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Effect of maturity on the phenolic compositions of pear juice and cell wall effects on procyanidins transfer

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A B S T R A C T

Perry pear polyphenols were characterized in fruit, juice and pomace for two cultivars and at two maturity stage. Cell walls were characterized only in fruits and pomaces. The phenolic contents and compositions of fruits did not change during overripening. However, their extraction to juice was modified. Juices of ripe fruits contained 38% (Plant de Blanc) and 28% (De Cloche) of initial polyphenols, whereas overripe pear juices contained only 26% and 15% respectively. Thus, procyanidins had more affinity for cell walls after overripening. Pear cell walls from De Cloche cultivar lost arabinose and galactose from pectic side chains during overripening promoting non-covalent interactions between procyanidins and cell walls.

Keywords:

Pyrus communis L.

Juice

Condensed tannins

Pectic side chains

Overripening

1. Introduction

Perry pears are used in the west Midlands (UK) and in the regions of Bretagne and Normandie in France for the production of perry, a fermented fizzy beverage close to cider. They are specific cultivars characterized by their high content in polyphenols. Phenolic compounds have an important role in food industry. They contribute to flavor and color characteristics of fruit juices and wines (Spanos & Wrolstad, 1990). Polyphenols are divided into several classes, i.e. phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), flavonoids (flavonols, flavones, flavanols, flavanones, isoflavones, proanthocyanidins), stilbenes, and lignans (Collin & Cruzet, 2011). Procyanidins are the major polyphenols class in pear (dessert and perry pear cultivars) (Guyot, Marnet, Le Bourvellec, & Drilleau, 2002; Le Bourvellec et al., 2013; Renard, 2005) where they contribute to astringency of perry. In perry processing, the use of fruit at the overripe stage is the normal practice to decrease the astringency and to increase colloidal

stability during storage. Further, preliminary studies have shown that the concentration of procyanidins in the juices varied with the fruit maturity at pressing (Alberti et al., 2016; Guyot, Marnet, Sanoner, & Drilleau, 2003; Spanos & Wrolstad, 1990). Low quantities of procyanidins are found in juices compared with the initial quantities measured in fruit (Guyot et al., 2002). Therefore, factors such as maturity that can induce change in the cell wall composition and structure can modulate the extractability of polyphenols and the organoleptic properties of perry.

The association between procyanidins and cell wall polysaccharides, especially pectins, can influence the transfer of procyanidins from fruit to juice (Guyot et al., 2003; Taira, Ono, & Matsumoto, 1997). The binding of condensed tannins to cell walls depends on one hand on the molecular characteristics of procyanidins, mainly their degree of polymerization but also the pyranic ring stereochemistry of the flavan-3-ols, and on the other hand on cell wall structure and composition. Associations differ depending on neutral sugar composition and the structure of pectic fractions. Arabinogalactan had the lowest affinity for procyanidins (Watrelet, Le Bourvellec, Imbert, & Renard, 2014). Watrelet, Le Bourvellec, Imbert, and Renard (2013) showed also that the strongest association was obtained with highly polymerized procyanidins and highly methylated pectins. The adsorption mechanism involves the establishment of non-covalent interactions, hydrogen bonds and hydrophobic interactions (Le Bourvellec, Bouchet, & Renard, 2005;

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Le Bourvellec, Guyot, & Renard, 2004; Lea & Arnold, 1978).

In general, cell wall modifications in ripening fruit involve hydrolysis of neutral sugars from side chains of pectin, depolymerization and increased solubilization of pectins (Brummell, 2006). Pear cell walls have a large heterogeneity compared to other fruits due to the presence of parenchyma and stone cells. Their cell walls have different polysaccharide compositions and evolve differently during ripening. In Spanish pears, changes were predominant in parenchyma cells and were accompanied by a decrease in pectic polysaccharides (arabinose, uronic acids) and an increase in their solubility (Martin-Cabrejas, Waldron, Selvendran, Parker, & Moates, 1994). Those changes can affect differently the affinity of cell walls for procyanidins thus the organoleptic properties of pear juice.

The aim of this work was to determine the polyphenol and cell wall compositions of two perry pear cultivars at two different ripeness stages and to investigate the impact of maturity on polyphenol transfers in pear juices.

2. Materials and methods

2.1. Chemicals and standards

Acetonitrile, dichloromethane and acetone were obtained from VWR (Leuven, Belgium). Methanol, acetic acid, hydrochloric acid, sodium tetraborate, sodium hydroxide and sodium hydrogen carbonate were from Merck (Darmstadt, Germany). Sodium borohydride, *N*-methyl imidazole, potassium hydroxide, 3-phenylphenol, lignin, acetic anhydride, acetyl bromide, perchloric acid, chlorogenic acid, (+)-catechin, (–)-epicatechin, quercetin, isorhamnetin, hydroxylamine hydrochloride and benzyl mercaptan were provided by Sigma Aldrich (Steinheim, Germany). Sugar standards were from Fluka-Biochemica (Sigma Aldrich, Steinheim, Germany).

2.2. Plant material

Perry pear cultivars, “Plant de Blanc” and “De Cloche” were harvested in the experimental orchard of Institut Français des Productions Cidricoles (IFPC, Sées, France) on September 16 and October 5, 2015, respectively. They were stored at 1 °C until reaching the desired stage of maturity. Plant de Blanc pears were stored until September 22, De Cloche pears until October 8, 2015 to reach the ripe stage. The overripe stage was reached on October 21 for Plant de Blanc fruits, and on December 11, 2015 for De Cloche fruits.

2.3. Preparation of samples

Juice preparation from ripe and overripe pears was carried out by IFPC (Le Rheu, France).

The sugars/acids ratio serves as an industrial indicator because the balance between sugars and organic acids influences the taste of some beverages (Alberti et al., 2016; Pal & Sampath Kumar, 1995). It was used in our case as a ripening index. Sugars and acids were determined as described by Le Bourvellec et al. (2015) and their ratio was then calculated. Overripening was marked by an increase of sugars/acids ratio especially for Plant De Blanc (Data not shown). Pears were washed and then crushed under CO₂ atmosphere and with added sodium fluoride to prevent oxidation. Pears were pressed on a small laboratory high-pressure press (model HP5, 5 L, Hafico, Fischer and Co., Dusseldorf, Germany) for 15 min. The hydraulic pressure was set at 4×10^7 Pa, corresponding to an effective pressure of 234 N/m² on the plant material. For each cultivar and maturity 3×1 mL of juice were collected after pressing, freeze-dried and stored at –20 °C until analysis. Pomace samples, collected under CO₂ atmosphere, were divided in two lots

for polyphenols and cell walls analysis. Fresh fruits were cored and freeze dried in our laboratory (SQPOV, INRA, Avignon) and stored at –20 °C.

2.4. Analysis of phenolic compounds

Polyphenols were determined in fruit, juice and pomace at two maturity stages by high performance liquid chromatography (HPLC/Diode Array Detection) with or without thioacidolysis as described by Guyot, Marnet, and Drilleau (2001) and Le Bourvellec et al. (2011).

2.5. Cell walls preparation and characterization

Alcohol Insoluble Solids (AIS) from fruit and pomace at the two maturity stages were prepared according to Renard (2005); neutral sugars, galacturonic acid and methanol were determined as described in Renard and Ginies (2009).

Lignin was analysed in AIS as described by Syros, Yupsanis, Zafiriadis, and Economou (2004) with some modifications. Samples (10–15 mg) were digested in 1 mL of buffer (250 mL/L acetyl bromide, 27 mL/L perchloric acid and 723 mL/L acetic acid). Then, samples were incubated for 30 min at 70 °C. 10 µL for each sample were added to 570 µL of a solution of [172.4 mL/L of NaOH 2 mol/L and 827.6 mL/L of acetic acid] and 20 µL of 7.5 mol/L hydroxylamine hydrochloride to stop the reaction. The volume was corrected to 2 mL with acetic acid and the absorbance was read at 280 nm using a spectrophotometer V-730 (Jasco, Tokyo, Japan).

2.5.1. Statistics

Results are presented as mean values, and the reproducibility of the obtained results was expressed by pooled standard deviation (Pooled SD) (Box, Hunter, & Hunter, 1978). One-way analysis of variance (ANOVA) was performed on perry pear fruit, juice and pomace polyphenol compositions concerning ripeness using the Excel Stat package of Microsoft Excel.

3. Results and discussion

3.1. Phenolic composition of perry pears, juice and pomace

The phenolic composition of fruits, juices and pomaces and their changes during overripening for Plant De Blanc and De Cloche cultivars are summarized in Table 1.

Procyanidins were the predominant phenolic class, with between 6 and 8 g/kg FW, higher than reported by Le Bourvellec et al. (2013) in William pears (dessert pears) and by Renard (2005) in Gieser Wildeman (cooking pears). Perry pear procyanidins had a high degree of polymerization (DP_n = 20 for De Cloche and DP_n = 33 for Plant de Blanc fruits), as reported by Guyot et al. (2002). The only flavan-3-ols monomer detected was (–)-epicatechin as observed by Le Bourvellec et al. (2013). Hydroxycinnamic acids were represented by 5-caffeoylquinic acid, which has been reported as the main hydroxycinnamic acid in pears (Galvis Sánchez, Gil-Izquierdo, & Gil, 2003; Le Bourvellec et al., 2013; Li et al., 2012; Yim & Nam, 2016). Flavonols, located in the peel, mainly quercetin and isorhamnetin glucosides, were present in low quantities <40 mg/kg FW for both cultivars. No significant changes in polyphenol concentrations, neither procyanidins nor flavonols, were detected during fruit overripening, irrespective of the cultivar.

Juice concentrations decreased almost by half for De Cloche and slightly less for Plant De Blanc after overripening. This decrease was mainly due to decrease in procyanidins, as observed by Guyot et al. (2002). Decrease of the degree of polymerization was observed for

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Table 1

Polyphenol compositions and concentrations in the fruits, pomaces (mg/kg of fresh weight FW) and in the juices (mg/L of fresh weight FW) of two perry pear cultivars.

Cultivars	Tissue	Maturity	EC	PCA	DPn	CQA	Flavonols	Total
Plant de Blanc	Fruit	Ripe	30	7390	33	796	29	8240
Plant de Blanc	Fruit	Overripe	26	8020	37	926	31	9000
<i>Pooled SD</i>			1.2	352	1.0	24.68	5.7	378
<i>Significance</i>			*	ns	*	*	ns	ns
Plant de Blanc	Juice	Ripe	7	4060	44	624	2	4690
Plant de Blanc	Juice	Overripe	n.d.	2780	38	573	2	3360
<i>Pooled SD</i>			0.1	67.1	1.1	13.7	0.5	80.1
<i>Significance</i>			***	***	***	*	ns	***
Plant de Blanc	Pomace	Ripe	109	14,000	28	1480	145	15 600
Plant de Blanc	Pomace	Overripe	110	16,300	26	1610	136	18,100
<i>Pooled SD</i>			6.5	302	1.0	82.0	15.2	392
<i>Significance</i>			ns	**	ns	ns	ns	*
De Cloche	Fruit	Ripe	60	6660	20	318	35	7080
De Cloche	Fruit	Overripe	40	5880	21	289	31	6240
<i>Pooled SD</i>			4.0	429	1.2	18.2	7.8	456
<i>Significance</i>			*	ns	ns	ns	ns	ns
De Cloche	Juice	Ripe	21	2660	20	260	4	2940
De Cloche	Juice	Overripe	16	1450	14	216	3	1690
<i>Pooled SD</i>			0.4	167	0.4	25.8	0.2	192
<i>Significance</i>			***	***	***	*	ns	***
De Cloche	Pomace	Ripe	93	10,700	13	500	66	11,400
De Cloche	Pomace	Overripe	93	12,700	16	387	48	13,300
<i>Pooled SD</i>			4.4	200	0.3	30.3	5.0	175
<i>Significance</i>			ns	**	**	*	*	**

EC: (–)–epicatechin, PCA: procyanidins, DPn: average degree of polymerization of procyanidins, CQA: 5'-caffeoylquinic acid, Flavonols: sum of flavonols, Total: sum of polyphenols, n.d.: not detected, Pooled SD: pooled standard deviation, ns: not significant (P > 0.05), ***: P < 0.0001, **: P < 0.01, *: P < 0.1.

both cultivars. The reduction in 5-caffeoylquinic acid was less marked than for procyanidins. Flavonol concentrations in the juice for both cultivars were about 10 times lower than in the fruit, but were not affected by overripening.

In parallel, some polyphenols were retained in overripe pear pomace. Procyanidins, with lower DP than in fruit and juice, increased to >10 g/kg FW. The sum of flavonols was higher than in fruit and juice, between 48 mg/kg FW and 145 mg/kg FW for overripe De Cloche and ripe Plant De Blanc respectively, linked to the enrichment of pomace in peel and their low extractability. 5-caffeoylquinic acid was present in higher amounts than fruit and juice (387–1610 mg/kg FW for overripe De Cloche pomace and overripe Plant De Blanc pomace respectively).

3.2. Composition of alcohol insoluble solids (AIS)

Yields, carbohydrate compositions of AIS from fruits and pomaces at the two maturity stages are presented in Table 2.

Fruit AIS represented 43–48 g/kg fresh weight (FW) in ripe and overripe pears. Yields found here were relatively high than those found in other pear cultivars because cell walls were extracted from whole fruits but were comparable to those reported by Raffo,

Ponce, Sozzi, Vicente, and Stortz (2011) for Bartlett pears. The AIS yields obtained by Le Bourvellec et al. (2013), Martin-Cabrejas, Waldron, & Selvendran (1994), Jermyn and Isherwood (1956) from the flesh of William, Blanquette and Conference pears were close to 30 g/kg and were relatively higher than yields obtained by Renard (2005) for Gieser Wildemann pears. The main sugars in the AIS of both pears cultivars at both maturity stages were glucose (192–284 mg/g), xylose (140–176 mg/g) and galacturonic acid (108–118 mg/g). Arabinose and galactose were found in lower amounts (34–79 mg/g and 36–70 mg/g respectively), whereas rhamnose, fucose and mannose were minor compounds. The degree of methylation of pectin was >50. The composition was close to that reported by Martin-Cabrejas, Waldron, Selvendran, Parker, et al. (1994) on Banquette pears and by Renard (2005) on Gieser Wildemann pears. During AIS preparation, procyanidins bind spontaneously to cell walls by H-bonds and hydrophobic interactions (Renard, Baron, Guyot, & Drilleau, 2001). There were high concentrations of retained procyanidins (20–43 mg/g) with high degree of polymerization varied between 41 and 65. Pear cell walls contained also high quantities of lignin (212–232 mg/g). The high concentrations of glucose, xylose and lignin reflected the presence of lignified cell walls rich in xylans and cellulose, typical of

Table 2

Yields (mg/g fresh weight) and carbohydrate compositions (mg/g dry weight) of alcohol soluble solids from fruits and pomaces for Plant De Blanc and De Cloche at two maturity stages.

Cultivar	Material	Maturity	Yields	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	MeOH (DM)	Lig	PCA (DP)
Plant de Blanc	Fruit	Ripe	46	5	2	57	161	7	25	284	108	11 (56)	212	20 (63)
		Overripe	43	6	2	56	173	7	24	194	118	11 (51)	232	43 (65)
	Pomace	Ripe	220	7	2	61	133	8	28	287	124	12 (54)	170	14 (31)
		Overripe	190	6	3	64	154	8	26	177	104	13 (65)	202	21 (39)
De Cloche	Fruit	Ripe	43	5	3	79	140	7	38	263	113	12 (57)	211	30 (48)
		Overripe	48	6	2	34	176	7	19	192	111	11 (55)	226	40 (41)
	Pomace	Ripe	209	7	2	70	161	6	35	233	120	13 (60)	167	14 (26)
		Overripe	162	7	2	36	172	7	19	178	120	13 (60)	237	27 (40)
<i>Pooled SD</i>			3.5	0.5	0.1	2.2	7.4	0.3	1.4	9.7	5.8	0.5 (3.5)	7.1	0.6 (1.4)

Rha: rhamnose, Fuc: fucose, Ara: arabinose, Xyl: xylose, Man: mannose, Gal: galactose, Glc: glucose, Gal A: galacturonic acid, MeOH: methanol, DM: degree of methylation, Lig: lignin, PCA: procyanidins, DP: degree of polymerization of procyanidins, Pooled SD: pooled standard deviation.

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stone cells (Ben-Arie, Kislev, & Frenkel, 1979; Martin-Cabrejas, Waldron, Selvendran, Parker et al., 1994). Le Bourvellec et al. (2013) detected more galacturonic acid and less xylose in the AIS from the flesh of William pears.

The AIS contents of the remaining pomace varied from 162 g/kg FW to 220 g/kg FW and were 5 times higher than in pears, as observed for plum pomace by Kosmala et al. (2013). Accumulation of peel and seeds in the pomace further increased the AIS, as both contain more AIS than parenchyma, as reported e.g. for apples during processing into applesauce (Le Bourvellec et al., 2011). The composition of pomace AIS was close to that of fruit, with slightly higher galacturonic acid and lower lignin and procyanidins.

Overripening was marked by a clear loss (about 50%) of arabinose and galactose for De Cloche cultivar. This was in agreement with Ahmed and Labavitch (1980) on Bartlett pears. Rhamnose, fucose and mannose did not vary during overripening, in accordance with previous work (Ahmed & Labavitch, 1980; Martin-Cabrejas, Waldron, Selvendran, Parker et al., 1994). For both perry pear cultivars, a significant decrease of the total glucose amount was observed due to solubilization during ripening. An increase of xylose content was observed mainly for De Cloche Cultivar. Lignin was found in higher amounts with ripening, consistent with the formation of lignified cell walls (Martin-Cabrejas, Waldron, Selvendran, Parker et al., 1994). For pomace, changes in the sugar compositions were not drastic and similar to those noted on fruit AIS. The amounts of bound procyanidins increased from ripe to overripe for both fruits and pomaces. Only highly polymerized procyanidins (DP > 40 for De Cloche and DP > 60 for Plant De Blanc) were bound essentially in fruit AIS.

3.3. Impact of cell walls on procyanidins transfer in juice

The lower quantities of phenolic compounds in juices compared to fruits could be explained by the retention of phenolic compounds by cell wall polysaccharides (Alberti et al., 2016; Le Bourvellec, Le Quere, & Renard, 2007), considering that mostly procyanidins remained in the pomace. Procyanidins can be selectively adsorbed by cell wall polysaccharides. This has been reported for apple and grape (Bindon, Smith, & Kennedy, 2010; Renard et al., 2001; Ruiz-García, Smith, & Bindon, 2014) where procyanidins complexation with cell walls was found to be spontaneous and rapid (Renard et al., 2001).

Three phenomena can explain increased binding of procyanidins to cell walls from overripe pears. First, the loss of arabinose and galactose indicated a loss of pectin side chains. Watrelot et al. (2014) show that these pectin side chains have low affinity for procyanidins, hence their elimination and better access to pectin's rhamnogalacturonic backbone can contribute to increase affinity. Second, during overripening solubilization and loss of polysaccharides from cell wall might increase its porosity (Bindon, Madani, Pendleton, Smith, & Kennedy, 2014) so that more surface become available for binding of procyanidins. Thirdly, overripening seemed to promote interaction between pear procyanidins and cell walls. In over ripened fruit some membrane breakdown might start to occur, so that procyanidins might come in contact with cell walls prior to pressing.

4. Conclusion

Overripening of perry pears was demonstrated to decrease the extraction of procyanidins to the juice in spite of constant concentration in the fruit. This justifies the use of overripe pears by the perry producers. The same mechanisms seem to be present in pears as in grape (for wine production) or apple (for cider production). This indicates a generic mechanism that can be used to manipulate

bitterness and astringency of fruit juices and might be an alternative to fining.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.lwt.2016.09.009>.

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