

Evaluation of Apple Pomace as a Raw Material for Alternative Applications in Food Industries

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Summary

In order to measure the potential of apple pomace as a raw material for manufacturing food-related products (such as lactic acid, fiber-rich concentrates and pectin), samples from cider industry were assayed for composition and enzymatic digestibility. Alcohol-soluble compounds (monosaccharides, oligosaccharides and malic acid) accounted for 32–45 mass percent of oven-dry pomace. Glucose and fructose were the major components of this fraction. The alcohol-insoluble fraction accounted for 55–68 mass percent of oven-dry pomace and was mainly made up of neutral detergent fiber (62–69 percent of the mass fraction) and pectin (16.2–19.7 percent of the mass fraction). The feedstock content of N, P and metal ions (K, Mg, Fe and Mn) was favourable for further manufacture of lactic acid fermentation media. Apple pomace showed a high susceptibility to enzymatic hydrolysis: in media with a cellulase loading of 8.5 FPU/g of apple pomace and a cellobiase loading of 5 IU/FPU, about 80 % of the total glucan was converted into glucose after 15 h. Considering the low enzyme charge, liquor to solid ratio employed, reaction time needed to achieve the maximal sugars concentration and N, P and metal ion (K, Mg, Fe and Mn) content of samples, it can be concluded that apple pomace is a promising raw material for lactic acid production.

Key words: apple pomace, enzymatic digestibility, minerals, sugars, lactic acid, pectin, dietary fiber

Introduction

Apple pomace is the main by-product resulting from pressing apples for juice or cider and it accounts for 25–35 % of the dry mass of apple. In Spain, more than 20 000 metric tonnes of apple pomace are produced every year, which is mainly used as a feed component. Pectin manufacture is the only utilisation currently carried out at an industrial level. According to Kennedy *et al.* (1), the ideal use for apple pomace has yet to be found.

In this context, lactic acid production from apple pomace is an attractive alternative. Lactic acid has a

number of different applications (2), and has an increasing market (3). Cost reduction in lactic acid manufacture can be achieved, for example, by using cheaper and easily hydrolysable raw materials. Apple pomace shows comparative advantages as a raw material for lactic acid manufacture, including: (i) high content of polysaccharides (mainly cellulose, starch and hemicelluloses), (ii) presence of monosaccharides, di- and oligosaccharides, citric acid and malic acid, which can be metabolised by lactic acid bacteria (4), and (iii) rich in metal ions (Mg, Mn, Fe and others), which could limit the cost of nutrient supplementation for fermentation media.

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Any assessment on the potential of apple pomace for further processing must be based on reliable data concerning its average composition and variation range. The composition of apple pomace depends on the varieties and origin of apples and on their ripening degree.

This work deals with the analysis of samples collected at different stages of ripening to measure both average compositional values and variation intervals, and their enzymatic digestibility by cellulase-cellobiase mixtures allowing the production of monosaccharide solutions suitable as fermentation media for lactic acid production.

Materials and Methods

Raw material

Nine samples of apple pomace (belonging to the varieties Pero invierno, Pero verano, Raxoi, Reineta, Reineta roja, Verdeña, and Xoaquina) were seasonally collected (from September 30, 2005 to October 28, 2005) from a local cider factory (Sidrería Gallega, Chantada, Lugo, Spain), dried at 60 °C for 24 h, milled and stored in polyethylene bags at -18 °C until use.

Analytical methods

The scheme of the analytical processing of samples is shown in Fig. 1.

Moisture and ashes were determined according to the methods ISO 638:1978 and ISO 776, respectively. Elemental nitrogen was determined with a Thermo Finnegan Flash EA™ 1112 analyzer, using 130 and 100 mL/min of He and O₂, respectively, and an oven temperature of 50 °C. All determinations were made in triplicate. Protein content was obtained by multiplying the elemental N content by the universal factor of 6.25. Metals and elemental P were analyzed using a fast sequential atomic absorption spectrometer. In selected experiments, samples were digested in an MLS-1200 Microwave Labstation mega with 5 mL of HNO₃ 65 %, 1 mL of H₂O₂ 30 % and 0.5 mL of HF 40 %. Soxhlet extraction with 80 % ethanol (operating at liquor to solid ratio of 30 g/g) led to extracts (E) and to an alcohol-insoluble fraction (AIF), which were processed separately.

Stream E in Fig. 1 was assayed for dry residue by oven-drying at 105 °C (method ISO 638:1978) and for sugars, oligosaccharides, L-malic acid and uronic acids. Monosaccharides were quantified by HPLC using a Hewlett-Packard chromatograph with a refractive index detector

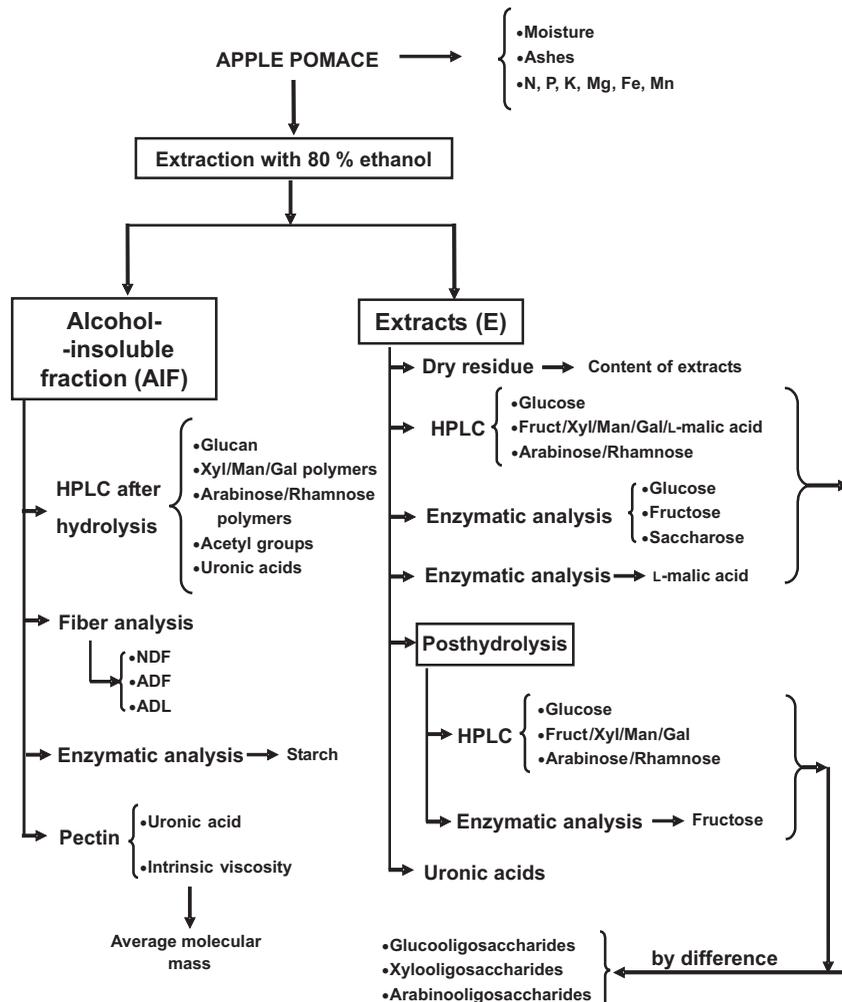


Fig. 1. Analytical procedure used to determine the composition of apple pomace samples

(temperature, 50 °C). Other analysis conditions were: column, ION-300 (Transgenomic, Inc., USA); mobile phase, 0.003 M H₂SO₄; flow, 0.6 mL/min. In HPLC chromatograms, glucose was eluted separately, fructose, xylose, mannose, galactose and malic acid were eluted together in a second peak, and arabinose and rhamnose were eluted together in a third peak. Additional determinations of glucose, fructose and saccharose were performed using the Boehringer Mannheim enzymatic kit reference number 10716260035. The L-malic acid content was measured using the Boehringer Mannheim enzymatic kit reference number 10139068035. Glucoligosaccharides, xylooligosaccharides and arabinooligosaccharides were measured by HPLC determination of the increase in sugar concentration caused by acid posthydrolysis of liquors (5). The results were corrected for fructose decomposition, because this sugar was partially degraded during posthydrolysis. Uronic acids were determined using the method of Blumenkrantz and Asboe-Hansen (6).

Stream AIF in Fig. 1 was subjected to quantitative acid hydrolysis (TAPPI T13m method), and liquors were assayed by HPLC as described above. The results allowed the determination of the contents of glucose polymers (here referred as glucan), hemicellulosic polysaccharides and acetyl groups. Hemicelluloses made up of xylose, galactose and manose units were quantified separately from hemicelluloses made up of arabinose and rhamnose units. Uronic acids were determined using the same method cited above. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the methods of Goering and Van Soest (7). Starch was determined enzymatically (Boehringer Mannheim kit reference number 10207748035). Pectins and pectin properties (intrinsic viscosity and anhydrogalacturonic acid content) were determined according to Hwang *et al.* (8).

Enzymatic hydrolysis

Enzymatic hydrolysis assays of sterilised samples (autoclave treatment at 121 °C for 20 min) were carried out at 48.5 °C in Erlenmeyer flasks with orbital agitation (150 rpm). pH was kept at 4.85 using 0.05 M citric acid/sodium citrate buffer. Enzyme concentrates were Cellu-

clast 1.5 L and NS50010 from Novozymes, Spain. The cellulase activity of Celluclast 1.5 L was measured by the filter paper assay (9) and the activity was expressed in terms of filter paper units (FPU). The β -glucosidase activity of NS50010 concentrates was measured by the *p*-nitrophenyl- β -D-glucopyranoside (pNPG) assay (10) and reported as International Units (IU). Experiments were carried out at liquor to solid ratio of 16 g/g with cellulase and cellobiase loadings of 8.5 UPF/g of apple pomace and 5 UI/UPF, respectively. The total volume of the mixtures was 50 mL. At selected reaction times, samples were withdrawn from the reaction media, centrifuged, filtered and analysed by HPLC by the same method cited above.

Results and Discussion

Sample composition and variability

During the 2005 season, nine samples of pomace from different apple varieties collected at known locations were taken from the press. Most samples came from the Lugo province, in the North-West of Spain, and corresponded to varieties Pero and Reineta.

Apple pomace samples were assayed for moisture, ashes, protein, P and metal ions (see Table 1). The moisture content of samples varied in a narrow range (71.5–78.6 mass percent of wet samples from press) with an average value of 74.4 mass percent. As the pressing conditions were the same in all cases, the variability of data was ascribed to factors such as type of apple and ripening degree. This local industry does not employ externally added enzymes in the process which could affect the juice extraction yields. Ash content was similar in all samples showing an average value of 1.53 %. The contents of protein, P and metal ions are important for this work because they are suitable components for making fermentation media, and their presence in hydrolysates would reduce the need for nutrient supplementation. The experimental data in Table 1 are in agreement with reported data (1), and confirm the expected low protein content (in the range 2.79–4.10 %). The results show that the concentrations of P, Fe and Mg in a suspension con-

Table 1. Compositional data of apple pomace samples

| No. | M ^a | SD | Ash ^b | SD | Prot. ^b | SD | P ^c | SD | K ^c | SD | Mn ^c | SD | Fe ^c | SD | Mg ^c | SD |
|-----|----------------|--------|------------------|-------|--------------------|-------|----------------|--------|----------------|-------|-----------------|-------|-----------------|-------|-----------------|--------|
| 1 | 74.3 | ±0.004 | 1.66 | ±0.03 | 2.94 | ±0.04 | 758 | ±5.30 | 676 | ±0.05 | 3.96 | ±0.16 | 31.8 | ±1.77 | 360 | ±22.87 |
| 2 | 74.4 | ±0.014 | 1.48 | ±0.01 | 2.79 | ±0.04 | 678 | ±27.15 | 578 | ±0.20 | 3.33 | ±0.25 | 29.7 | ±1.08 | 331 | ±5.36 |
| 3 | 72.3 | ±0.001 | 1.47 | ±0.02 | 2.92 | ±0.18 | 756 | ±17.65 | 662 | ±0.18 | 4.09 | ±0.13 | 24.8 | ±0.73 | 381 | ±2.04 |
| 4 | 71.5 | ±0.008 | 1.47 | ±0.01 | 3.40 | ±0.13 | 793 | ±30.46 | 649 | ±0.11 | 4.72 | ±0.14 | 29.7 | ±0.49 | 350 | ±3.54 |
| 5 | 78.6 | ±0.005 | 1.62 | ±0.04 | 3.79 | ±0.04 | 805 | ±30.25 | 654 | ±0.06 | 5.27 | ±0.26 | 27.4 | ±0.99 | 402 | ±8.79 |
| 6 | 75.4 | ±0.002 | 1.50 | ±0.00 | 3.63 | ±0.06 | 861 | ±10.11 | 685 | ±0.20 | 5.00 | ±0.04 | 27.8 | ±0.67 | 435 | ±6.40 |
| 7 | 74.8 | ±0.003 | 1.49 | ±0.04 | 3.77 | ±0.20 | 925 | ±17.43 | 660 | ±0.17 | 5.18 | ±0.10 | 29.7 | ±1.35 | 418 | ±1.82 |
| 8 | 73.3 | ±0.002 | 1.57 | ±0.03 | 3.65 | ±0.04 | 982 | ±25.40 | 729 | ±0.06 | 5.78 | ±0.09 | 27.5 | ±1.48 | 480 | ±6.82 |
| 9 | 74.9 | ±0.004 | 1.53 | ±0.07 | 4.10 | ±0.22 | 1090 | ±44.12 | 678 | ±0.14 | 5.49 | ±0.07 | 33.3 | ±0.35 | 489 | ±6.07 |

^aData expressed as mass percent of pomace as received directly from the press

^bData expressed as mass percent of oven-dry pomace

^cData expressed as mg/kg

SD: standard deviation; N=3; M: moisture; Prot.: protein

taining 100 g pomace/L would exceed the requirements for MRS culture media (usually employed for acid bacteria).

Further information on the composition of different types of pomace was obtained by fractionating the substrates with 80 % ethanol under standard conditions (see Fig. 1). Table 2 shows the experimental data determined for the content in ethanol-soluble compounds of apple pomace (stream E in Fig. 1). The results varied in a

Table 2. Mass fraction of extracts in apple pomace samples

| No. | Mass fraction (percent of oven-dry apple pomace) | SD |
|-----|--|-------|
| 1 | 38.1 | ±0.22 |
| 2 | 31.7 | ±0.09 |
| 3 | 40.5 | ±0.05 |
| 4 | 34.9 | ±0.04 |
| 5 | 44.4 | ±0.18 |
| 6 | 39.2 | ±0.22 |
| 7 | 35.8 | ±0.12 |
| 8 | 40.6 | ±0.29 |
| 9 | 39.7 | ±0.08 |

SD: standard deviation; $N=2$

broad range (from 31.7 ± 0.09) to 44.4 ± 0.18 mass percent of dry matter), with an average value of 38.5 mass percent of dry matter. The practical importance of these data for the purposes of this work comes from the fact that the ethanol-soluble fraction includes sugars and L-malic acid, which would also be extracted in the enzymatic hydrolysis media.

Complementary information on the components of the ethanol-soluble fraction is given in Table 3. Fructose was the major component, accounting for 41.8–52.5 mass

percent of ethanol-soluble compounds. This amount corresponded to 14.9–20.0 mass percent of oven-dry apple pomace, with an average value of 18.1 mass percent. These data are within the range reported by Kennedy *et al.* (1) (variation range, 13–27 mass percent of oven-dry apple pomace). The second most important extractable component was glucose, which accounted for 14.9–22.1 mass percent of ethanol-soluble compounds, corresponding to 5.2–9.0 mass percent of oven-dry apple pomace (average value, 7.4 mass percent). These results are in the range of reported data (1).

Minor amounts of arabinose and rhamnose (joint contribution, 2.4–3.1 mass percent of oven-dry apple pomace), xylose, galactose and mannose (0.3–4.2 mass percent of oven-dry apple pomace) and saccharose (1.3–2.8 mass percent of oven-dry apple pomace) were also present in the samples. Malic acid accounted for 1.0–1.3 mass percent of oven-dry apple pomace. The relative amounts of other components as arabinooligosaccharides are negligible for the purposes of this work. The above results confirm that a substantial part of ethanol-soluble compounds (83–91 mass percent) corresponds to monosaccharides, saccharose and malic acid, which are potential carbon sources for lactic acid bacteria.

The ethanol-insoluble fraction (stream AIF in Fig. 1) accounted for 55.6–68.3 mass percent of apple pomace, and contained mainly polysaccharides (cellulose, starch and hemicelluloses), pectin and lignin (see composition in Table 4). This fraction measures the potential of apple pomace as a raw material for dietetic fiber production or as a substrate for enzymatic hydrolysis of polysaccharides. Owing to the procedure employed for polysaccharide analysis (total saccharification and further HPLC quantification of sugars), no distinction can be made between cellulose and starch (both polysaccharides are converted into glucose upon hydrolysis). In the same way, the glucose-making part of xyloglucan also contributes to the total glucose content of polysaccharides. Be-

Table 3. Composition of the extracts (E)

| Sample | Mass fraction (percent of extract, o.d.b.) | | | | | | | | | | | | | | | | | |
|-------------------------------|--|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| | 1 | SD | 2 | SD | 3 | SD | 4 | SD | 5 | SD | 6 | SD | 7 | SD | 8 | SD | 9 | SD |
| Saccharose | 3.8 | ±0.04 | 4.2 | ±0.15 | 6.8 | ±0.12 | 5.9 | ±0.01 | 4.2 | ±0.07 | 4.3 | ±0.17 | 3.7 | ±0.07 | 5.1 | ±0.04 | 4.2 | ±0.01 |
| Glucose | 19.5 | ±0.02 | 17.6 | ±0.07 | 19.0 | ±0.07 | 14.9 | ±0.06 | 19.2 | ±0.15 | 18.1 | ±0.03 | 18.5 | ±0.04 | 22.1 | ±0.15 | 20.2 | ±0.03 |
| Fructose | 52.5 | ±0.26 | 46.4 | ±0.16 | 49.2 | ±0.39 | 43.7 | ±0.03 | 45.4 | ±0.20 | 47.5 | ±0.05 | 41.8 | ±0.01 | 50.2 | ±0.39 | 47.5 | ±0.12 |
| Xylose, mannose and galactose | 1.2 | ±0.04 | 5.5 | ±0.06 | 0.6 | ±0.08 | 10.2 | ±0.13 | 5.2 | ±0.02 | 4.6 | ±0.18 | 11.7 | ±0.88 | 3.7 | ±0.06 | 1.9 | ±0.06 |
| L-malic acid | 2.6 | n.d. | 3.3 | n.d. | 2.9 | n.d. | 3.1 | n.d. | 2.8 | n.d. | 2.8 | n.d. | 3.1 | n.d. | 3.2 | n.d. | 2.9 | n.d. |
| Arabinose and rhamnose | 7.9 | ±0.25 | 7.2 | ±0.22 | 7.7 | ±0.28 | 7.3 | ±0.13 | 6.7 | ±0.02 | 7.6 | ±0.11 | 8.1 | ±0.06 | 6.9 | ±0.48 | 6.0 | ±0.01 |
| Glucoligosaccharides | 3.8 | ±0.45 | 5.2 | ±0.22 | 5.9 | ±0.18 | 5.1 | ±0.14 | 3.0 | ±0.28 | 6.6 | ±0.07 | 3.3 | ±0.17 | 5.0 | ±0.14 | 6.1 | ±0.19 |
| Xylooligosaccharides | 3.7 | ±0.27 | 2.1 | ±0.15 | 2.6 | ±0.17 | 1.2 | ±0.42 | 1.5 | ±0.34 | 1.8 | ±0.43 | 1.8 | ±0.19 | 1.3 | ±0.31 | 1.4 | ±0.08 |
| Arabinooligosaccharides | 0.4 | ±0.11 | 0.4 | ±0.06 | 0.2 | ±0.01 | 0.5 | ±0.03 | 0.4 | ±0.05 | 0.3 | ±0.02 | 0.4 | ±0.04 | 0.2 | ±0.01 | 0.2 | ±0.03 |
| Uronic acids | 2.7 | ±0.12 | 3.1 | ±0.07 | 2.8 | ±0.15 | 3.2 | ±0.06 | 2.3 | ±0.09 | 2.2 | ±0.16 | 3.3 | ±0.21 | 2.9 | ±0.04 | 3.3 | ±0.20 |

SD: standard deviation; n.d.: no data; $N=3$; o.d.b.: oven dry basis

Table 4. Composition of the alcohol-insoluble fraction (AIF)

| Sample | Mass fraction (percent of AIF, o.d.b.) | | | | | | | | | | | | | | | | | |
|-------------------------------|--|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|
| | 1 | SD | 2 | SD | 3 | SD | 4 | SD | 5 | SD | 6 | SD | 7 | SD | 8 | SD | 9 | SD |
| Glucan | 42.9 | ±0.12 | 49.1 | ±0.24 | 47.4 | ±0.33 | 44.2 | ±0.35 | 34.3 | ±0.18 | 37.3 | ±0.04 | 41.2 | ±0.12 | 38.9 | ±0.15 | 37.0 | ±0.05 |
| Starch | 17.1 | ±0.29 | 22.4 | ±0.85 | 22.1 | ±0.38 | 15.9 | ±0.60 | 10.5 | ±0.53 | 10.7 | ±0.38 | 12.1 | ±0.51 | 10.3 | ±0.46 | 9.8 | ±0.58 |
| XOn ¹ | 13.0 | ±0.18 | 13.4 | ±0.12 | 12.8 | ±0.13 | 14.2 | ±0.08 | 13.4 | ±0.09 | 13.7 | ±0.04 | 13.6 | ±0.25 | 14.2 | ±0.13 | 13.9 | ±0.05 |
| ARn ² | 8.1 | ±0.10 | 8.2 | ±0.22 | 8.8 | ±0.10 | 9.3 | ±0.16 | 9.7 | ±0.13 | 8.7 | ±0.07 | 9.1 | ±0.12 | 9.1 | ±0.24 | 9.6 | ±0.02 |
| Acetyl groups | 1.2 | ±0.04 | 0.0 | – | 0.0 | – | 1.3 | ±0.13 | 1.6 | ±0.09 | 1.5 | ±0.05 | 1.4 | ±0.06 | 1.5 | ±0.04 | 1.6 | ±0.03 |
| Acid-insoluble residue | 17.3 | ±0.08 | 15.5 | ±0.13 | 15.8 | ±0.11 | 17.2 | ±0.14 | 19.0 | ±0.09 | 18.8 | ±0.11 | 19.0 | ±0.08 | 19.1 | ±0.10 | 19.1 | ±0.06 |
| Uronic acids | 14.1 | ±0.21 | 10.8 | ±0.15 | 13.7 | ±0.38 | 13.7 | ±0.19 | 16.9 | ±0.22 | 16.1 | ±0.19 | 14.9 | ±0.11 | 15.5 | ±0.19 | 16.0 | ±0.04 |
| Neutral detergent fiber (NDF) | 68.8 | ±0.08 | 64.6 | ±0.24 | 66.5 | ±0.18 | 66.5 | ±0.24 | 62.1 | ±0.17 | 65.7 | ±0.06 | 65.6 | ±0.23 | 63.5 | ±0.25 | 65.4 | ±0.18 |
| Acid detergent fiber (ADF) | 40.4 | ±0.27 | 36.6 | ±0.22 | 36.6 | ±0.16 | 40.4 | ±0.16 | 42.2 | ±0.08 | 41.8 | ±0.10 | 41.2 | ±0.21 | 40.7 | ±0.06 | 41.4 | ±0.26 |
| Acid detergent lignin (ADL) | 15.8 | ±0.14 | 13.8 | ±0.09 | 16.4 | ±0.14 | 14.6 | ±0.19 | 16.8 | ±0.05 | 16.4 | ±0.13 | 14.8 | ±0.08 | 16.6 | ±0.06 | 17.1 | ±0.09 |

¹XOn: polysaccharides made up of xylose, mannose and galactose

²ARn: polysaccharides made up of arabinose and rhamnose

SD: standard deviation; N=3; o.d.b.: oven dry basis

cause of this, the joint contribution of cellulose, starch and glucose components of xyloglucan are referred to as glucan in this work. Glucan accounted for 34.3–49.1 mass percent of oven-dry AIF, corresponding to 19.1–33.5 mass percent of oven-dry apple pomace (average value, 25.0 mass percent).

Xylose, mannose and galactose moieties, making part of hemicellulosic polymers (*e.g.* arabinoxylan and arabinogalactan), varied in narrow range (12.8–14.2 mass percent of oven-dry AIF), whereas arabinose and rhamnose units (this latter making part of rhamnogalacturonan) accounted jointly for 8.1–9.7 mass percent of AIF. The same data corrected to express the corresponding contents as mass percent of oven-dry pomace led to average values of 8.5 for xylose, mannose and galactose moieties and 5.2 for arabinose and rhamnose moieties.

The results obtained for fibers and starch provided further insight in order to assess the relative proportions of glucose determined by HPLC coming from cellulose, starch or hemicellulosic polymers. Fiber analysis has been used in literature to assess the composition of pomace

(1,11). From data in Table 4, the proportion of cellulose in stream AIF (calculated as the difference between the contents of acid detergent fiber (ADF) and acid detergent lignin(ADL)) varied in the range from 20.2 to 26.4 mass percent of AIF, corresponding to 42.6 to 73.8 percent of the glucan contained in this fraction. Comparatively, lower proportions of glucose came from starch, which accounted for 9.8–22.4 mass percent of AIF. On the other hand, the hemicellulose content of pomaces (calculated as the difference between NDF and ADF) varied in the range 20.0–29.9 mass percent of AIF.

Stream AIF also contained pectin, a backbone of (1→4)- α -D-galacturonan with ramified rhamnogalacturonan regions highly substituted by neutral sugar-rich side chains (12). Table 5 shows the experimental data concerning the pectin content of apple pomace samples, which varied in the range 9.2–12.8 mass percent of dry apple pomace. These results are in good agreement with reported data (1,13). Galacturonic acid accounted for 73.5–79.0 mass percent of pectins, a range of values above the reported data (8,13). Determination of the in-

Table 5. Pectin fraction in pomace samples and parameters measuring the quality of pectins

| Parameter | Sample | | | | | | | | | | | | | | | | | |
|-----------------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | SD | 2 | SD | 3 | SD | 4 | SD | 5 | SD | 6 | SD | 7 | SD | 8 | SD | 9 | SD |
| Pectin fraction ¹ | 11.7 | n.d. | 11.6 | n.d. | 9.7 | n.d. | 12.8 | n.d. | 9.2 | n.d. | 10.3 | n.d. | 11.2 | n.d. | 10.2 | n.d. | 10.4 | n.d. |
| AGA fraction ² | 73.5 | ±0.90 | 76.1 | ±1.29 | 76.6 | ±1.08 | 75.0 | ±1.10 | 77.0 | ±0.35 | 77.1 | ±0.56 | 75.6 | ±1.14 | 75.4 | ±1.98 | 78.9 | ±0.65 |
| Intrinsic viscosity/ (mL/g) | 76.7 | n.d. | 67.2 | n.d. | 70.1 | n.d. | 63.0 | n.d. | 79.6 | n.d. | 90.5 | n.d. | 71.6 | n.d. | 88.4 | n.d. | 79.7 | n.d. |
| Average molecular mass/(g/mol) | 31100 | n.d. | 26300 | n.d. | 27800 | n.d. | 24300 | n.d. | 32400 | n.d. | 38500 | n.d. | 28600 | n.d. | 37400 | n.d. | 32400 | n.d. |

¹Data expressed as mass percent of oven-dry apple pomace

²Anhydrogalacturonic acid fraction in pectins: data expressed as mass percent of oven-dry pectin

SD: Standard deviation; nd: no data; N=3

trinsic viscosity of pectins (with results in the range 63.0–90.5 mL/g), slightly lower than the ones reported by Hwang *et al.* (8), allowed the determination of their mean molecular mass, which was calculated using the equation of Mark-Houwink:

$$\eta = 0.0216 \times M_r^{0.79} \quad /1/$$

where η is the intrinsic viscosity (mL/g) and M_r the average molecular mass (g/mol). This equation is suitable for pectins with M_r values in the range 20 000–200 000 g/mol (14). The experimental results (M_r in the range 24 300–38 500) corresponded to the lower part of the variation range of commercial pectins (20 000–200 000 g/mol) according to Pagan (15). It can be noted that the acidic media employed in the pectin extraction step result in random breakdown of glycosidic bonds (8,16).

Enzymatic digestibility of pomace

The manufacture of culture media from apple pomace with maximal monosaccharide concentrations involves the saccharification of polysaccharides, which can be performed by using enzymes. In this work, a mixture of Celluclast 1.5 L cellulases (with low β -glucosidase activity) and NS50010 β -glucosidase was used in order to achieve the enzymatic hydrolysis of cellulose without ac-

cumulation of cellobiose in the reaction media. Besides β -glucosidase activity, this last complex also contains amylolytic activity, being able to cause the saccharification of starch. Considering different compositions of pomaces, the maximum concentrations of the desired products achieved after total saccharification upon enzymatic hydrolysis (here denoted 'potential concentrations') for a given liquor to solid ratio can be calculated. Table 6a lists the potential concentrations obtainable under the operational conditions employed in this work (liquor to solid ratio, 16 g/g). The potential glucose concentrations varied in a broad range (18.3–26.2 g/L), with an average value of 22.1 g/L. The joint potential concentrations of fructose, xylose, mannose, galactose and malic acid accounted for 18.3 g/L as an average, whereas the joint potential concentrations of arabinose and rhamnose accounted for about 5.4 g/L. As it can be seen, the values for this two last fractions were similar in all samples.

Fig. 2 shows the time course of sugar concentrations corresponding to enzymatic hydrolysis experiments. Significant amounts of sugars were present in the hydrolysis media at the beginning of the reaction, owing to the pomace content of free monosaccharides and malic acid. The experimental results confirmed the high susceptibility of substrates towards enzymatic hydrolysis,

Table 6. Data on enzymatic hydrolysis of apple pomace

a) Potential composition of enzymatic hydrolysates using a liquor to solid ratio of 16 g/g

| No. | $\gamma(\text{glucose})/(\text{g/L})$ | $\gamma(\text{FXO})^1/(\text{g/L})$ | $\gamma(\text{AR})^2/(\text{g/L})$ |
|-----|---------------------------------------|-------------------------------------|------------------------------------|
| 1 | 22.6 | 18.8 | 5.2 |
| 2 | 26.2 | 16.7 | 5.1 |
| 3 | 24.4 | 18.2 | 5.4 |
| 4 | 22.9 | 18.0 | 5.6 |
| 5 | 18.3 | 19.3 | 5.4 |
| 6 | 20.5 | 18.6 | 5.4 |
| 7 | 21.9 | 18.1 | 5.7 |
| 8 | 21.6 | 19.5 | 5.3 |
| 9 | 20.7 | 18.1 | 5.3 |

b) Enzymatic saccharification yields measured at 48 h

| No. | $Y_G^3/\%$ | SD | $Y_{\text{FXO}}^4/\%$ | SD | $Y_{\text{AR}}^5/\%$ | SD |
|-----|------------|------------|-----------------------|------------|----------------------|------------|
| 1 | 84.7 | ± 2.24 | 80.3 | ± 0.88 | 58.6 | ± 2.22 |
| 2 | 81.5 | ± 0.47 | 76.0 | ± 0.44 | 50.6 | ± 0.01 |
| 3 | 87.6 | ± 0.03 | 89.4 | ± 1.50 | 58.5 | ± 1.63 |
| 4 | 81.7 | ± 0.60 | 71.3 | ± 0.76 | 46.6 | ± 3.47 |
| 5 | 86.4 | ± 1.60 | 90.1 | ± 1.96 | 55.0 | ± 0.47 |
| 6 | 82.7 | ± 1.42 | 79.8 | ± 0.06 | 55.4 | ± 0.49 |
| 7 | 85.1 | ± 0.63 | 78.2 | ± 0.32 | 51.6 | ± 0.42 |
| 8 | 80.0 | ± 1.13 | 80.8 | ± 0.46 | 56.0 | ± 0.89 |
| 9 | 82.5 | ± 0.84 | 86.5 | ± 1.25 | 52.0 | ± 0.15 |

¹FXO includes fructose, xylose, mannose and galactose

²AR includes arabinose and rhamnose

³Calculated as percentage of potential glucose concentration

⁴Calculated as percentage of the sum of potential concentrations of fructose, xylose, mannose, galactose and malic acid

⁵Calculated as percentage of the sum of potential concentrations of arabinose and rhamnose

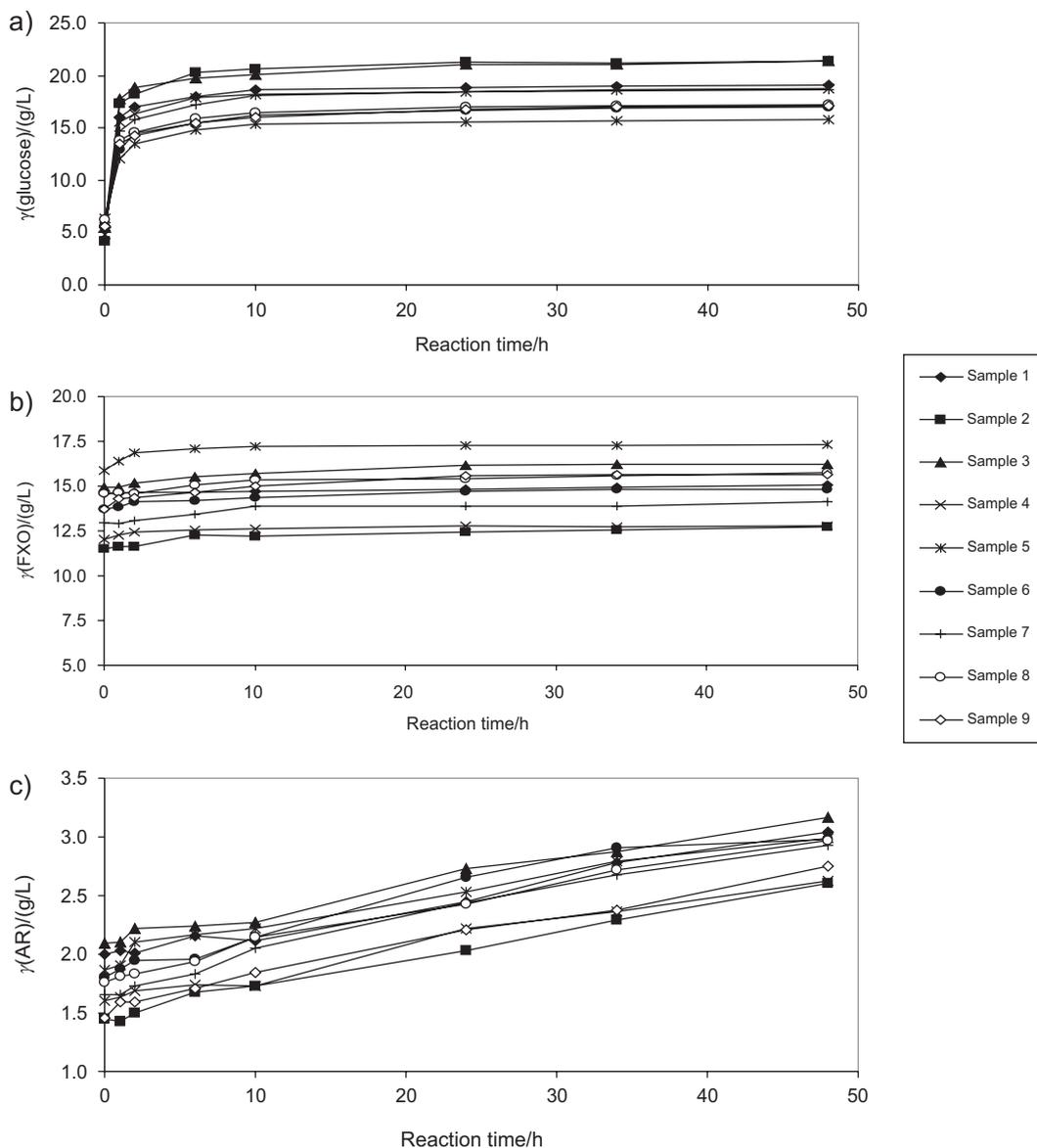


Fig. 2. Time course of sugars and malic acid concentration in enzymatic hydrolysates: a) glucose, b) fructose, xylose, mannose, galactose and malic acid, c) arabinose and rhamnose, $N=2$

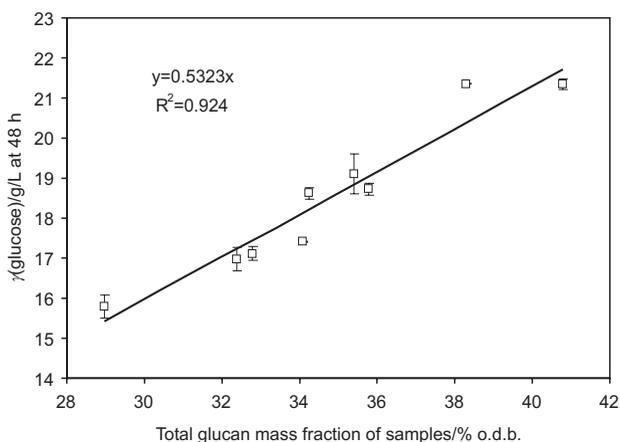


Fig. 3. Dependence of glucose concentration measured at 48 h in hydrolysates on total glucan mass fraction in samples, $N=2$

with a high initial rate and flat concentration profiles for glucose after 10–15 h. Different profiles were observed for FXO and AR fractions.

Table 6b lists the enzymatic saccharification yields measured at 48 h that can vary in a narrow range (80–87.6 % of the total glucan mass fraction). The experimental data showed a fairly linear interrelationship between the total glucan mass fraction of the samples and the glucose concentration achieved at 48 h of reaction, as it is shown in Fig. 3. The conversion of the fraction made up of fructose, xylose, mannose, galactose and malic acid accounted for 71.3–90.1 % of the potential values, whereas for fraction containing arabinose and rhamnose accounted for 46.6–58.6 % of the theoretical amounts. Taking into account the increase in glucose concentrations, it can be deduced that between 73 and 86 % of glucan (present as polysaccharide) was hydrolysed. Xylan and arabinan presented lower enzymatic digestibility.

Conclusions

The composition of apple pomace, its variability and enzymatic digestibility were determined in order to evaluate its potential as a raw material for manufacturing food-related products (such as lactic acid, fiber-rich concentrates and pectin). Alcohol-soluble compounds were mainly made up of glucose and fructose (two carbon sources readily fermentable by lactic acid bacteria), whereas the alcohol-insoluble fraction was mainly made up of neutral detergent fiber. The feedstock contents of N, P and metal ions (K, Mg, Fe and Mn) were favourable for further manufacture of lactic acid fermentation media. Apple pomace showed high susceptibility to enzymatic hydrolysis. Owing to the compositional differences among the samples, the final glucose content of enzymatic hydrolysates obtained under the same operational conditions may differ in up to 35 %. The results derived from this experimental study confirm that apple pomace is a promising raw material for lactic acid production.

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