1.9×10^{-6}	1.2×10^{-4}
R^2		92.9	86.6	80.3		81.1	73.8	87.6

mash (S1). This was not significantly different from the total polyphenol yield obtained from the large mash (6.2%).

The difference in polyphenol yield between the mashes (S1 and S2) was closely related to their juice extraction. Juice yield increased by 23% (S1) and 33% (S2) for treated mash. PEF treatment of S1 and S2 mash caused 46.5% and 8.3% of total polyphenol concentration loss, respectively. These changes had a direct effect on the polyphenolic yield in raw juices (see eq 4). In this study, a complex behavior of polyphenol extraction was observed due to mash size change and electric treatment.

To estimate the significance of the effects and the interaction of the two factors (mash size and electric field strength) on the concentration of polyphenols, a polynomial regression was carried out according to eq 5. The significance rate of the factors and the model coefficients are shown in **Table 3**. The linear effects and the interaction were generally significant ($P < 0.05$) except in the cases of flavan-3-ols' aDP for all the factors and in the case of flavonols for only the size factor. Total polyphenols and each polyphenol family concentration were then described by a polynomial model, in which only significant effects were included.

Increasing mash size (S) had a negative effect ($a_1 < 0$) on the native polyphenols content in the juices, especially for hydroxycinnamic acids and flavan-3-ol families (**Figure 2**). Increasing mash size reduced the number of damaged cells, and so a lot of highly polymerized procyanidins (PCD) were not released. Decreasing the oxidative area by using a large mash prevents the loss of native hydroxycinnamic acids by oxidation. Electric treatment (E) had a negative effect on the native polyphenol content of each family ($a_2 < 0$). The interaction between mash size and electric treatment for all polyphenolic families was positive ($a_{12} > 0$).

Oxidation was privileged when raw juices were left without any precautions (addition of ascorbic acid, sodium fluoride, carbon dioxide, ...) for one hour during mechanical pressing. 5CQA, which is the main substrate of polyphenoloxydase (PPO), was partially lost by oxidation (*43*). This observation was more pronounced for the small mash. On the contrary, PCQ, which is not a preferential substrate of PPO activity, was not decreased as much as 5CQA in PEF treated samples (*44*). This can explain the higher extraction yield (five to 6-fold) for this second compound. The CQA quinone oxidized, in turn, the other polyphenols, resulting in a further loss of native phenolic compounds, especially the catechins (*45*). This chain of reaction leads to the formation of the colored molecules of apple juices(*46*).

In this study, a correlation ($r^2 = 0.74$) could be established between the decrease of light absorption (**Table 1**) and the decrease of native polyphenol concentration due to PEF treatment. Higher absorbance and higher native polyphenol concentration in the juices were observed for control small mash. Treating small mash caused an important decrease of both light absorbance and native polyphenol concentration in juices.

A recent study showed insignificant changes in native polyphenol content when apple mash was treated with PEF (*29*). The enzymatic browning reaction was limited by mixing apple mash with ascorbic acid. In the present work, PPO activity was not restrained. PEF applied to small size mash (S1) caused a significant decrease in native phenolic compounds. For large size mash (S2), no difference in the native polyphenols content of juices was observed between the control and the treated mash. A hypothesis can be propounded considering the presented results: PEF treatment provided enough energy to permeabilize the inner membrane of apple cells. It promoted the contact between PPO in plastids and native polyphenols from the vacuole (*47*). The oxidized phenolic compounds remained caught in the cell by reaction with proteins and cell wall polysaccharides (*48*, *49*). The phenolic concentration and the absorbance of juice were low. In control samples, more cells remained intact and more native phenolics compounds were released. The oxidation occurred mainly in the juice. This phenomenon was more distinguished for small mash sizes, which contain a bigger number of damaged cells than large mash.

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