Phenolic and polysaccharidic composition of applesauce is close to that of apple flesh

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1. Abstract.

Apple fruit is well known for its health properties, ascribed to its content in both phenolics and fibres. As more and more apples are consumed after processing, there is a need to understand the impact of the conventional process on the nutritional potential of applesauce. We have investigated the variation in composition between fresh and processed apples of 12 cultivars, focussing on polysaccharides and polyphenols. The polysaccharide content estimated in the alcohol insoluble solid (AIS) of the apple flesh varied from 17 (cv. Greensleeves, obtention TMR 7 A 82) to 25 (cv. Jolyne) mg/g fresh weight. The AIS contents varied from 15 to 32 mg/g fresh weight in applesauce. The applesauces were fractionated into serum, containing low concentrations (< 5 mg/g) of soluble fibers, and particles concentrating the fibers (40-60 mg/g) and the cellulose (> 30% of the AIS). Total phenolic concentrations varied in the flesh from 578 (cv. Golden Delicious) to 1596 mg/kg fresh weight (cv. 3535), and in the peel from 1658 (cv. Greensleeves) to 7658 mg/kg fresh weight (cv. Sinta). The applesauces contained flavonols, coming from the skin, and slightly higher concentration of dihydrochalcones coming from the pips than the flesh. A dihydrochalcone oxidation product was also detected. The concentration of hydroxycinnamic acids and flavan-3-ols were decreased in proportions depending on the cultivar. The flavan-3-ols were concentrated in the particles of the applesauce. The composition of applesauce thus appeared to be very close to that of apple flesh.

Keywords: *malus domestica*, polyphenols, fibers, fruit processing, procyanidins.

2. Introduction.

The health effects of apples, especially their cholesterol-lowering properties, are ascribed to the combined fiber and polyphenol fractions. Aprikian et al., (2003) found that apple pectin and polyphenol fractions are more effective together than are either apple pectin alone or apple polyphenol alone for lowering plasma and liver cholesterol and triglycerides. Thus the beneficial effect of apple on health may be better achieved in products which are rich in fiber and phenolics. An example of this kind of product is applesauce.

Both fiber and phenolic compositions of apple are fairly well known in terms of structure (Renard, Voragen, Thibault, & Pilnik, 1990; Renard & Thibault, 1991; Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994; Massiot, Baron & Drilleau, 1994; Massiot & Renard, 1997; Guyot, Marnet, Laraba, Sanoner, & Drilleau 1998; Sanoner, Guyot, Marnet, Molle, & Drilleau, 1999; Guyot, Le Bourvellec, Marnet, & Drilleau, 2002; van der Sluis, Dekker, de Jager, & Jongen, 2001; Tsao, Yang, Young, Zhu, 2003; Vrhovsek, Rigo, Tonon, & Mattivi, 2004; Renard, 2005a; Marks, Mullen, & Crozier, 2007; Khanizadeh, Tsao, Rekika, Yang, Charles, & Rupasinghe, 2008). Five classes of phenolic compounds are present in apple fruit : hydroxycinnamic acids derivatives, monomeric and oligomeric flavan-3-ols named procyanidins, dihydrochalcones, flavonols and anthocyanins only present in the peel of some red varieties (Nicolas et al., 1994; Guyot et al., 1998; Sanoner et al., 1999; Guyot et al., 2002; van der Sluis et al., 2001; Tsao et al., 2003; Vrhovsek et al., 2004; Marks et al., 2007; Khanizadeh et al., 2008). Apple cell wall is mainly composed of cellulose, highly methylated pectins relatively rich in xylogalacturonans, the hemicelluloses fucogalactoxyloglucan and mannan (Renard et al., 1990; Renard & Thibault, 1991 ; Massiot et al., 1994; Massiot & Renard, 1997; Renard, 2005a). However,

the effects of the whole processing for production of apple applesauce on fiber and phenolic are not well known.

In industrial apple applesauce production, the fruits are either crushed, refined and cooked in a cold-break process or cooked then refined in a hot-break process. There are thus three potential mechanisms for modification of the phenolic and fiber compositions: oxidation, selectivity at refining, and heat treatment.

Oxidation can occur during crushing step, during which polyphenols, polyphenol oxidase, oxygen and cell walls, initially segregated, come into contact and may react (Nicolas et al., 1994, Renard, Baron, Guyot, & Drilleau, 2001). Effects of these reactions are well known for apple juice: oxygenation of the juice or crushed apples during processing result in a significant decrease of phenolic compounds variable depending on the phenolic class and the variety (Guyot, Marnet, Sanoner, & Drilleau, 2003). Catechins and procyanidins are particularly affected by oxidation, whereas 5'-caffeoylquinic acid is partly preserved suggesting the involvement of a coupled oxidation mechanism (Cheynier & Ricardo da Silva, 1991). As oxidation can lead to browning, it is usually palliated by addition of ascorbic acid at crushing.

A major cause for loss of polyphenols and fibers is the refining step, which eliminates the peel and pips. Concerning polyphenols, interest has been focused on flavonols, concentrated in peels (Guyot et al., 1998; Sanoner et al., 1999; Guyot et al., 2002; Tsao et al., 2003; Marks et al., 2007; Khanizadeh et al., 2008). Peel elimination is thus a major cause of losses (Oszmianski & Wojdylo, 2008). Apple peel and carpels also contain more cell walls, and thus more fibers, than apple flesh (Massiot et al., 1994; Massiot & Renard, 1997). Moreover, apple flesh contains a higher content in pectic components while carpels are richer in xyloglucans and glucans. Colin-Henrion,

96 Mehinajic, Renard, Richomme, & Jourjon (2009) thus recorded a highly significant
97 decrease in the insoluble fibre content of a hot-break applesauce after the refining step.

98 Last, heating may also be detrimental for polyphenol and polysaccharide content.
99 On pear, Renard (2005a) shows that after 1 hour of cooking, 65% of the original flavan-3-
100 ols and 40% of the original 5'-caffeoylquinic acid are still detectable in the pear tissue.
101 During cooking the pectic fractions are degraded becoming more soluble and of lower
102 molecular weight (Renard, 2005a, Colin-Henrion et al., 2009), while xylans and cellulose
103 are not affected (Renard, 2005a).

104 The objectives of the present study were to evaluate the impact of conventional
105 cold-break process production of apple applesauce on the nutritional properties. To reach
106 this aim polysaccharide and polyphenol profiles (qualitative and quantitative) of twelve
107 apple varieties were investigated focussing on their variation between fresh apples and
108 applesauce produced using a cold-break process.

109

3. Materials and methods

3.1. Standards and chemical

Chlorogenic acid, (+)-catechin, (-)-epicatechin were obtained from Sigma-Aldrich (Deisenhofen, Germany). 4-coumaric acid and quercetin were obtained from Extrasynthese (Lyon, France). Phloridzin was obtained from Fluka (Buchs, Switzerland). Sugar standards were from Fluka (Buchs, Switzerland). d3-methanol was from Acros organics (Geel, Belgium). NaBH₄, N-methyl imidazole, acetic anhydride, toluene- α -thiol were from Sigma-Aldrich (Deisenhofen, Germany). Acetonitrile was analytical grade and from Fisher Scientific (Fair Lawn, New Jersey, USA).

3.2. Plant material

Apple fruits (*Malus domestica* Borkh.) of the 2007 crops were obtained from the experimental orchard of INRA in Angers (49, France) as part of an investigation of the French Association of Fruit and Vegetables Processing (AFIDEM), into the potential of various varieties for applesauce production. 12 cultivars were used: Baujade, Chanteline, Freiberg, Golden Delicious, Greensleeves, Granny Smith, Jolyne, New Gold, Newton Pippin, Sinta, TMR 7 A 82, 3535. Three triplicates of 10 apples were constituted. Each apple was peeled and cored (**Figure 1a**) and then divided as follows: each apple was cut vertically into 12 equal portions (**Figure 1b** and **Figure 1c**). Portions 2, 5, 8 and 11 were discarded (**Figure 1d**). Each of the other 8 portions was cut in two vertically giving 16 pieces per apple (**Figure 1e**). The 16 portions were divided in two containers A and B in such a way that container A received 2 left and 2 right quarters from portions 1, 4, 7 and 10 and container B received 2 left and 2 right quarters from portions 3, 6, 9 and 12 (**Figure 1f**). The two containers were freeze-dried but only container A was used.

3.3. Industrial processing and sample preparation

Applesauce processing was carried out by the Centre Technique de la Conservation des Produits Agricoles (CTCPA), using a cold-break process. The process was composed of several steps, summarized in **Figure 2**. The fruits were washed, and crushed. A solution of ascorbic acid at 250 g/l was then added to the crushed apple (1 liter for 50 kg). Crushed apples were refined (sieve opening 1.25 mm) before cooking (95°C, 2 min). A saccharose syrup was then added in order to obtain a Brix degree of 16° +/- 0.5°. Applesauce was hot conditioned and pasteurised (90°C, 5 min) then cooled (water at 20 °C, 20 min). For each apple variety two cans of applesauce were collected.

Serum and particles were separated by centrifugation. 80 g of applesauce were centrifuged at 13 600 g for 10 minutes. The supernatant was removed and filtered in a G3 sintered glass filter. Serum and particles were frozen and stored at -20°C before use.

3.4. Alcohol insoluble solid (AIS)

AIS were prepared according to Renard et al., (1990) and Renard (2005b).

Approximately 2 g of freeze-dried apple powder, in a 50 ml empty Sep-pack column (Interchim, France) equipped with a sinter of porosity 20 µm, were suspended in 30 ml of boiling 96% ethanol and left 20 minutes. The supernatant was removed by filtration. The material was resuspended in 70% ethanol and extracted at room temperature still absence of sugars as shown by negative reaction in the phenol sulfuric test (Dubois et al., 1956). Samples were then washed twice with acetone : water : acetic acid (v/v/v 60 : 39 : 1), then twice with acetone: water solution (v/v 80 : 20), then with pure acetone until discolouration of the supernatant. The residue was dried at 40°C during 24 hours and weighed (AIS). For each apple variety three replicates were performed.

For each apple variety AIS were prepared from each of the two cans for applesauce, serum and particles. Approximately 20 g of fresh applesauce or fresh particles were suspended in 80 ml of 96% ethanol and left 24 hours. The supernatant was removed by

filtration and a portion of 70% ethanol was added. The same washing and drying procedures were used as for apple. For each apple variety AIS from applesauce were performed on each of the two cans.

40 ml of serum were poured in 120 ml of 96% ethanol and left to stand at 4°C during 24 hours. Samples were first centrifuged at 4200 g for 20 minutes, the supernatant was removed and a portion of 70% ethanol was added. The samples were then centrifuged at 8900 g for 10 min. The washings were continued till absence of sugars as shown by negative reaction in the phenol sulfuric test (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The same drying procedure was used as for apple.

3.5. Analytical

Polyphenols were measured by HPLC after thioacidolysis as described by Guyot et al., 2001. The average degree of polymerisation was measured by calculating the molar ratio of all the flavan-3-ol units (thioether adducts plus terminal units) to (-)-epicatechin and (+)-catechin corresponding to terminal units. Polyphenols were separated in an Agilent 1050 separation system (Agilent Technologies) including a quaternary pump coupled to a diode array detector and controlled by Chemstation A.10.02 software. Separations were achieved using a (250 × 4mm i.d.) Licrospher PR-18 5 µm column (Merck, Darmstadt, Germany) with a guard column (Licrospher PR-18 5 µm column, Merck, Darmstadt, Germany) operated at 30 °C. The mobile phase consisted of water/acetic acid (97.5:2.5, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 1 ml/min. The elution programme was as follows: 3–9% B (0–5 min); 9–16% B (5–15 min); 16–50% B (15–45 min); 50–90% B (45–48 min); 90–90% B (48–52 min); 90–3% B (52–55 min); 3–3% B (55–60 min). For apple the three batches were analysed and for applesauce, serum and particles each of the two cans were analysed. Samples were injected at a level of 20 µl. The

column effluent was monitored at 280, 320 and 350 nm. Quantification was achieved by injection of standard solutions of known concentrations.

UPLC-MS analyses were performed on the Acquity Ultra performance LCTM (UPLCTM) apparatus from Waters (Milford, MA, USA), equipped with an UV-visible diode array detector and coupled with a Bruker Daltonics (Bremen, Germany) HCT ultra ion trap mass spectrometer with a negative electrospray ionization mode. Separations were achieved using a (150 × 2.1 mm i.d.) 1.7 µm Acquity UPLC HSS T3 column (Waters, Milford, MA, USA) operated at 30 °C. The mobile phase consisted of water/formic acid (99.95:0.05, v/v) (eluent A) and acetonitrile (eluent B). The flow rate was 0.6 ml/min. The elution programme was as follows: 3–9% B (0–1.4 min); 9–16% B (1.4–4 min); 16–50% B (4–15 min); 50–97% B (15–15.5 min); 97–97% B (15.5–18 min). Samples were injected at a level of 2 µl. The column effluent was monitored at 280, 320 and 365 nm. The mass spectra were generated in the Ultrascan mode in the m/z range 100–900. Nitrogen was used as the nebulising gas. The ion source parameters were : nebuliser pressure, 40 psi; drying gas flow, 10 L/min; drying gas temperature, 365°C; capillary voltage, 2 kV. Helium was used as the damping gas. Data were collected and processed using Bruker Compass Data Analysis software.

Neutral sugars, galacturonic acids and methanol were analysed as described by Renard & Ginies (2009). Dry matter was estimated by the loss of weight recorded after freeze-drying.

3.6. Statistical analysis

Results are presented as mean values, and the reproducibility of the results was expressed as pooled standard deviation. Pooled standard deviations were calculated for each series of replicates using the sum of individual variances pondered by the individual degrees of freedom (Box, Hunter & Hunter, 1978). The variability of the composition

208 between varieties was expressed calculating the average of the mean values for each
209 variety and the standard deviation of the mean.

210 Two-way analysis of variance (ANOVA) by Fisher 's test (F) was used to compare
211 the means and performed using the Excelstat package of Microsoft Excel. Differences
212 were considered to be significant at $P < 0.05$.

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4. Results and discussion

4.1. Cell wall characterisation

Table 1 summarized the yields and compositions of the AIS of apple flesh. The AIS content of apple flesh varied from 17 mg/g (cv Greensleeves) to 25 mg/g (cv Jolyne) fresh weight (FW), which was consistent with previously published works (15-24 mg/g of fresh weight) (Renard & Thibault, 1991; Massiot & Renard, 1997; Renard, 2005b; Colin-Henrion et al., 2009). The sugar composition of the AIS of the different apple varieties were very close (**Table 1**). They reflected the macromolecular composition of the apple cell wall (Renard et al., 1990; Massiot et al., 1994; Massiot & Renard, 1997; Renard, 2005b): cellulose, highly methylated pectins relatively rich in xylogalacturonans, fucogalactoxyloglucan and mannan. The two main sugars were glucose (from 233 mg/g (cv. Freiberg) to 349 mg/g (cv. Golden Delicious)) and galacturonic acid (from 209 mg/g (cv. Freiberg) to 273 mg/g (cv. Granny Smith and TMR 7 A 82)). Glucose came essentially from cellulose (Renard et al., 1990). Pectic substances were present in high amount as shown by the high galacturonic acid content, and were highly methylated (from 71% (cv. Granny Smith, New Gold and Sinta) to 80% (cv. Jolyne)). In previous work (Voragen et al., 1980; Renard et al., 1990; Renard, 2005b), degrees of esterification from 63 to 75% were obtained in the AIS preparations from apple. Besides glucose and galacturonic acid, the neutral sugars arabinose (from 68 mg/g (cv. Greensleeves) to 133 mg/g (cv. Sinta)), xylose (from 50 mg/g (cv. Newton Pippin) to 66 mg/g (cv. New Gold)) and galactose (from 45 mg/g (cv. Greensleeves) to 80 mg/g (cv. Freiberg)) dominated. Arabinose and galactose enter in the composition of the side chains of the hairy region of the pectic substances (de Vries, Rombouts, Voragen, & Pilnik, 1982), while xylose, together with fucose (from 7 mg/g (cv. Newton Pippin) to 11 mg/g (cv. Golden Delicious)), part of the

galactose and glucose, constitute fucogalactoxyloglucan, which is the main hemicellulose of apple cell wall (Renard et al., 1990, Renard, Voragen, Thibault, & Pilnik, 1991). Mannose (from 10 mg/g (cv. new Gold) to 22 mg/g (cv. Sinta)) enter in the composition of mannan (Renard et al., 1991).

The yields and the composition of the AIS of applesauces are summarised in **Table 2**. After processing into applesauce the AIS contents varied from 15 to 32 mg/g FW and were either higher (Freiberg, Golden Delicious, Greensleeves, Jolyne, New Gold and Sinta) or lower (Newton Pippin, Chanteline, Granny Smith, 3535, TMR 7 A 82) than for the corresponding raw apple. Few studies report the polysaccharide content of applesauces, yet values obtained here were slightly higher than those reported by Marlett & Wollendorf (1994) (14 mg/g) or contained that reported by Colin-Henrion et al., (2009) (18 mg/g). During processing into applesauce, four main mechanisms may affect the AIS content :

- a dilution during addition of sugar and ascorbic acid,
- the temperature applied (90°C) may have contributed to the solubilization by depolymerisation of pectins (Ng & Waldron, 1997; Renard, 2005b; Colin-Henrion et al., 2009) by acid hydrolysis and β -elimination (Sajjaanantakul, van Buren, & Dowling, 1989; Fraeye, de Roeck, Duvetter, Verlent, Hendrickx, van Loey 2007),
- the presence of particles coming from skin and pips, both containing more AIS material than parenchyma (Massiot et al., 1994; Massiot & Renard, 1997),
- co-precipitation of proteins and polyphenols from the apple fruit (Colin-Henrion et al., 2009). Cutting and crushing could favour the formation of processing-induced complexes between polyphenols, especially procyanidins, and cell wall polysaccharides (Renard et al., 2001, Le Bourvellec, Guyot, & Renard, 2004). Renard (2005a) reported a similar effect of cooking on AIS content of boiled pear wedges. As here the Brix chosen

for the puree was very close to that obtained for most of the apples, there was no dominant dilution effect.

AIS from applesauce mainly contained less galacturonic acid and rhamnose and more galactose than those of the apple flesh (**Table 2**). The applesauces were fractionated into serum and particles. The serum contained low concentrations (< 5 mg/g FW) of soluble fibers mainly containing galacturonic acid, rhamnose, arabinose and galactose, i.e. primarily pectic polymers. Particles concentrated the fibers (40-60 mg/g FW) and the major component was glucose coming from cellulose ($> 30\%$ of the AIS), but high amounts of galacturonic acid were also detectable. Glucose and xylose content were higher than in apple flesh, coming probably from carpel zone (Massiot et al., 1994; Massiot & Renard, 1997). The same trends for AIS and soluble and insoluble fibres of applesauce has also been reported by Colin-Henrion et al. (2009).

Two-ways ANOVAs (variety and “type of product” effects, with interactions) were performed on apple flesh and applesauce AIS compositions. The variety effect was significant ($P < 0.0001$) for all variables, except degree of methylation and the “type of product” for all variables except degree of methylation, mannose and galacturonic acid (**Table 3**). The variety effect was more important for arabinose, mannose, glucose, and galacturonic acid whereas the 'type of product' effect was more important for rhamnose, fucose, xylose, galactose and methanol.

4.2. Polyphenol characterisation

Apple peel and flesh

Composition and concentrations of the major phenolics found in the peel and in the flesh of the 12 apple varieties are shown in **Table 4** and **5** respectively. Five major phenolic groups with a total of fourteen identified individual compounds were quantified. The sum of phenolics determined by HPLC ranged from 1658 mg/kg (cv. Greensleeves) to 7658 mg/kg FW (cv. Sinta) in the peel. For all varieties, in the flesh, the total polyphenols

were significantly lower than in the peel, ranging from 539 mg/kg (cv. Newton Pippin) to 1596 mg/kg FW (cv. 3535). In our study, total polyphenols in the flesh were slightly higher, but in the range of previous works for dessert and cider apples (228-6999 mg/kg FW)(Sanoner et al., 1999; van der Sluis et al., 2001; Guyot et al., 2002; Tsao et al., 2003; Vrhovsek et al., 2004, Khanizadeh et al., 2008). The different varieties of apples studied come from a collection of INRA Angers and are intended for different use and therefore have significant biodiversity.

Among the five major groups, procyanidins were the predominant class in both the skin (73%) and the flesh (69%) (**Table 4** and **5**). These data are in agreement with other authors (Guyot et al., 1998; Sanoner et al., 1999; Guyot et al., 2002; Tsao et al., 2003; Vrhovsek et al., 2004; Khanizadeh et al., 2008). For all varieties, in the flesh, the procyanidins' average degrees of polymerisation ($\overline{DP}_n$) were significantly lower than those of the peel, ranging respectively from 2.9 (cv. Baujade) to 3.8 (cv. Chanteline) and from 1.4 (cv. Sinta) to 5.6 (cv. Granny Smith) as observed in previous work (Sanoner et al., 1999; Guyot et al., 2002). However, $\overline{DP}_n$ in the flesh were slightly lower than the $\overline{DP}_n$ observed by Sanoner et al., (1999) and Guyot et al., (2002) in the flesh of dessert variety with $\overline{DP}_n$ from 5.7 to 6.0. The flavan-3-ols monomers were detected as (-)-epicatechin and (+)-catechin, the former being highly preponderant and the later sometimes only in traces amounts (**Table 4** and **5**).

Hydroxycinnamic acids accounted for only 4% of the total polyphenols in the peel while they constituted the second polyphenol class in the flesh where they accounted for circa 20% of the total polyphenols. Hydroxycinnamic acids were lowest in Granny Smith and particularly concentrated in the peel and flesh of TMR 7 A 82 with about half a g per kg FW. The main hydroxycinnamic acid was always 5'-caffeoylquinic acid. The highest concentrations of *p*-Coumaroylquinic acid were only circa 50 mg/kg FW (**Table 4** and **5**).

For all varieties, dihydrochalcones were a minor group which accounted for an average of 3% and 1.5% of the total polyphenols in the peel and in the flesh, respectively (**Table 4** and **5**). Higher concentration were found in the peel, up to about 300 mg/kg FW, than in the flesh (up to 40 mg/kg FW). Phloridzin and phloretin-2-xyloglucoside were the major constituent of this class, and in all the cultivar tested here, phloridzin was preponderant.

For all variety no compounds of the flavonol class could be detected in the flesh. Flavonols represented the second highest class in apple peel with 14% of the total polyphenols (**Table 5**). Six quercetin glycosides were found and quantified in quercetin equivalent : quercetin 3-galactoside > quercetin 3-arabinopyranoside > quercetin 3-rhamnoside > quercetin 3-glucoside > quercetin 3-xyloside > quercetin 3-rutinoside. Depending on the variety, total flavonols ranged from 300 mg/kg (cv. Golden Delicious) to 901 mg/kg FW (cv. TMR 7 A 82) in the peel.

All these concentrations and relative composition of each class are consistent with previous works. Previous studies have also shown a wide variation in the phenolic content of different cultivars (Guyot et al., 1998; Sanoner et al., 1999; Guyot et al., 2002; Tsao et al., 2003; Vrhovsek et al., 2004; Marks et al., 2007; Khanizadeh et al., 2008). These different works deal with the characterization of phenolic profiles of dessert apples (Guyot et al., 2002; Tsao et al., 2003; Vrhovsek et al., 2004), of selected apple genotypes for processing with high polyphenol content (Khanizadeh et al., 2008) while having, however, less extreme concentrations than those achieved by cider apple (Guyot et al., 1998; Sanoner et al., 1999; Marks et al., 2007).

Applesauces

Composition and concentrations of the major phenolics found in the apple applesauces are shown in **Table 6**. The composition and concentrations of phenolics

compounds in the applesauce were closely related to their contents in the raw material (Oszmianski, Wolniak, Wojdylo, & Wawer, 2008; Oszmianski & Wojdylo, 2008). The total phenolics ranged from 182 mg/kg (cv. Greensleeves) to 1334 mg/kg FW (cv. 3535) and were significantly lower than in the flesh as previously shown by Dragovic-Uzelac, Pospisil, Levaj, & Delonga (2005). Total native polyphenols in the applesauce accounted for 37% (cv. New Gold) to 94% (cv. Golden Delicious) of those in the initial flesh. The applesauce polyphenol retention was mainly related to those of flavan-3-ols. As in apple flesh, most of the flavan-3-ols of the applesauce were procyanidins (**Table 6**). The total procyanidins ranged from 72 mg/kg (cv. Greensleeves) to 814 mg/kg FW (cv. Granny Smith). They represented from 18% (cv. New Gold) to 90% (cv. Granny Smith) of the procyanidins initially present in the flesh. $\overline{DP}_n$ of the procyanidins essentially did not change from apple to applesauce, ranging respectively from 2.3 (cv. Newton Pippin) to 3.7 (cv. Jolyne). (-)-Epicatechin was the preponderant flavan-3-ol monomer, but also the least stable. (-)-Epicatechin retention ranged from 27% (cv. Greensleeves) to 90% (cv. Granny Smith) and applesauce concentrations ranged from 12 mg/kg (cv. Greensleeves) to 93 mg/kg FW (cv. Granny Smith). (+)-Catechin retention ranged from 41% (Baujade) to 78% (cv. Jolyne) and applesauce concentrations ranged from traces (cv. Freiberg, Golden Delicious, Greensleeves, New Gold, Newton Pippin) to 93 mg/kg FW (cv. Granny Smith).

Total hydroxycinnamic acids content of applesauces decreased, compared to that of the flesh, only by a small amount for most varieties except for cultivars Freiberg, Golden Delicious and Granny Smith where total hydroxycinnamic acids content increased. Concentrations ranged from 45 mg/kg (cv. Greensleeves) to 433 mg/kg FW (cv. 3535) (**Table 6**). The increase in hydroxycinnamic acids content may be due to facilitating the diffusion of phenolic compounds favorized by cooking. As in apple flesh, among hydroxycinnamic acids, the main compound in applesauce was always 5'-caffeoylquinic

acid, the retention of which ranged from 53% (cv. Greensleeves) to 125% (cv. Granny Smith) .*p*-Coumaroylquinic acid concentrations represented from 33% (cv. New Gold) to 108% (cv. 3535) of those in the flesh (**Table 6**).

Total dihydrochalcones content of applesauces increased compared to that of the flesh, with concentration ranging from 9 mg/kg (cv. Granny Smith) to 57 mg/kg FW (cv. TMR 7 A 82) and representing from 98% (cv. New Gold) to 844% (cv. Chanteline) of those in the flesh (**Table 6**). The increase observed was mainly due to an increased phloridzin content. During applesauce processing dihydrochalcones, abundant in peel and seeds (Sanoner et al., 1999; Guyot et al., 2002), may have diffused before refining. Oszmianski & Wojdylo (2008) have shown that applesauce prepared from peeled apple slices devoid of seeds had lower content of phloretin derivatives than applesauce prepared from non-peeled apples. The main dihydrochalcone in applesauce was always phloridzin which retention ranged from 98% (cv. Granny Smith) to 192% (cv. Jolyne) of those in the flesh.. Phloretin-2-xyloglucoside retention ranged from 64% (cv. New Gold) to 168% (cv. Greensleeves)) of those in the flesh. (**Table 6**).

Quercetin derivatives were detected in applesauce (**Table 6**) whereas they were not present in apple flesh. Quercetin derivatives, abundant in peel (Sanoner et al., 1999; Guyot et al., 2002), may have diffused during applesauce processing and before refining or could be trapped within small peel particles. Like in apple peel six quercetin glycosides were found in applesauce and quantified in quercetin equivalent. The same order of importance was observed : quercetin 3-galactoside > quercetin 3-arabinopyranoside > quercetin 3-rhamnoside > quercetin 3-glucoside > quercetin 3-xyloside > quercetin 3-rutinoside. Depending on the variety, total flavonols ranged from 9 mg/kg (cv. 3535) to 35 mg/kg FW (cv. TMR 7 A 82). The concentrations of flavonols in applesauce were not directly correlated to those in the flesh of the corresponding apples. This phenomenon might be

explained by differences in the skin of apples, and notably different structures, mechanical resistance and cuticle thickness.

Two-ways ANOVAs (variety and “type of product” effects, with interactions) were performed on apple flesh and applesauce phenolic compositions. The variety and type of product effect were always highly significant ($P < 0.0001$) (**Table 7**) except for phloretin-2-xyloglucoside for which the type of product effect was not significant ($F = 0.38$ and $P = 0.54$). There was more variability with variety for hydroxycinnamic acids (5'-caffeoylquinic acid and *p*-coumaroylquinic acid) whereas for other phenolic classes and total phenolics the variability was more important with “type of product”. This phenomenon might be explained by the decrease in flavan-3-ols between apple flesh and applesauce and by the increase in dihydrochalcones coming from the peel and seeds and flavanols coming from the peel.

At the beginning of the applesauce chromatograms (**Figure 4**) the onset of a new compound was observed. This component was assigned to the dihydrochalcone group because of its UV-spectra, which maximum absorbance was 292 nm. This compound was further analysed by HPLC-DAD/ESI-MS. It had a deprotonated molecular ion $[M-H]^-$ at $m/z = 467$ which fragmented upon MS^2 to yield a major ion at $m/z = 305$. The loss of 162 mass units on MS^2 experiment correspond to the glucose moiety. The MS^3 fragmentation of the 305 aglycon ion showed a main fragment at $m/z = 261$ that corresponded to the loss of the carbonyl group (44 mass units). This compound was identified as the colourless compound resulting from the oxidation of phloridzin named POPi (colourless phloridzin oxidation product) (Guyot, Serrand, Le Quéré, Sanoner, & Renard, 2007; Le Guernevé, Sanoner, Drilleau, & Guyot, 2004). Its amount was expressed in phloridzin equivalent and its concentration ranged from 13 mg/kg (cv. Freiberg) to 30 mg/kg FW (cv. Baujade) in applesauce. presence of POPi and absence of further compounds in the oxidation chain of

phloridzin, i.e. primarily POPj, the coloured compounds (Guyot et al., 2007; Le Guernevé et al., 2004) indicated that a limited enzymatic oxidation had occurred during processing.

The decrease in phenolic content was probably a result of the processing technology and more specifically of oxidation of polyphenols by polyphenoloxidase. Thus, the phenolic composition of apple applesauce depends first on the initial composition of the fruit and second on the processing conditions of the applesauce and the response of each variety to the technology used. Oxidation of polyphenols during applesauce processing, despite the addition of ascorbic acid, led to the formation of new phenolic compounds not, one of which (POPi) was identified on the chromatogram.

A linear relationship was obtained (**Figure 3**) between hydroxycinnamic acids retention and procyanidin retention in applesauces. The more hydroxycinnamic acids were consumed by oxidation, the more procyanidins were also. Two groups were observed: a group relatively protected from oxidation with procyanidin concentration in the applesauce ranging from 71% (cv. Jolyne) to 90% (cv. Granny Smith) of those in the flesh and one other without protection where this ratio applesauce to flesh ranged from 29% (cv. Greensleeves) to 57% (cv. Baujade). Only New Gold variety did not follow this trend with a an applesauce to flesh ratio for hydroxycinnamic acids of 90% and of 18% for procyanidins. This close relationship between the concentrations of the hydroxycinnamic acids and procyanidins in apple flesh and apple sauce is probably related to secondary oxidation reactions. In apple, the main substrate of PPO is caffeoylquinic acid (Janovitz-Klapp, Richard, & Nicolas, 1989). It is first enzymatically oxidised to its highly reactive *o*-quinones, with the simultaneous consumption of oxygen, the second substrate of PPO (Mayer & Harel 1979; Mayer, 1987). Then, *o*-quinones react following different pathways according to their electrophilic and/or oxidant character with nucleophilic compounds present in the medium such as polyphenols - or proteins, to produce secondary products by

covalent interactions (Jervis & Pierpoint 1989). Procyanidins are not substrates of PPO (Goodenough & Lea 1979), but they can be converted into corresponding *o*-quinones by coupled oxidation/reduction involving primary *o*-quinones formed by PPO catalysis. During this reaction the primary *o*-quinones are reduced back to their *o*-diphenol form (Cheynier & Ricardo da Silva, 1991). During applesauce processing the *o*-quinone of 5'-caffeoylquinic acid may react with an *o*-diphenol like 5'-caffeoylquinic acid or flavan-3-ol monomers to yield a condensation product by a mechanism analogous to a Michael 1,4 nucleophilic addition (Oszmianski & Lee, 1990) or with a procyanidin by a coupled oxidation/reduction (Cheynier & Ricardo da Silva, 1991). This mechanism may explain the correlation between 5'-caffeoylquinic acid and procyanidins consumption. The existence of two groups, one protected from oxidation and the other not may be due to a higher PPO activity in the second group.

Particles and serums

The polyphenols were not equally distributed between particles and serum, they were mainly concentrated in the solid part of the applesauce (**Table 8 and 9**) with different proportions between the different classes. Total phenolics in the particles ranged from 228 mg/kg (cv. Greensleeves) to 2690 mg/kg FW (cv. 3535) and in the serum from 123 mg/kg (cv. Greensleeves) to 863 mg/kg FW (cv. 3535). Flavan-ols in particular were concentrated in the particles of the applesauce (**Table 9**). This could be explained as particles concentrate the fibers (4-6 mg/g FW) (**Table 2**), which are able to absorb selectively procyanidins (Renard et al., 2001; Le Bourvellec et al., 2004). A selective partition of the procyanidins between the particles and the serum was observed both in concentration and in degree of polymerisation. The concentration of of procyanidins in the particles ranged from 1757 mg/kg FW (3535) to 99 mg/kg FW (Newgold), and those in the serum from 366 mg/kg FW (Granny Smith) to 19 mg/kg FW (Newgold). The $\overline{DP}_n$ of the procyanidins of

the particles (**Table 8**) ranged from 2.4 to 3.8 and were for each individual variety higher than those of the procyanidins of the serum (**Table 9**), ranging from 2.3 to 3.1. By studying the adsorption of procyanidins on cell wall material, Renard et al., (2001) and Le Bourvellec et al., (2004) have also shown a selective partition of procyanidins between the soluble phase and the insoluble material: the $\overline{DP}_n$ of the bound procyanidins were higher than those of the initial fraction and of the free procyanidins. Unlike procyanidins, which were selectively distributed between particles and serum, flavan-3-ols monomers were distributed equally over particles and serum (**Table 8 and 9**). The same was observed for hydroxycinnamic acids, flavonols and dihydrochalcones. For example, (-)-epicatechin concentrations ranged from 11 mg/kg (cv. Greensleeves) to 95 mg/kg FW (cv. Granny Smith) in the particles and from 4 mg/kg (cv. Greensleeves) to 82 mg/kg FW (cv. Sinta) in the serum.

Like the other polyphenol classes flavanols were distributed equally between particles and serum (**Table 8 and 9**). Flavonols concentrations ranged from 9 mg/kg (cv. 3535) to 42 mg/kg FW (cv. Baujade) in the particles and from trace mg/kg (cv. 3535) to 24 mg/kg FW (cv. Baujade) in the serum.

5. Conclusions

We have investigated here the polysaccharidic and phenolic composition of 12 apple varieties and their applesauces. Fiber composition varied little between apple cultivars and processing to applesauce induced little modification, with however a solubilisation of pectins in the serum. The polyphenol compositions were much more variable, so that variety was the main determinant of phenolic composition in applesauces. Though applesauce phenolic compositions were still very close to those of apple flesh, there were significant increases in dihydrochalcones and flavonols. In spite of added ascorbic acid during processing, there could be marked losses in hydroxycinnamic acids and procyanidins between the fresh apple and the applesauce. Further in the applesauce the procyanidins were concentrated in the particles while the smaller molecules were more equally distributed between serum and particles.

The phenolic compound concentrations in applesauce were results of their content of raw materials and the diffusion and enzymatic oxidation reactions which phenolics undergo during applesauce processing. On average concentration of phenolics in the applesauce represented 70% of those in the flesh. Processing of apples into applesauces caused limited changes in phenolic content compared to processing into juices where only 42% of fruit total phenolics were found in juices (Guyot et al., 2003). The nutritional properties of applesauce can therefore be expected to be close to those of apple flesh.

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Figure Captions

Figure 1 : Scheme of fractionation of apples into twelve sample, with a systematic repartition of right and left faces.

Figure 2 : Applesauce processing

Figure 3 : Comparison of the relative variations in the concentrations of 5'-caffeoylquinic acid and procyanidins between the fruit and the applesauce.

Figure 4 : Typical RP-18 chromatograms at 280 nm of apple flesh (Var. 3535, A) and applesauce (Var. 3535, B). Peak identification : 1, POPI; 2, (+)-catechin; 3, 5'-caffeoylquinic acid; 4, procyanidin B2; 5, (-)-epicatechin, 6, para-coumaroylquinic acid; 7, phloretin-2-xyloglucoside; 8, phloridzin.

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Fig 1

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Fig 4

Comment citer ce document :

Le Bourvellec-Samour, C., Bouzerzour, K., Ginies, C., Regis, S., Plé, Y., Renard, C. (2011). Phenolic and polysaccharidic composition of applesauce is close to that of apple flesh. Journal of Food Composition and Analysis, 24 (4-5), 537-547. , DOI : 10.1016/j.jfca.2010.12.012

Tables

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Table 1 : Yields from fresh weight (mg/g fresh weight) and compositions (mg/g dry matter) of the different apple flesh AIS.

Variety	Yields	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	MeOH (DM)
Baujade	19.3	9	8	124	52	14	51	286	247	33 (79)
Chanteline	18.8	9	8	101	62	16	53	338	238	33 (75)
Freiberg	27.0	10	8	88	52	14	80	233	209	28 (73)
Golden D.	22.0	11	11	104	63	21	58	349	255	33 (72)
Granny Smith	24.5	9	8	126	51	19	59	301	273	36 (71)
Greensleeves	17.4	10	8	68	53	11	45	258	236	32 (74)
Jolyne	25.2	11	9	117	52	18	50	320	255	37 (80)
New Gold	22.3	11	9	98	66	10	65	310	256	33 (71)
Newton Pippin	23.0	8	7	118	50	16	71	246	237	32 (75)
Sinta	22.9	11	9	133	60	22	77	319	236	31 (71)
TMR 7 A 82	17.2	10	8	104	54	20	54	285	273	33 (72)
3535	18.4	9	9	119	59	17	62	308	230	31 (74)
SDM	0.07	0.1	0.1	1.1	0.5	0.2	0.5	2.6	2.6	0.1 (0.8)

AIS, alcool insoluble solid. Rha, rhamnose. Fuc, Fucose. Ara, arabinose. Xyl, xylose. Man, mannose. Gal, galactose. Glc, glucose. Gal A, galacturonic acid. MeOH, methanol. DM, degree of methylation. SDM, standard deviation of the mean n= 72, except for the yields n = 24.

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3 **Table 2 :** Yields from fresh weight (mg/g fresh weight) and compositions (mg/g

4 dry matter) of the AIS of applesauces, particles and serum.

Variety	P.	Yields	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	MeOH (DM)
Baujade	AS	22 2	6 0.2	7 0.3	91 1.8	38 1.8	14 0.5	62 1.8	243 10.3	311 5.3	33 0.2 (59 2)
	Pl	45 2	8 0.2	11 0.2	138 2.4	60 1.2	23 0.4	90 1.4	385 7.9	326 5.0	28 0.2 (47 3)
	S	4 0.3	7 0.2	2 0.1	81 1.8	6 0.2	0 0.0	79 1.7	0 0.0	502 10.8	70 0.4 (77 2)
Chanteline	AS	29 2	6 0.2	7 0.3	99 1.8	56 1.8	15 0.5	67 1.8	310 10.3	250 5.3	28 0.2 (64 2)
	Pl	53 2	7 0.2	7 0.2	85 2.4	48 1.2	15 0.4	62 1.4	292 7.9	183 5.0	25 0.2 (75 3)
	S	3 0.3	17 0.2	4 0.1	147 1.8	16 0.2	0 0.0	113 1.7	0 0.0	456 10.8	77 0.4 (94 2)
Freiberg	AS	21 2	8 0.2	6 0.3	78 1.8	41 1.8	14 0.5	77 1.8	229 10.3	178 5.3	27 0.2 (85 2)
	Pl	57 0.2	9 0.2	7 0.2	88 2.4	49 1.2	17 0.4	89 1.4	290 7.9	199 5.0	25 0.2 (70 3)
	S	1 0.3	6 0.2	1 0.1	43 1.8	4 0.2	0 0.0	46 1.7	0 0.0	407 10.8	55 0.4 (75 2)
Golden D.	AS	20.2	9 0.2	8 0.3	88 1.8	48 1.8	18 0.5	51 1.8	296 10.3	205 5.3	32 0.2 (86 2)
	Pl	42 2	10 0.2	9 0.2	93 2.4	52 1.2	20 0.4	53 1.4	332 7.9	216 5.0	28 0.2 (72 3)
	S	3 0.3	6 0.2	2 0.1	52 1.8	6 0.2	2 0.0	41 1.7	0 0.0	356 10.8	64 0.4 (100 2)
Greensleeves	AS	31 2	6 0.2	6 0.3	83 1.8	32 1.8	15 0.5	101 1.8	253 10.3	170 5.3	22 0.2 (73 2)
	Pl	59 2	5 0.2	6 0.2	96 2.4	38 1.2	17 0.4	116 1.4	307 7.9	76 5.0	20 0.2 (100 3)
	S	4 0.3	3 0.2	1 0.1	41 1.8	6 0.2	0 0.0	45 1.7	0 0.0	505 10.8	77 0.4 (85 2)
Granny	AS	15 2	10 0.2	5 0.3	76 1.8	41 1.8	12 0.5	39 1.8	217 10.3	237 5.3	30 0.2 (70 2)
	Pl	49 2	8 0.2	6 0.2	59 2.4	43 1.2	12 0.4	38 1.4	258 7.9	171 5.0	27 0.2 (88 3)
	S	2 0.3	11 0.2	2 0.1	73 1.8	8 0.2	0 0.0	76 1.7	0 0.0	271 10.8	67 0.4 (100 2)
Jolyne	AS	24 2	9 0.2	9 0.3	139 1.8	56 1.8	21 0.5	71 1.8	340 10.3	245 5.3	33 0.2 (74 2)
	Pl	53 2	7 0.2	6 0.2	97 2.4	45 1.2	16 0.2	51 1.4	259 7.9	233 5.0	28 0.2 (67 3)
	S	5 0.3	5 0.2	2 0.1	50 1.8	9 0.2	0 0.0	35 1.7	0 0.0	465 10.8	67 0.4 (80 2)
New Gold	AS	18 2	10 0.2	9 0.3	114 1.8	61 1.8	12 0.5	80 1.8	349 10.3	215 5.3	31 0.2 (79 2)
	Pl	47 2	11 0.2	10 0.2	118 2.4	65 1.2	12 0.2	78 1.4	367 7.9	133 5.0	28 0.2 (100 3)
	S	3 0.3	4 0.2	1 0.1	27 1.8	2 0.2	0 0.0	29 1.7	0 0.0	421 10.8	60 0.4 (80 2)
Newton	AS	32 .2	6 0.2	5 0.3	73 1.8	28 1.8	12 0.5	65 1.8	198 10.3	292 5.3	26 0.2 (49 2)
	Pl	59 2	8 0.2	8 0.2	112 2.4	45 1.2	20 0.4	97 1.4	359 7.9	137 5.0	22 0.2 (91 3)
	S	5 0.3	4 0.2	1 0.1	39 1.8	6 0.2	0 0.0	37 1.7	0 0.0	334 10.8	59 0.4 (75 2)
Sinta	AS	21 2	11 0.2	9 0.3	140 1.8	58 1.8	26 0.5	102 1.8	352 10.3	225 5.3	29 0.2 (71 2)

TMR 7 A 82	Pl	53 2	10 0.2	8 0.2	118 2.4	53 1.2	23 0.4	84 1.4	320 7.9	199 5.0	23 0.2 (63 3)
	S	5 0.3	6 0.2	1 0.1	76 1.8	5 0.2	0 0.0	53 1.7	0 0.0	521 10.8	76 0.4 (80 2)
	AS	18 2	11 0.2	8 0.3	113 1.8	54 1.8	25 0.5	62 1.8	333 10.3	215 5.3	30 0.2 (79 2)
	Pl	51 2	6 0.2	6 0.2	78 2.4	41 1.2	19 0.4	41 1.4	256 7.9	195 5.0	23 0.2 (66 3)
3535	S	4 0.3	6 0.2	1 0.1	60 1.8	4 0.2	0 0.0	38 1.7	0 0.0	421 10.8	67 0.4 (88 2)
	AS	22 2	9 0.2	11 0.3	132 1.8	69 1.8	21 0.5	89 1.8	374 10.3	222 5.3	31 0.2 (77 2)
	Pl.	54 2	8 0.2	10 0.2	120 2.4	67 1.2	21 0.4	79 1.4	380 7.9	186 5.0	25 0.2 (80 3)
	S	3 0.3	2 0.2	1 0.1	27 1.8	4 0.2	0 0.0	25 1.7	0 0.0	560 10.8	71 0.4 (72 2)

- 1 AIS, alcool insoluble solid. P. : Product. AS. : Applesauce. Pl. : Particles. S : Serum. Rha, rhamnose. Fuc,
- 2 Fucose. Ara, arabinose. Xyl, xylose. Man, mannose. Gal, galactose. Glc, glucose. Gal A, galacturonic acid.
- 3 MeOH, methanol. DM, degree of methylation. Values in italic correspond to the standard deviation of the
- 4 mean n= 24, except for the yields n = 12.

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Table 3 : Fishers' F-values and *p*-values associated with two-way ANOVAs (type of product and variety effects, with interactions) performed on apple flesh and applesauce AIS compositions (mg/g dry matter).

Variable	Variety effect		Type of product effect		Variety x type of product effect	
	F	P	F	P	F	P
Rha	16.9	<0.0001	40.9	<0.0001	3.03	<0.0001
Fuc	13.0	<0.0001	30.9	<0.0001	4.6	<0.0001
Ara	14.5	<0.0001	8.38	<0.0001	6.5	<0.0001
Xyl	11.2	<0.0001	41.0	<0.0001	5.2	<0.0001
Man	39.7	<0.0001	1.04	0.311	5.4	<0.0001
Gal	20.1	<0.0001	45.47	<0.0001	9.4	<0.0001
Glc	11.0	<0.0001	1.35	<0.0001	3.9	<0.0001
Gal A	5.8	<0.0001	3.16	0.078	7.1	<0.0001
MeOH	12.1	<0.0001	44.4	<0.0001	7.4	<0.0001
DM	1.5	0.154	2.4	0.121	5.146	<0.0001

AIS, alcool insoluble solid. Rha, rhamnose. Fuc, Fucose. Ara, arabinose. Xyl, xylose. Man, mannose. Gal, galactose. Glc, glucose. Gal A, galacturonic acid. MeOH, methanol. DM, degree of methylation.

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2 **Table 4** : Polyphenol composition and concentrations (mg/kg of fresh weight) and $\overline{DP}_n$ in the peel of the twelve apple varieties.

Variety	CAT	EC	PCA	$\overline{DP}_n$	XPL ^a	PLZ	TotalFl ^b	CQA	pCoQA ^c	Total
Baujade	24	363	3279	3.9	17	52	616	187	5	4542
Chanteline	24	173	2879	5.3	6	47	331	90	5	3555
Freiberg	t	198	1966	3.4	91	222	799	162	9	3447
Golden Delicious	t	250	2397	4.0	17	75	300	100	6	3146
Granny Smith	t	171	3337	5.6	18	21	763	10	t	4321
Greensleeves	24	94	1021	3.7	15	47	373	81	3	1658
Jolyne	26	424	3154	4.1	24	51	704	46	12	4440
New Gold	T	244	1816	5.0	14	43	500	78	3	2698
Newton Pippin	20	165	1333	3.8	t	32	381	103	2	2037
Sinta	29	265	6080	1.4	6	33	426	20	t	7658
TMR 7 A 82	45	359	3228	3.5	32	230	901	517	7	5315
3535	55	301	3558	4.2	t	98	481	400	47	4942
Pooled SD	0.8	5.7	112.2	0.8	0.5	3.0	9.6	1.4	0.1	117.1
mean	20	251	2904	4.0	20	79	548	150	8	3980
%	1	6	73		1	2	14	4	0.2	100
SDM	17.5	97.2	1509.5	1.1	24.4	71.5	202.6	154.9	12.7	1619.4

3 CAT : (+)catechin, EC : (-)-epicatechin, PCA : procyanidins, $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins), , XPL : phloretin-2-
 4 xyloglucoside, PLZ : phloridzin, TotalFl : total flavonols, CQA : 5'-caffeoylquinic acid, pCoQA : para-coumaroylquinic acid, Total : total polyphenols, SD : standard
 5 deviation, n= 24, SDM : standard deviation of the mean, n = 12, t = traces. a : quantified as phloridzin, b : quantified as quercetin c : quantified as p-coumaric acid. ..

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2 **Table 5** : Polyphenol composition and concentrations (mg/kg of fresh weight) and $\overline{DP}_n$ in the flesh of the twelve apple varieties.

Variety	CAT	EC	PCA	$\overline{DP}_n$	XPL ^a	PLZ	CQA	pCoQA ^b	Total
Baujade	35	162	926	2.9	8	t	336	15	1482
Chanteline	39	98	1128	3.8	2	t	169	19	1456
Freiberg	t	82	474	3.1	6	t	47	11	620
Golden Delicious	t	54	414	3.6	3	t	98	8	578
Granny Smith	42	103	902	3.5	4	5	44	1	1101
Greensleeves	t	43	244	3.2	4	9	86	2	637
Jolyne	19	76	910	3.6	8	13	86	47	1159
New Gold	t	52	355	3.1	12	16	77	27	539
Newton Pippin	23	97	501	3.6	2	16	230	6	874
Sinta	28	175	1075	2.6	3	13	64	6	1364
TMR 7 A 82	15	102	679	3.3	6	32	519	6	1358
3535	43	71	1006	3.5	3	20	430	23	1596
Pooled SD	0.6	1.8	14.0	0.05	0.2	0.5	5.1	0.4	21.4
mean	20	93	718	3.3	5	10	182	14	1043
%	2	9	69	-	0.5	1	17	1	100
SDM	17.3	40.9	308.8	0.4	3.0	9.9	162.0	13.3	426.0

3 CAT : (+)catechin, EC : (-)-epicatechin, PCA : procyanidins, $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins), XPL : phloretin-2-
 4 xyloglucoside, PLZ : phloridzin, CQA : 5'-caffeoylquinic acid, pCoQA : para-coumaroylquinic acid, Total : total polyphenols, SD : standard deviation, n = 24, SDM :
 5 standard deviation of the mean, n=12, t : traces. a : quantified as phloridzin, b : quantified as *p*-coumaric acid.

1 **Table 6** : Polyphenol composition and concentrations (mg/kg of fresh weight) and $\overline{DP}_n$ in the applesauce of the twelve apple varieties.

Variety	CAT	EC	PCA	$\overline{DP}_n$	XPL ^a	PLZ	POPi ^a	TotalFl ^b	CQA	pCoQA ^c	Total
Baujade	14	73	527	2.8	11	36	30	32	256	13	991
Chanteline	16	45	554	3.5	2	17	18	26	126	16	871
Freiberg	t	62	408	3.4	5	18	13	32	55	9	603
Golden Delicious	t	37	346	3.3	4	24	18	20	106	8	562
Granny Smith	31	93	814	3.2	4	5	21	18	55	t	1042
Greensleeves	t	12	72	3.2	7	16	17	14	45	t	182
Jolyne	15	50	647	3.7	9	24	27	32	85	39	930
New Gold	t	16	65	2.6	8	19	15	13	70	9	214
Newton Pippin	t	38	212	2.3	2	23	21	15	140	3	452
Sinta	15	89	595	3.1	2	20	19	20	49	3	812
TMR 7 A 82	9	59	364	3.0	8	49	18	35	384	4	929
3535	27	50	755	3.5	3	33	25	9	408	25	1334
Pooled SD	0.7	1.3	15.2	0.7	0.7	1.0	0.5	1.9	3.7	0.5	17.3
mean	11	52	446	3.1	5	24	20	22	148	11	743
%	1	7	60	-	1	3	3	3	20	1	100
SDM	11.0	25.3	246.8	0.4	3.2	11.3	5.1	9.0	129.5	11.6	346.8

- 2 CAT : (+)-catechin, EC : (-)-epicatechin, PCA : procyanidins, $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins), XPL : phloretin-2-
- 3 xyloglucoside, PLZ : phloridzin, POPi : colourless phloridzin oxidation product, TotalFl : total flavonols, CQA : 5'-caffeoylquinic acid, pCoQA : para-coumaroylquinic acid, ,
- 4 Total : total polyphenols, SD: standard deviation, n = 24, SDM : standard deviation of the mean, n=12, t : traces. a : quantified as phloridzin.. b : . quantified as quercetin. c :
- 5 quantified as *p*-coumaric acid.

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2 **Table 7** : Fishers' F-values and *p*-values associated with two-way ANOVAs (type
3 of product and variety effects, with interactions) performed on apple flesh and applesauce
4 phenolic compositions (mg/kg of fresh weight).

Variable	Variety effect		Type of product effect		Variety x type of product effect	
	F	P	F	P	F	P
(+)-Catechin	154.5	<0.0001	201.3	<0.0001	15.3	<0.0001
(-)-Epicatechin	98.9	<0.0001	434.5	<0.0001	15.0	<0.0001
Procyanidin	73.2	<0.0001	203.9	<0.0001	5.4	0.0004
$\overline{DP}_n$	11.6	<0.0001	26.0	<0.0001	2.5	0.015
Phloretin-2-xyloglucoside	27.9	<0.0001	0.40	0.54	2.9	0.0008
Phloridzin	63.1	<0.0001	388.8	<0.0001	17.2	<0.0001
5'-caffeoylquinic acid	250.3	<0.0001	37.8	<0.0001	5.8	<0.0001
Para-coumaroylquinic acid	313.8	<0.0001	77.3	<0.0001	12.8	<0.0001
Total polyphenols	92.6	<0.0001	162.20	<0.0001	6.1	<0.0001

5 $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins).

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2 **Table 8** : Polyphenol composition and concentrations (mg/kg of fresh weight) and $\overline{DP}_n$ in the particles of the twelve apple varieties.

Variety	CAT	EC	PCA	$\overline{DP}_n$	XPL ^a	PLZ	POPi ^a	TotalFl ^b	CQA	pCoQA ^c	Total
Baujade	16	80	792	3.1	12	43	25	42	275	13	1298
Chanteline	18	48	660	3.5	2	19	15	28	128	17	936
Freiberg	10	65	517	3.5	5	19	11	37	49	8	721
Golden Delicious	t	35	505	3.7	4	25	16	20	96	8	709
Granny Smith	34	95	1029	3.4	4	11	19	14	55	1	1262
Greensleeves	t	11	108	3.3	3	17	17	26	46	t	228
Jolyne	9	53	772	3.9	8	25	26	20	83	38	1035
New Gold	t	16	99	2.4	8	23	13	28	72	10	270
Newton Pippin	11	47	262	2.4	1	27	20	17	152	5	541
Sinta	11	87	756	3.3	2	22	17	40	49	4	988
TMR 7 A 82	t	68	1103	3.4	61	20	30	32	698	9	2020
3535	39	69	1757	3.8	31	46	28	9	659	42	2690
Pooled SD	2.2	2.2	13.7	0.7	0.4	0.4	0.7	2.1	3.5	0.3	13.3
mean	12	56	697	3.3	12	25	21	26	197	13	1058
%	1	5	66		1	2	2	2	19	1	100
SDM	13.0	26.4	465.4	0.5	17.5	10.1	7.7	10.4	234.1	13	708.4

3 CAT : (+)-catechin, EC : (-)-epicatechin, PCA : procyanidins, $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins), XPL : phloretin-2-
 4 xyloglucoside, PLZ : phloridzin, POPi : colourless phloridzin oxidation product, TotalFl : total flavonols, CQA : 5'-caffeoylquinic acid, pCoQA : para-coumaroylquinic acid,
 5 Total : total polyphenols, SD: standard deviation, n = 24, SDM : standard deviation of the mean, n=12, t : traces. a : quantified as phloridzin.. b : quantified as quercetin. c :
 6 quantified as *p*-coumaric acid.

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2 **Table 9** : Polyphenol composition and concentrations (mg/kg of fresh weight) and $\overline{DP}_n$ in the serum of the twelve apple varieties.

Variety	CAT	EC	PCA	$\overline{DP}_n$	XPL ^a	PLZ	POPi ^a	TotalFl ^b	CQA	pCoQA ^c	Total
Baujade	12	58	269	2.4	10	31	31	24	237	12	684
Chanteline	14	37	303	2.8	2	15	15	21	122	16	546
Freiberg	t	50	215	2.6	5	14	11	19	51	8	372
Golden Delicious	t	27	189	2.9	3	19	16	10	92	8	364
Granny Smith	22	68	366	2.5	4	8	18	10	42	t	537
Greensleeves	t	4	45	3.0	2	12	16	5	39	t	123
Jolyne	t	43	359	3.0	7	20	24	21	79	37	590
New Gold	t	43	19	3.1	7	17	14	20	70	7	198
Newton Pippin	t	13	224	2.3	8	43	17	7	365	3	679
Sinta	12	82	354	2.6	2	18	18	10	47	3	545
TMR 7 A 82	t	23	156	2.3	t	19	20	5	127	3	343
3535	15	33	399	3.0	2	27	21	t	344	22	863
Pooled SD	0.3	1.9	4.7	0.7	0.1	0.2	0.3	1.5	0.8	0.1	4
mean	6	40	241	2.7	4	20	18	13	134	10	469
%	1	8	50		1	4	4	3	28	2	100
SDM	8.1	22.3	124.5	0.3	3.0	9.3	5.2	7.9	116.5	10.7	213

3 CAT : (+)-catechin, EC : (-)-epicatechin, PCA : procyanidins, $\overline{DP}_n$: average degree of polymerisation of flavanols (catechins + procyanidins), XPL : phloretin-2-
 4 xyloglucoside, PLZ : phloridzin, POPi : colourless phloridzin oxidation product, TotalFl : total flavonols, CQA : 5'-caffeoylquinic acid, pCoQA : para-coumaroylquinic acid,
 5 Total : total polyphenols, SD: standard deviation, n = 24, SDM : standard deviation of the mean, n=12, t : traces. a : quantified as phloridzin. b : quantified as quercetin. c :
 6 quantified as *p*-coumaric acid.