Comparing the impact of high pressure, pulsed electric field and thermal pasteurization on quality attributes of cloudy apple juice using targeted and untargeted analyses

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ABSTRACT

The impact of low-oxygen spiral-filter press technology combined with thermal pasteurization (TP), pulsed electric field (PEF) and high pressure processing (HPP) on cloudy apple juice quality was investigated immediately after the treatments and after 3 weeks of storage at 4 °C. Based on equivalent levels of microbial safety and desired shelf-life, low and high processing intensities were selected: TP (72 °C/15 s; 85 °C/30 s), PEF (12.5 kV/cm, 76.4 kJ/L; 12.3 kV/cm, 132.5 kJ/L), and HPP (400 MPa/3min; 600 MPa/3 min). High intensity thermal treatment resulted in a bright, yellowish color which was maintained during storage. PPO and POD activities were largely reduced by high intensity PEF and TP yet showed high resistance to HPP. The highest vitamin C content was provided by fresh juice followed by PEF-treated juices. Due to oxidative degradation reactions, vitamin C of all treated samples significantly decreased during storage. Immediately after processing, high cloud stability values were obtained in all samples; however, cloud stability decreased during storage particularly for HPP juices with high residual PME. No significant changes were observed in pH, titratable acidity, organic acid and sugar content which also corresponded to sweet and sour taste. Results from untargeted volatile profiles showed that esters increased after PEF and were better retained after HPP. Contrary to TP treatment where ester degradation reactions occurred together with the formation of off-flavors. Most of the volatiles decreased during storage which could be linked to oxidation and ester hydrolysis reactions.

Industrial relevance

Being one of the most popular fruit juices consumed worldwide, cloudy apple juice can still undergo quality changes such as color degradation, cloud loss (fast sedimentation) and flavor changes during processing and storage. This study evaluates the potential of low-oxygen spiral-filter press in combination with different preservation technologies to obtain a maximal quality of cloudy apple juice. Results shows that high intensity thermal pasteurization can effectively inactivate quality-degrading enzymes, therefore it is useful to obtain an optimal cloudy apple juice product in terms of color and cloud stability. Although HPP has minimal impact on aroma of the juice, shelf-life of the juice may be limited due to incomplete enzyme inactivation. In the case of PEF treatment, thermal effects may contribute to maintain apple juice quality.

Keywords:

Cloudy apple juice, quality, HPP, PEF, pasteurization, storage

1 INTRODUCTION

Cloudy apple juice is one of the most popular fruit juices consumed worldwide due to its fresh-like taste, mouthfeel and nutritional value (De Paepe et al., 2015). It is usually produced by thermal processing which aims at inactivation of spoilage microorganisms and enzymes hence increasing shelf-life of these products

(Aguilar-Rosas, Ballinas-Casarrubias, Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007). However, this treatment affects the quality of the juice during processing and storage leading to quality degradative reactions such as color changes, cloud loss and loss of flavor (Krapfenbauer, Kinner, Gössinger, Schönlechner, & Berghofer, 2006; Su & Wiley, 2006; Aguilar-Rosas et al., 2007). In recent times, the growing awareness of the consumption of healthy foods have driven consumers to patronize fresh-like foods with high nutritional value, minimally processed and free from additives. This trend for healthy, minimally processed foods has urged the food industry and researchers to explore novel techniques to produce foods with fresh-like quality at the same time ensuring microbial safety to satisfy the needs of consumers. In this regard, the fruit juice industry has been investigating alternative, non-thermal processing technologies including pre-treatment techniques that result in fresh-like products with longer shelf-life. Among these techniques, high-pressure processing (HPP) has been recognized as one of the frequently applied alternative techniques to conventional thermal processing (Barba et al., 2012; Landl, Abadias, Sárraga, Viñas, & Picouet, 2010; Terefe, Buckow, & Versteeg, 2014). Moreover, the potential of pulsed electric field (PEF) for commercial applications in the processing of fruit and vegetables had been investigated (Schilling et al., 2008; Kempkes, 2010).

Studies over the past two decades have proposed that HPP and PEF provide products with quality attributes comparable to freshly squeezed juices with extended shelf-life (Bi et al., 2013; Landl et al., 2010; Nienaber & Shellhammer, 2006; Vervoort et al., 2011; Turk, Vorobiev, & Baron, 2012). From the available literature, it is a common practice for food industry to use food additives to prevent quality changes and thereby extending shelf-life (Ephrem, Najjar, Charcosset, & Greige-Gerges, 2018). To satisfy the consumer needs for fresh-like, minimally processed and clean label juice without using additives, use of alternative pre-treatment technology such as low-oxygen spiral-filter press processing in combination with HPP and PEF technologies could be an interesting option. It has been reported that this juice pressing technology is beneficial to prevent discoloration, retain flavor and bioactive compounds and provide juice of high yield (Kips et al., 2017; De Paepe et al., 2015), thus it could be seen as a promising technology in fruit juice processing.

The aim of this study is to compare the impact of low-oxygen pre-treatment combined with PEF, HPP and conventional thermal processing on quality attributes of cloudy apple juice, produced at pilot scale. Furthermore, an analysis of the quality attributes under refrigerated storage was conducted. High and low levels of processing intensity were selected depending on the targeted shelf-life (refrigerated or shelf-stable products) and equivalent microbial safety. Based on the FDA guidelines, a 5-log reduction must be targeted to the "pertinent pathogen" that is the most resistant microorganism of public health significance likely to be present in the juice (FDA, 2001). In apple juice, the pertinent microorganism is *Escherichia coli* O157:H7 (McLellan M.R & Padilla-Zakour O.I., 2005).

Quality changes of apple juice were investigated using targeted and untargeted approaches. In the targeted approach, particular attributes selected at a starting point of the investigation were focused on. In this study, the targeted quality attributes included color, cloud and taste related attributes. The

targeted approach can provide valuable information, in addition to that, it is of interest to understand other possible unexpected and unknown effects due to treatment or storage on food quality attributes. This is of particular importance in the case of 'novel processing', where (pre-)processing effects have not been completely explored yet. This approach is known as untargeted fingeprinting approach, which considers all detected, not predetermined compounds and thus it is a more hypothesis-free technique (Grauwet, Vervoort, Colle, Van Loey, & Hendrickx, 2014). In the current study, the untargeted approach was performed by analyzing changes in the volatile fraction of apple juice samples using a headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technique. This approach has been demonstrated to be able to obtain insight into chemical reactions which are influenced by processing or storage (Wibowo, Grauwet, Kebede, Hendrickx, & Van Loey, 2015a; Kebede et al., 2014)

2 MATERIALS AND METHODS

2.1 Cloudy apple juice preparation and processing.

Belgian apple cultivars ('Keuleman'~12.3%, 'Pomme Henri'~19.6%, 'Boskoop'~18.0%, 'Court Pendu'~3.6%, 'Bellefleur'~6.4%, 'Cgascoigner'~5.0%, 'Zaailing'~5.3%, and 'Jonagold'~29.9%) were purchased from a local supplier and stored in a cold room until use. Each cultivar, in total around 530 kg, was emptied into plastic container filled with fresh tap water. Apples were washed, mixed and sorted before dividing into three batches in which were each subjected to a different type of treatment: thermal pasteurization (TP), high pressure processing (HPP) and pulsed electric field (PEF) treatments (**Table 1**).

In the production of cloudy apple juice, at first, apples were shredded into mash with a Multicut system (Bruckner Liquid Food Tech, VaculiQ GmbH & Co. KG, Hamminkeln, Germany). The shredding was performed under an inert atmosphere using a nitrogen gas to minimize the deteriorative effect of oxygen on quality attributes during juicing. Subsequently, the mash was immediately transferred into a buffer tank before being pressed and extracted under vacuum conditions with a one-stage low-oxygen spiral-filter press system (VaculIQ 1000, VaculIQ, Hamminkeln, Germany). Optimized process parameters were used for obtaining cloudy juice with high solids content: feed pump frequency 12 %, spiral frequency 100 % or 55.8 rpm, vacuum pump frequency 100 % or 750 mbar, pore size of the filter element 100 µm and 4 channels of the spiral with a shaft inclination of 25 to 38°. Juice was collected in a buffer tank and the apple pomace was ejected at the top of the spiral. Untreated juice and juice for HPP were filled into 500 mL polyethylene terephthalate (PET) bottles with UV blocker (Resilux NV, Wetteren, Belgium). All bottles and caps used in this study were sterilized by gamma irradiation (Synergy Health, Etten-Leur, The Netherlands).

2.1.1 Thermal pasteurization (TP) treatment

Thermal treatments were conducted in a multipurpose UHT pilot plant unit (APV SPP, SPX Corporation, Gatwick, United Kingdom). Two levels of processing intensity were selected: a low intensity (72 °C/15 s)

aiming at destruction of pathogenic and spoilage microorganisms therefore intended for cold storage and a high intensity (85 °C/30 s) to destroy pathogens and inactivate endogenous enzymes resulting in shelf-stable juice. For low intensity treatment (TP1), juice from the buffer tank was preheated to 65 °C, and subsequently heated in a tubular heat exchanger to 72 °C for 15 s. For the second treatment (TP2), apple juice was pasteurized at 85 °C for 30 s which aimed at the destruction of pathogenic microorganisms and the inactivation of endogenous enzymes. After passing the holding tube, the juice was cooled down to 4 °C and manually packed into 500 mL PET bottles.

2.1.2 High pressure processing (HPP) treatment

Previous studies indicate that pressure treatments of 350-500 MPa lasting 1-5 min is required to inactivate *E. coli* in apple juice (Bayindirli, Alpas, Bozoglu, & Hizal, 2006; Ramaswamy, Riahi, & Idziak, 2006; Jordan, Pascual, Bracey, & Mackey, 2001). In the food industry, treatments of 500 – 600 MPa have yielded good quality and safety of food products thus 400 and 600 MPa for 3 min were selected for low (HPP1) and high intensity (HPP2) treatments, respectively. HPP treatments were conducted in an industrial Wave 6000/55 unit (55 L, 20 cm inner diameter, Hiperbaric, Burgos, Spain). First, untreated bottled juice was loaded into perforated cylindrical horizontal vessels (polyethylene, 18 cm outer diameter, 85 cm outer length). Next, the pressure vessel begins to fill with water with the aid of a pressure pump until the targeted pressure is achieved. Both conditions were set at room temperature.

2.1.3 Pulsed electric field (PEF) treatment

Regarding PEF treatments, it has been reported that treatment of 25-40 kV/cm for 100-400 µs on apple juice could be used to inactivate *E. coli*. (Garcia, Hassani, Manas, Condon, & Pagan, 2005; Sen Gupta, Masterson, & Magee, 2003). In this study, the selection of the processing parameters was based on microbial and enzymatic inactivation. A continuous flow pilot scale unit (HVP 5 kW Elea, Quakenbrueck, Germany), consisting of two collinear treatment chambers with 10 mm electrogap and a 10 mm diameter, was used for PEF processing. A spiral feed pump was connected to the system (Seepex GmbH, bottrop, Germany) providing a flow rate of 24.5-27.6 L/h at a frequency of 94 and 62 Hz, respectively. Electric field strength of 12.5 kV/cm and energy input of 76.4 kJ/L were applied to the cloudy apple juice with inlet and outlet temperatures of 37.6 and 59.5 °C, respectively, for the low intensity treatment (PEF1). For the high intensity treatment (PEF2), electric field strength at 12.3 kV/cm and energy 132.5 kJ/L were selected with inlet and outlet temperatures of 37.3 and 73.8 °C, respectively. A pulse width of 2 µs in bipolar mode was applied. Given the importance of enzyme inactivation in case of apple juice, processing conditions resulting in a temperature increases close to thermal processing were deliberatedly chosen.The treated juice was immediately cooled down to 4 °C and the juice was filled into a 500 mL of PET bottle under hygienic conditions in a laminar air flow cabinet.

2.1.4 Storage and sampling

Untreated samples were stored at −40 °C serving as the control samples. All treated samples were stored in a refrigerator at 4 °C for 3 weeks. At the end of the storage period, bottles were randomly sampled and the juice was transferred to smaller tubes which were frozen in liquid nitrogen and stored at −40 °C. At the time of analysis, the frozen samples were thawed in a circulating water bath at 25 °C and once more homogenized. After treatments and during storage, the microbiology load (total aerobic psychrotrophic, lactic acid bacteria, aerobic sporeforming bacteria, yeasts and molds) was evaluated to ensure the microbial quality of the samples (**Supplementary 1**).

2.2 Targeted quality analyses

2.2.1 Color measurement

Color of apple juice was measured using a UV–Vis spectrophotometer (Sensing Unveils CM-5, Konica MinoltaSensing, Osaka, Japan). Around 40 mL of juice was poured into a glass cylindrical container and placed on the top-port. The CIE color coordinates *L**, *a** and *b** components were recorded. The *L** value represents the degree of lightness, varying from 0 (black) to 100 (white). The *a** value gives the degree of greenness (negative) to redness (positive) and the *b** value indicates the degree of blueness (negative) to yellowness (positive). Color can also be expressed as chroma (C^*_{ab}) or saturation index which is propotional to its intensity and as hue angle (h_{ab}) in which 0° or 360° for red, 90°, 180° and 270° for yellow, green and blue, respectively.

$$C^*_{ab} = \sqrt{a^{*2} + b^{*2}} \quad \text{(Eq. 1)}$$

$$h_{ab} = \arctan b^*/a^* \quad \text{(Eq. 2)}$$

2.2.2 Turbidity and cloud stability determination

Turbidity or cloudy appearance and the degree of cloud stability of juice samples was determined according to the procedure by Bhat & Goh (2017) with slightly modification. The turbidity was measured at 660 nm using a spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences) and calculated according to **Equation 3**, where distilled water was used as a blank. For the cloud stability, 10 mL of juice was centrifuge at at 4,200×*g* for 10 min at 25 °C. Subsequently, the supernatant was collected and measured at 660 nm using a spectrophotometer. The cloud stability was reported as relative turbidity (%T) where T_0 and T_c are the juice turbidities before and after centrifugation (De Paepe et al., 2015a). Measurements were performed in triplicate.

$$Transmittance = 100 \times 10^{-Abs} \quad \text{(Eq. 3)}$$

$$Turbidity\ (T) = 100 - Transmittance \quad \text{(Eq. 4)}$$

$$\%T = (T_c/T_o \times 100) \quad \text{(Eq. 5)}$$

2.2.3 Particle size distribution determination

The particle size distribution (PSD) was determined using a laser diffraction particle size analyzer (LS 13320, Beckman Coulter Inc., Brea, CA) equipped with a Universal Liquid Module. All samples were shaken before adding dropwise into a stirred tank filled with demineralized water until a polarization intensity differential scattering (PIDS) obscuration of 40% was reached. Subsequently, the diluted sample was pumped into the measuring cell. The volumetric PSD was calculated based on the intensity profile of the scattered light (wavelength main illumination source: 750 nm; wavelengths halogen light for PIDS: 450 nm; 600 nm; 900 nm) using the Fraunhofer model. In this work, the particle sizes were expressed as D[v,0.1], D[v,0.5] and D[v,0.9] values which indicate the particle diameter at which 10, 50 and 90 vol.% of the particles have a smaller diameter, respectively.

2.2.4 Enzyme activity measurements

2.2.4.1 Polyphenol oxidase (PPO) activity

PPO activity was analyzed according to the method applied by Liu, Wang, Li, Bi, & Liao (2014) with some modifications. The enzyme was extracted from the apple juice by mixing 1 mL juice with 400 μL 0.2 M sodium phosphate buffer pH 6.5 containing 1 M NaCl and 1% PVPP. After vortexing, the mixture was centrifuged at 16,000×*g* for 15 min at 4 °C (Microfuge 22R, Beckman Coulter). The supernatant was collected and analyzed for enzyme activity. The extraction was performed in triplicate. The PPO activity of the enzyme extract was measured spectrophotometrically by adding 2.8 mL substrate solution (0.05 M catechol in 0.2 M phosphate buffer, pH 6.5) and 200 μL enzyme extract to a 1 cm path cuvette. A UV/Vis spectrophotometer was used to monitor the changes in the absorbance at 420 nm at 25 °C for 3 min. The PPO activity was determined from the linear section of the activity curve.

2.2.4.2 Peroxidase (POD) activity

POD activity was determined based on the method of Yi et al. (2017) with some modifications. The enzyme was extracted in triplicate in a similar way as PPO extraction. The reaction mixture consisted of 200 μL extract and 2.8 mL substrate solution containing 0.2 M sodium phosphate buffer (pH 6.5), 0.3% (w/v) *o*-phenylenediamine and 0.1% (v/v) hydrogen peroxide. The formation of the colored oxidation product (2,3-diaminophenazine) was measured immediately using a spectrophotometer at 485 nm at 25 °C for 3 min. The POD activity of each extract was measured in duplicate.

2.2.4.3 Pectin methylesterase (PME) activity

PME was assayed using the method reported by Vervoort et al. (2011). First, 1 mL juice was added to 30 mL of a 0.35% (w/v) apple pectin solution, containing 0.117 M NaCl. The pH of the mixture was maintained constant by addition of 0.01 N NaOH using an automatic pH-stat titrator (718 STAT titrino, Metrohm, Herisau, Switzerland). The PME activity was determined by the amount of enzyme required to release 1 μmol of carboxyl group per min during the pectin hydrolysis as a function of time at pH 7.0 and 22 °C. The PME activity of each sample was measured in triplicate. Relative residual activities of PPO, POD and PME were evaluated as:

$$\%\ Residual\ activity\ = \frac{Enzyme\ activity\ after\ treatment}{Enzyme\ activity\ in\ the\ untreated\ juice} \times 100\% \qquad \text{(Eq. 6)}$$

2.2.5 Total soluble solid (TSS) and sugar profile determination

Total soluble solids content (°Brix) was measured in triplicate using a digital refractometer (RX-7000α, Atago, Tokyo, Japan) at 20 °C.

The sugar profile was analyzed according to the method of Wibowo et al. (2015b). First, 10 mL juice was mixed with 500 µL of each Carrez I (15% w/v K4[Fe (CN)6]) and Carrez II (30% w/v ZnSO4). After resting for 30 min, the mixture was centrifuged at 24,000×g for 15 min at 4 °C. The supernatant was filtered through a 0.45 µm syringe filter (Chromafil A-45/25, Macherey-Nagel, Düren, Germany). A 10-fold dilution of the filtrate in milli-Q water was made prior to analysis in RP-HPLC system (Agilent 1200 series, Diegem, Belgium) coupled with evaporative light scattering detection (Alltech 3300 ELSD, Grace, Deerfield, IL, USA). Sugar extract (5 µL) was separated on a Prevail carbohydrate ES column (250 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, IL, USA) coupled to a guard cartridge using an isocratic elution (75% (v/v) acetonitrile/water) at 30 °C. The flow rate was set at 1 mL/min. Analyzes were carried out in triplicate. For identification, retention times were compared with glucose monohydrate, fructose and sucrose standard solutions. For quantification, calibration curves of standard solutions were used.

2.2.6 pH, titratable acidity (TA) and organic acid profile determination

The pH measurements were performed in triplicate at room temperature using a pH meter (Meterlab PHM210, Radiometer Analytical, Villeurbanne, France).

Titratable acidity, expressed as percent malic acid, was determined based on AOAC method 962.12 (AOAC, 1998) and calculated based on **Equation 7**. Ten gram of juice was diluted with 250 mL deonized water. After adding one to two drops of phenolphthalein indicator, analysis was done by titrating juice samples with 0.1 N NaOH until the juice color changed to pale pink (pH 8.2). Samples were analyzed in triplicate.

$$\%\ malic\ acid = \frac{Volume\ NaOH\ (ml)\ \times 0.1(N\ NaOH)\ \times 0.067}{Juice\ weight\ (g)} \times 100\% \qquad \text{(Eq. 7)}$$

The determination of organic acids was following the procedure of Wibowo et al. (2015b). The extraction procedure of organic acids was the same as that of sugar profile analysis. Two microliters of the extract was analyzed using RP-HPLC (Agilent 1200 series, Diegem, Belgium) equipped with a Prevail Organic Acid column (250 mm × 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, USA) protected with a guard cartridge (7.5 mm× 4.6 mm, 5 µm particle size, Alltech Grace, Deerfield, USA). Separation occurred at 25 °C by isocratic elution (25 mM potassium dihydrogen phosphate buffer pH 2.5) at a flow rate of 1 mL/min. A UV-DAD detector at 210 nm was used. All samples were analyzed in triplicate. Identification and quantification were performed based on retention times and a calibration curve of standard solutions.

2.2.7 Vitamin C determination

Total vitamin C was determined using the method of Wibowo et al. (2015b). Five milliliters of juice was mixed with 15 mL extraction buffer (1% w/v meta-phosphoric acid with 0.5% oxalic acid adjusted to pH 2.0). After a centrifugation step at 24,000×*g* for 15 min at 4 °C, the supernatant was filtered through a syringe filter and stored at −80 °C. Extraction was performed in triplicate. Five mL of the supernatant was adjusted to pH 3.5 and then divided into two parts. For ascorbic acid analysis, 2 mL of phosphate buffer (20 mM NaH_2PO_4 + 1 mM Na_2EDTA, pH 3.5) was added into 1 mL of the pH-adjusted supernatant. To analyze the total vitamin C, 2 mL TCEP (2.5 mM tris (2-carboxyl-ethyl) phosphine in phosphate buffer, pH 3.5) was added into 1 mL of the pH-adjusted supernatant. The mixture was centrifuged at 19,900×*g* for 15 min at 23 °C. Both extracts were filtered through a 0.45 µm syringe filter before injection to RP-HPLC/UV detection (DionexBioLC, Sunnyvale, CA). A prevail C18 column (250 mm × 4.6 mm, 5 µm particle size, Grace, Columbia, MD) coupled to corresponding guard column was used for chromatographic separation. An isocratic elution (1 mM Na_2EDTA and 10 mM CH_3COONH_4) was applied at a flow rate of 0.8 mL/min (25 °C). The injection volume was 25 µL and detection of the compounds was performed at 245 nm. For quantification, calibration curves of external standard solutions of ascorbic acid (99% Acros organics, Geel, Belgium) were used.

2.2.8 Sensory analysis

The panel consisted of 34 people (7 men and 27 woman) with age ranging from 23 to 58 years old, all working at the Institute of Agriculture, Fisheries and Food Research. The sensory evaluation was conducted in a room at constant temperature and panel members were seated in individually partitioned booths. Red light was used to masked color differences between the samples which were produced the week before. The panel was asked to score the taste of apple juice on a 10 cm line scale (0 meaning absent and 10 meaning very noticeable present) in regards to sweet, sour, and bitter taste. The sample treated with the lowest PEF was not included in the sensory analysis due to a high amount of total aerobic psychotropic colony-forming units. The results were analyzed by the software Fizz Calculation (BioSystèmes, Couternon, France) using the Duncan test on a 95% significance level.

2.3 Headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) analysis

Headspace fractions were analyzed using gas chromatography (GC) system (7890N, Agilent technologies, Diegem, Belgium) coupled to a mass selective detector (MSD) (5977N, Agilent Technologies, Diegem, Belgium) and equipped with a CombiPAL autosampler (CTC analytics, Zwingen, Switzerland). Apple juice (1.5 mL) and a saturated NaCl solution (1.5 mL) were pipetted into an amber glass vial (10 mL, VWR International, Radnor, PA, USA). The vials were tightly closed using screw-caps with silicone septum seal (GRACE, Columbia, MD, USA), vortexed and placed in the cooling tray of the autosampler (10 °C). Samples were equilibrated in the incubator at 40 °C for 10 min under agitation at 500 rpm. Subsequently, volatiles

were extracted using a SPME fiber coated with 85 µm CAR/PDMS (StableFlex, Supelco, Bellefonte, PA, USA) for 5 min at 40 °C. The volatiles were injected into the GC column in split-mode with a split-ratio of 1/5 and separated on HP-INNOWAX capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent Technologies, Santa Clara, CA, USA) using helium as carrier gas at a flow of 1.27 mL/min. Starting temperature in the GC-oven was set at 40 °C, for 2 min, following increase at a rate of 4 °C/min to 120 °C and then ramped to 200 °C at 7 °C/min and finally ramped to 250 °C at 50 °C/min where it is kept constant for 2 min at before cooling down to 40 °C. The mass spectra were obtained by electron ionization (EI mode) at 70 eV with a scanning range of 35 to 400 m/z. The ion source and quadrupole temperatures were 230 and 150 °C, respectively. A new fiber was used for each storage condition (before and after storage). During the analysis, the samples were randomized as a function of treatment per storage time. The GC-MS analysis of each sample was repeated six times.

2.4 Statistical data analysis

To evaluate the impact of the different processing treatments on targeted quality parameters, one way analysis of variance (ANOVA) was performed. Differences between the means were compared according to Tukey's multiple comparison test at level of significance of 95% ($p < 0.05$). Both analyses were carried out using JMP software (JMP Pro 13.1 statistical software, SAS Institute, Chicago, IL).

2.5 Data pre-processing and multivariate data analysis (MVDA)

GC-MS chromatograms were analyzed with automated mass spectral deconvolution and identification system (AMDIS Version 2.72, 2014, National Institute of Standards and Technology, Gaithersburg, MD, USA) for peak deconvolution and with Mass Profiler Professional (MPP) (Version 12.0, 2012, Agilent Technologies, Diegem, Belgium) for filtering and peak alignment. For a detailed explanation in the integrated data pre-processing steps, AMDIS and MPP, the reader is referred to the works of Wibowo, Grauwet, Kebede, Hendrickx, & Van Loey (2015a).

A data table combining the information on peak area for every peak detected per sample was used as an input for MVDA. The data analysis was performed using Solo software (Version 8.5.2, 2017, Eigenvector Research, Wenatchee, WA, USA). First, all data were mean-centered and the variables were weighed by their standard deviation to give them equal variance. To evaluate each data set and to detect potential outliers, principal component analysis (PCA) was applied. To compare processing impact, partial least squares discriminant analysis (PSL-DA) was performed with the volatile compounds as *X*-variables and untreated (control), thermal pasteurization (TP), high pressure processing (HPP), and pulsed electric field (PEF) treatments as categorical *Y*-variables. Latent variables (LVs) were added to the model until they at least contributed more than 2% of the *Y*-variance of the selected model. Biplots were constructed for graphical representation of differentiation among the classes using OriginPro 8 (Origin Lab Corporation, Northampton, MA, USA). To quantitatively select discriminant volatiles, Variable Identification (VID) coefficients were subsequently calculated. Volatiles with an absolute VID coefficient higher than 0.80 were considered important (i.e. markers) and were further identified by comparing the deconvoluted mass spectrum with the reference mass spectra from NIST spectral library (NIST14, version 2.2, National

Institute of Standards and Technology, USA) and Wiley spectral library (Wiley 2010, version 9, USA). Compounds with a match and reverse match of above 80% were used together with visual inspection of the spectral matching between the detected compound and the match from the library. Each marker was plotted individually and to test for significant differences between mean peak areas, Tukey's multiple comparison test was used.

3 RESULTS AND DISCUSSIONS

3.1 Comparing the impact of treatments and storage on quality attributes of cloudy apple juice: a targeted approach

3.1.1 Impact on color related attributes

3.1.1.1 Color

Table 2 shows that high intensity thermally-pasteurized (TP2) juice had the highest level of lightness followed by low intensity TP- and PEF-treated juices. In contrast, untreated (control) sample had the darkest color which attributed to the enzymatic browning reaction by polyphenol oxidase (PPO) and/or peroxidase (POD). This finding suggests that color of apple juice was retained due to inactivation of oxidative enzymes by thermal processing. Apple juice contains polyphenolic compounds including components such as quercetin glycosides, catechins, chlorogenic acid and anthocyanins (mainly cyanidin-3-galactoside) which serve as substrates of oxidative enzymes to produce highly reactive o-quinones, which further polymerise and lead to the formation of brown pigments (Février, Le Quéré, Le Bail, & Guyot, 2017; Candrawinata, Golding, Roach, & Stathopoulos, 2013; Schilling et al., 2008).

With regard to the impact of PEF on color, PEF-treated juices differed from the untreated juice with higher lightness (L^*) and redness (a^*) and no significant differences in yellowness (b^*) and color intensity (C^*_{ab}). On the other hand, compared to control, HPP-treated samples had less pronounced increases in L^* and a^*. HPP-treated juices also showed higher yellowness and color intensity compared to other samples (**Table 2**). Effect of PEF processing on apple juice color was also investigated by Bi et al. (2013) who reported that higher field strength at 30 and 35 kV/cm resulted in a significant increase in lightness and yellowness compared to untreated sample. In their study, higher degree of lightness was atributed to inactivation of PPO and POD by the treatment. Similar to our findings, insignificant increases in the L^* and a^* values of apple purée were observed after HPP treatments (Landl et al. 2010). Oey, Lille, Van Loey, & Hendrickx (2008) reported that HPP could preserve color due to its minimal effect on the covalent bonds of low molar-mass compounds such as color compounds. However, relatively high residual PPO and POD activities at HPP-treated samples induce enzymatic browning and off-flavor compound formation during storage (**section 3.1.1.2**). During storage at 4 °C, it was observed that L^*, C^*_{ab} and h_{ab} values were decreasing, while a^* was increasing. It indicated that the samples changed towards a reddish-brown color. The formation of brown pigments in HPP- and PEF-treated juices during storage may be attributed to

action of PPO and POD on phenol compounds in the presence of oxygen (Schilling et al., 2008; Yi et al., 2017). High intensity TP juice on the contrary had a better color stability. The addition of ascorbic acid prior to HP and thermal treatments can delay oxidation enzymatic browning (Yi et al., 2017; Juarez-Enriquez, Salmeron-Ochoa, Gutierrez-Mendez, Ramaswamy, & Ortega-Rivas, 2015; Krapfenbauer et al., 2006). However, it should be taken into account that the amount of ascorbic acid added should be proportional as reactive carbonyl compounds produced in ascorbic acid degradation could lead to browning in a later stage of storage (Roig, Bello, Rivera, & Kennedy, 1999).

3.1.1.2 Polyphenol oxidase (PPO) and Peroxidase (POD) activities

The effect of treatments on the residual PPO and POD activities is shown in **Figure 1**. Among different treatments, severe thermal pasteurization (TP2) lead to complete PPO and POD inactivation. More than 90% inactivation of apple juice PPO and POD was observed after mild pasteurization (TP1) and severe PEF treatment (PEF2). On the other hand, applying PEF treatment at low energy (PEF1) resulted in 36% and 49% reduction in PPO and POD activity, respectively. It seems that the process temperature played an important role in the inactivation of the enzymes since the outlet temperature after the PEF1 and PEF2 treatment was around 60 and 73 °C, respectively. The PEF2 outlet temperature was similar to the temperature of TP1, which could indicate the same inactivation effect. In comparison with this study, complete inactivation of POD was seen in apple juice treated at 25 kV/cm, 65 kJ/kg with inlet and outlet temperatures of 60 and 73.9 °C, respectively (Schilling et al., 2008). Moreover, the inactivation of PPO and POD were more effective when a PEF treatment is combined with moderate heat, which may be attained by high energy input or by preheating. Riener, Noci, Cronin, Morgan, & Lyng (2008) reported that both PEF and preheat treatments (40 kV/cm, 100 μs, 50 °C) showed higher inactivation up to 71% than the conventional pasteurization treatment (72 °C/26 s) which was 48%. This thermal effect resulted in structural changes in the enzyme and eventual loss of activity (Terefe, Buckow, & Versteeg, 2015; Van Loey, Verachtert, & Hendrickx, 2001).

In the non-stored samples, HPP juices showed high residual enzyme activities (RA ≥ 100% for PPO and RA ≥ 98% for POD) compared to the other treated juices. The observed increase in HP-treated samples could be attributed to either pressure-induced modification of the secondary and tertiary structure of the enzymes or release of the membrane-bound form of the enzymes from the juice (Terefe et al., 2014). An increase in PPO activity after HPP has been reported in several studies (Anese, Nicoli, Dall'aglio, & Lerici, 1994; Bayindirli et al., 2006; Buckow, Weiss, & Knorr, 2009). Buckow et al. (2009) observed a 65% increase of PPO apple juice activity after HP treatment at 400 MPa for 5 min at 20 °C. For POD, Vervoort et al. (2011) reported 90% RA in orange juice after HPP and decreased during refrigerated storage.

Further decreases in PPO and POD activities were observed during refrigerated storage for 3 weeks (**Figure 1**). It has been suggested that polyphenol compounds could either interact with the protein molecules to form an inactive enzyme-substrate complex or they could alter the catalytic site of the enzyme hence preventing reaction of the enzyme with the substrate (Vámos-Vigyázó & Haard, 1981). Le Bourvellec, Le

Quéré, Sanoner, Drilleau, & Guyot (2004) also attributed this decrease to the oxidation of phenolic compounds (procyanidins, caffeoyl quinic acids and (-)epicatechin). They explained that oxidation reduces the amount of substrate present in the product and the oxidized products could inhibit the enzyme's activity. Similarly, low POD activity observed during storage may be explained by the oxidation of phenol compounds such as catechin by POD leading to the formation of oxidation products, e.g. dehydrodicatechin, which might have prevented further reaction of the enzyme (López-Serrano & Ros Barceló, 2002).

3.1.1.3 Vitamin C

Figure 1 presents the changes in vitamin C content of cloudy apple juices after processing and during storage. Total vitamin C content of untreated sample was 63.85 mg/L, which was higher than the observation by Suárez-Jacobo et al. (2012) with 13.59 mg/L. Varming, Petersen, & Toldam-Andersen (2013) reported that, in commercial apple juices, the vitamin C content ranged from 1.2 to 2.6 mg/100 mL. In the current study, no significant differences can be observed between PEF1 and untreated sample. High intensity PEF and low intensity TP resulted in relatively high vitamin C retention (90%), however, vitamin C decreased more substantially after HPP at 600 MPa. Likewise, Landl et al. (2010) reported that pressure at 600 MPa yielded in 78.5% vitamin C retention, in contrast, apple juice treated with 400 MPa and mild pasteurization at 75 °C had almost no changes of the vitamin C content (93.5% and 100% retention, respectively). Apple contains polyphenolic compounds which could protect vitamin C against oxidative degradation (Miller & Rice-Evans, 1997). As mentioned in **section 3.1.1.2**, a relative high PPO and POD activity in HPP-treated juice was observed, which may result in a decrease in the amount of total phenolics in apple juice; therefore this might be related to the lower content of vitamin C in HPP samples. In the study of Landl et al. (2010), total phenolic content was retained at 75% after 600 MPa HPP, in comparison with 87% retention after pasteurization. They reported that the amount of phenolic compounds were related to residual PPO activity after the treatments.

Besides fruit juice composition, vitamin C stability is influenced by different factors such as oxygen exposure during processing and storage, type of packaging as well as storage conditions (Wibowo et al., 2015b; Ros-Chumillas, Belissario, Iguaz, & Lòpez, 2007; Bi et al., 2013; Varming, Petersen, & Toldam-Andersen, 2013). With increasing storage time, the total vitamin C of all samples decreased (**Figure 1**). Oxygen can difuse into the juice from the entrapped air, from headspace and/or through the PET bottles, thus allowing some oxygen to enter the juice and driving oxidative degradation of ascorbic acid. Via this pathway, ascorbic acid is oxidised to dehydroascorbic acid and further degraded to 2,3-diketogulonic acid. Loss of vitamin C during storage can be also correlated with the formation of furfural and 3-hydroxy-2-pyrone (3OH2P) (Shinoda, Komura, Homma, & Murata, 2005). After refrigerated storage for 3 weeks, HPP- and PEF-treated samples had higher retention of vitamin C compared to thermally-treated samples. Barba et al. (2012) reported that after 56-days of refrigerated storage, HPP blueberry juice (600 MPa/5 min) maintaned higher ascorbic acid content compared to PEF (36 kV/cm, 100 μs) and untreated juices. In terms of shelf-life determination based on vitamin C degradation rate, an estimated half-time was

464 determined to be 10.3, 10.0, and 9.3 days for pasteurized, 400 MPa and 600 MPa HPP-treated samples,
465 respectively (Landl et al., 2010). In contrast, Polydera, Stoforos, & Taoukis (2003) reported a longer shelf-
466 life for HPP orange juice sample compared to pasteurized juice.

467 **3.1.2 Impact on cloud stability related attributes**

468 **3.1.2.1 Cloud stability and particle size distribution**

469 Cloud stability plays an important role in the appearance and mouthfeel of cloudy apple juice. It is
470 governed by Stokes' law indicating that particle diameter, particle density and viscosity are among other
471 factors influence the sedimentation rate and thereby the cloud stability (Beveridge, 2002). The impact of
472 different preservation technologies on the cloud stability and the PSD of apple juice is shown in **Figure 2**.
473 Prior to storage, all samples showed a high cloud stability above 95%. PEF and TP-treated juice samples
474 have comparable stability with no significant differences. The observed high values could be due to
475 thermal effects associated with PEF-treatment and high temperature applied in thermal processing,
476 respectively; thereby inactivating the PME to a great extent (Beveridge & Wrolstad, 1997).
477 At the end of the storage period (3 weeks, 4 °C), cloud stability of the samples decreased. In the TP juices,
478 the change was not significant. Similarly, Krapfenbauer et al. (2006) found no significant difference in
479 cloudiness after cold storage of thermally-treated apple juice for six months. On the other hand, in HPP-
480 treated juice, a significant decrease in cloud stability may be due to high residual PME activity after the
481 treatment (**section 3.1.2.2**). The methoxy groups of pectin molecules may have been de-esterifies by the
482 enzyme and in the presence of divalent cations such as calcium or magnesium, form cross-linkages with
483 these ions resulting in gel formation and consequently cloud loss (Croak & Corredig, 2006).
484
485 As for PSD, a unimodal distribution was observed for all samples, with maximum peaks around 200 µm
486 diameter. This could indicate the uniformity and homogeneity of juices pretreated with the spiral-filter
487 press. Other authors observed a multimodal distribution of untreated cloudy apple juice with main peaks
488 were around 0.6 and 200 µm and a smaller peak was around 20 µm (Illera et al., 2018). Espinosa et al.
489 (2011) reported a bimodal PSD with maxima at ~200 µm and ~1000 µm. Differences in PSD can be
490 influenced by different grinding steps, shearing rate, and homogenisation pressure (Betoret, Betoret,
491 Carbonell, & Fito, 2009; Espinosa et al., 2011; Layal, Michèle, Julien, Emilie, & Christelle, 2018).
492 Throughout storage, the changes in particle size were relatively small (**Supplementary 2**). The average
493 particle size or the median diameter, expressed as D[v,0.5], was in range of 196-207 µm. Moreover, two
494 additional parameters, D[v,0.1] and D[v,0.9] were 101-110 µm and 330-366 µm, respectively. Because the
495 particle size was not changing too much during storage, it seems that differences in the cloud stability was
496 influenced more by the PME activity rather than particle diameter.

497 **3.1.2.2 Pectin methylesterase (PME) activity**

498 After processing, PME was completely inactivated by PEF2 and TP2 (**Figure 2**). Low intensity TP1 and PEF1
499 treatments resulted in about 90% and 50% RA, respectively. Conversely, PME seemed to be highly

resistant to HPP, as the residual activities were remained high after the treatments (RA > 90%), which eventually results in cloud loss during storage (**section 3.1.2.1**). The high residual enzyme activity in HP-treated juices may be attributed to the presence of pressure stable isoenzymes (Terefe et al., 2014). The observed decrease in PME by PEF could be largely attributed to thermal effects associated with PEF treatment which alter the secondary and tertiary structure resulting in loss of activity (Terefe et al., 2015; Zhao & Yang, 2010). Inactivation of the enzyme by thermal treatment may be due to denaturation of the enzyme which is consistent with the results of Krapfenbauer et al. (2006). In their study, the PME activity of cloudy apple juice significantly decreased at 80 and 90 °C (20-100 s), while relatively stable at 70 °C.

After storage, no enzyme activity was observed in PEF2 and thermally-treated juice due to the inactivation of the enzyme during processing. On the other hand, a slight decrease in enzyme activity was observed in PEF1 juice. Although PEF1 could not result in complete enzyme inactivation, it is likely that irreversible structural conformations of the enzyme might have occurred leading to a decrease in enzyme activity during storage (Agcam, Akyildiz, & Evrendilek, 2014). Meanwhile, the observed increase in HPP2 juice might be explained by pressure-induced structural changes of the enzyme such that during storage, it was able to regain its activity (Oey, 2010).

3.1.3 Impact on taste related attributes

3.1.3.1 Sugar and organic acid profile

The overall taste of apple juice is provided by a good balance of sugars and organic acids; sugars contribute to sweetness while the acids contribute to sourness. In **Table 3**, little effects of the processing technologies on sugars and organic acids can be observed. The TSS ranged from 12.8 to 13.2 °Brix which meet the minimum Brix level of 11.2° by the EU regulation (Directive 2012/12/EC). The predominant sugars in apple juice are fructose, accounted for ~60% of the total sugar content, followed by glucose ~23% and sucrose ~16%, respectively. Acidity in apple juice is attributed to malic, citric and quinic acids. In freshly extracted (untreated) juice, malic acid serves as the main organic acids (4.63 g/L) with pH 3.32 and TA 0.47%. The sensory evaluation results showed that there were slight differences of sweet taste among samples, in which PEF2 was perceived sweeter than HPP1 and TP samples. However, the panelists could not distinguish a difference between all samples for sour and bitter taste (**Supplementary 3**). This could be an indication that the treatments seem to have a minimal impact on the sugar and acid concentrations. Similarly, PEF treatments at different electrical field strengths (15, 25, and 35 kV/cm) and energy inputs (8.5-65.5 kJ/kg) had not affected the concentrations of glucose, fructose, and sucrose (Schilling et al., 2008). In the work by Lee, Kebede, Lusk, Mirosa, & Oey (2017), increasing sourness and cooked flavor on thermally-processed apple juice was reported. In contrast, PEF- and HPP-treated juices were perceived as fresh, natural, sweet and balanced flavor.

Concerning the sugar changes during storage, a significant decrease was observed only for sucrose content. TSS, fructose and glucose were not significantly changed ($p > 0.05$), although the concentrations

of fructose and glucose increased. Suárez-Jacobo et al. (2012) reported that the sucrose content of ultra high-pressured apple juice (300 MPa) was significantly decreased during storage at 30 °C for 60 days and no significant increases for glucose and fructose contents. The possible explanation could be that sucrose was hyrolyzed into monosaccharides, resulting in an increase in fructose and glucose. The acidity of the juice remained stable during refrigerated storage, which was in agreement with the result obtained by Juarez-Enriquez et al. (2015), who observed no significant changes in variables such as pH, sugar content (°Brix), and malic acid of HPP-treated apple juice (430 MPa/7 min) after 34 days of storage at 4 °C. However, a decrease in pH at higher storage temperature 20 °C was reported.

3.2 Comparing the impact of treatments and storage on aroma of cloudy apple juice: an untargeted GC-MS fingerprinting approach

Aroma is one of the sensory properties affecting quality perception and consumer acceptance of fruit juices to a large extent. It consists of a complex mixture of a large number of volatile compounds. The headspace volatile fraction of cloudy apple juice was analyzed using an untargeted HS-SPME-GC-MS technique. A representative example of GC-MS total ion chromatogram of the headspace fraction of untreated juice at the beginning of storage is presented in **Figure 3**. The three most abundant peaks could be identified as ethyl butanoate (esters), butyl acetate (esters) and hexanal (aldehydes). Previous studies reported that key aroma compounds in apple juice belong to esters, aldehydes and alcohols groups (Dixon & Hewett, 2000; Komthong, Katoh, Igura, & Shimoda, 2006). Factors affecting differences in volatile profiles of apple juice include cultivar, stage of maturity, geographic region, climate, processing and storage conditions (Schmutzer, Magdas, David, & Moldovan, 2014; Hashizume, Gordon, & Mottram, 2007; Su et al., 2006).

As described in **section 2.5**, the resulting chromatograms were analyzed first with data pre-processing techniques AMDIS and MPP prior to MVDA. After exploring the data with PCA (data not shown), PLS-DA was performed with volatile components considered as *X*-variables and untreated (control), thermal pasteurization (TP), high pressure processing (HPP), and pulsed electric field (PEF) treatments as categorical *Y*-variables. As mentioned earlier, PLS-DA is a multivariate technique used to classify different groups of samples. For visual representation of comparison of different processing impacts compared to untreated sample, a PLS-DA biplot was constructed. **Figure 4** shows PLS-DA biplots of the first two latent variables (LV1 and LV2) for different refrigerated storage time (week 0 and week 3). In these biplots, groupings and/or separations between differently processed apple juice classes can be observed. Clearly, there are differences in the volatile profile among thermal treatments, PEF treatments and HPP treatments. On the contrary, untreated samples are located closely to HPP samples. On the biplots, classes that are closer to each other are considered as similar, while classes that are far away from each other are considered as different (Vervoort et al., 2012). Moreover, the importance of the volatiles for classification can be indicated by their location and their distance from the centre. The inner and outer ellipses represent correlation coefficients of 70% and 100%, respectively. For a volatile located between the two ellipses, more than 70% of its variability is explained by the first two LVs. Also, volatiles projected

far from the center and close to a certain group of classes are respectively highly positively correlated to the corresponding class and vice versa. Taking into account the vectors' length and a relatively small percentage of the *Y*-variances explained by the first two LVs (31% and 38% for week 0 and week 3, respectively) (**Figure 4**), it can be concluded that higher LVs contain important additional information on discriminative volatiles. In this work, six LVs were included for the comparison over all processing intensities before storage (week 0), explaining 83% of *Y*-variance, while five LVs were needed to explain 93% of *Y*-variance for after storage (week 3), since LVs were added to the model until they at least contributed more than 2% of the *Y*-variance of the selected model.

Although some information can be deducted from biplots, it is not a straightforward method to indicate the most important volatiles for a specific class (compared to the other classes). Therefore, VID coefficients were calculated and only volatiles with absolute value higher than 0.80 were selected and considered as potential discriminant markers for a specific class. This approach was used to identify which compounds are correlated the most to a particular class, as well as to rank the importance of volatiles based on their discriminative power. **Table 4** shows the discriminant volatile compounds listed per treatment in a decreasing order of VID value, for week 0 and week 3, respectively. Also, the corresponding individual plots of these compounds are displayed in **Figures 5**.

In total, there were 16 markers, in which 9 of them were reported as key aroma volatiles in apple juice (compounds in italic). At week 0, only one discriminant compound was selected for untreated class belonging to aldehydes group (hexanal). After HPP treatment, no volatile was found that met the VID procedure, whereas ten volatiles were selected for other treated samples, five for each PEF and TP treatments. Five discriminant compounds selected for PEF class are four esters (2-methylbutyl acetate, isobutyl acetate, amyl acetate, and butyl acetate) and one terpene hydrocarbons (limonene). For thermal pasteurization, selected volatiles can be categorized as hydrocarbons (pentane and heptane), ether (ethyl ether) and esters (ethyl butanoate and ethyl 2-methylbutanoate). Most of the compounds have a positive VID coefficient indicating a higher concentration after the corresponding treatment(s). Only three compounds in thermal class had negative VID values which means that the decrease of these compounds was more pronounced after severe thermal treatment (TP2).

Interpretation of the selected discriminant markers can be made by linking with different (bio)-chemical reactions described in literature. In this way, insight into chemical reactions behind quality changes of apple juice can be obtained.

A higher concentration of one aldehyde (hexanal) in the untreated class compared to other treatments could be due to enzyme-catalysed reactions initiated during the blending and preparation of the juice (Aganovic et al., 2014). Lipoxygenase and hydroperoxide lyase are naturally present in many fruits; when fruits are homogenised, polyunsaturated fatty acids, such as linoleic and linolenic are oxidised to various C_6- and C_9-aldehydes (Dixon et al., 2000). Signifcant decrease in aldehydes (hexanal) after thermal processing was similar with previous study by Aguilar-Rosas et al. (2007), who observed a considerable

615 decrease of apple juice hexanal content (62%) after thermal pasteurization (90 °C, 30 s) whereas only 7%
616 decrease after PEF treatment (35 kV/cm, 1200 pps). After HPP treatment in our study, aldehydes (hexanal)
617 significantly decreased. On the contrary, Mastello, Janzantti, Bisconsin-Júnior, & Monteiro (2018)
618 observed two aldehydes, hexanal and octanal, were significantly higher in the HPP-treated orange juice
619 than in untreated juice. Hexanal is known as the contributor of green apple, grass like odour (Komthong
620 et al., 2006).
621
622 Ester compounds are known for their fruity characteristics, for example hexyl acetate have a fruity, sweet
623 and herbal aroma while ethyl butanoate is described as having typical fruity apple odour (Dixon et al.,
624 2000; Qin, Petersen, & Bredie, 2018). A decrease of esters would decrease the pleasant fruity aroma of
625 apple juice. The effect of PEF, HPP, and TP treatments on ester compounds was shown in **Figure 5**. The
626 concentration of esters (ethyl acetate, isobutyl acetate, amyl acetate, butyl acetate, 2-methylbutyl
627 acetate, and ethyl 2-methylbutanoate) were significantly higher after the application of PEF. On the
628 contrary, a substantial decrease of esters was found after thermal pasteurization. Increase in esters after
629 PEF treatments could be due to electropermeabilization effect, thereby enhancing the release of the
630 compounds immediately after the treatment (Sotelo et al., 2015). Aguilar-Rosas et al. (2007), examining
631 the effects of PEF treatment on flavor compounds in fruit juice, observed that flavor compounds may not
632 be degraded after the treatment and were better retained compared to the fresh juice. In their study,
633 hexyl acetate and butyl hexanoate were decreasing respectively by 8% and 18% after PEF (35 kV/cm,
634 12000 pps), while higher loss of these compounds respectively by 23% and 36% were observed after heat
635 treatment at 90 °C for 30 s. Moreover, Jia, Howard Zhang, & Min (1999) stated that volatiles can be also
636 removed during vacuum degassing rather than the PEF treatment itself.
637
638 No sigificant changes in ester compounds was observed after HPP treatments (**Figure 5**). It is generally
639 known that HPP could retain natural flavor of food products because it has a limited effect on the covalent
640 bonds of the low-molecular-weight compounds. However, HPP can have an effect enhancing or inhibiting
641 enzymatic and chemical reactions which could indirectly change the content or the composition of aroma
642 compounds (Oey, Lille, Van Loey, & Hendrickx, 2008). Some authors reported also significant changes in
643 volatiles after HP processing. For example, González-Cebrino, García-Parra, & Ramírez (2016) observed a
644 lower content of most esters in HPP-treated plum puree although the overall aroma was not affected.
645 Lambert, Demazeau, Largeteau, & Bouvier (1999) reported both positive and negative changes in the ester
646 contents (e.g. ethyl butanoate, butyl acetate, and methyl hexanoate) under pressure conditions at 200,
647 500 and 800 MPa for 20 min. A decrease in esters of apple juice could be explained by esterase catalyzed
648 hydrolysis reaction (Yi et al., 2017). Nevertheless, during HPP treatment, the pressure, temperature and
649 time of treatment are important factors to consider to avoid deterioration of aroma compounds (Jiménez-
650 Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017).
651
652 In the TP class, hydrocarbons (pentane and heptane) were induced by the thermal treatments, in
653 particular at high temperature (85 °C). Formation of these compounds can be linked to fatty acid
654 degradation reactions (Kebede et al., 2014). Compounds such as pentane, hexane, and heptanal were

identified as off-flavor compounds which tended to increase during high temperature storage at 70 °C (Lee & Choe, 2012).

In respect to changes during storage, a decline in most of the volatile compounds was observed for all treatments indicating further degradation of the volatiles (**Figure 5**). During storage, changes in aroma compounds can be caused by factors such as storage time and temperature, oxygen content, light exposure and type of packaging (Hashizume et al., 2007; Wibowo et al., 2015a). Changes in the volatile compounds during refrigerated storage were reported in several studies. Baxter et al. (2005) compared the quality of untreated orange juice with pasteurized juice (85 °C/25 s) and HPP-treated juice (600 MPa/60 s) over 12 weeks of storage at 4 and 10 °C. They discovered that at the end of storage, the volatile contents of HPP and pasteurized juice ranged from 6% to 38% of the initial levels. In the case of PEF strawberry juice, the concentrations of most esters and hexanal were maintained during 21 days of storage. However, a substantial loss of methyl butanoate and butyl acetate was observed (Aguiló-Aguayo et al., 2009). They referred to ascorbic acid degradation as one of the responsible reactions for flavor changes occurring during storage. Studies reported that a decrease in aldehydes concentration during storage can be linked to oxidation reaction to its corresponding acid and absorption by packaging materials (van Willige, Linssen, Legger-Huysman, & Voragen, 2003; Wibowo et al., 2015a). It is possible that oxygen can penetrate the PEF bottles, diffuse into the juice and induced chemical reactions. Decrease in esters can be associated to acid-catalyzed hydrolysis (Wibowo et al., 2015a). Moreover, volatiles can be degraded if enzymes are not completely inactivated (Buckow et al., 2009; Yi et al., 2017; Aguiló-Aguayo et al., 2009).

4 CONCLUSION

The impact of low-oxygen spiral-filter press in combination with PEF, HPP and thermal processing on apple juice quality (e.g. color, cloud stability, taste and aroma attributes) was compared after processing and during refrigerated storage. Changes in quality attributes were investigated by integrating targeted and untargeted approach. Severe thermal processing (TP2) produced the brightest color compared to the other treatments. PEF2 and both TP inactivated the PPO, POD and PME enzymes, reducing their activity to a large extent. This was atttributed to thermal effects of PEF since the outlet temperature after PEF2 treatment was similar to the temperature of low intensity thermal pasteurization (TP1) and therefore indicated the same inactivation effect. On the contrary, all enzymes showed considerably high residual activity after HPP signifying their apparent resistance to pressure inactivation. Browning was observed in the HPP- and PEF-treated samples which could be ascribed to both enzymatic, as PPO and POD were not inactivated completely, and non-enzymatic reactions. Moreover, oxidative ascorbic acid degradation could occur during storage as oxygen can diffuse from the environment into the product through the PET bottles. This observation was, indeed, confirmed by the lower amount of vitamin C in all stored samples. Cloud stability seemed affected more by residual PME activity rather than by particle size; in which its stability was maintained in TP1, TP2 and PEF2 samples with inactivated PME activity. This experiment

confirmed that no impact of treatments and storage on total sugar, total organic acids, TA and pH, although a significant difference in taste (sweetness) was observed between some samples. During storage, fructose and glucose showed an increase in concentration while sucrose concentration decreased for all samples which could be ascribed to the hydrolysis of sucrose into fructose and glucose due to the acidic nature of the juice.

In terms of the aroma profiles, using an untargeted approach, some key aroma volatiles belonging to the aldehydes and esters groups were selected as discriminant volatiles. High concentration of aldehydes (hexanal) in the untreated class can be related to enzymatic unsaturated fatty acid degradation. Esters, responsible for the fruity aroma, were enhanced after PEF treatments which could linked to the changes of structural tissue by the treatments. HPP treatments can also maintain these compounds similar to the untreated fresh samples. In contrast, conventional thermal pasteurization resulted in a decrease of most volatiles and induced formation of off-odor compounds. In general, the decrease in concentration of compounds during storage could be linked to oxidative reactions and acid-catalyzed hydrolysis of esters.

This study demonstrated that the application of low-oxygen spiral-filter press in combination with high intensity thermal pasteurization can inactivate quality-degrading enzymes, therefore the color and cloud stability of cloudy apple juice could be maintained. However, the aroma profile was significantly reduced by the treatment. On the other hand, HP treatments carried out here produced apple juice with aroma comparable to the fresh juice. Nevertheless, enzymes were resistant to HPP which can induce discoloration as well as cloud loss formation during storage. High intensity PEF processes, designed to allow enzyme inactivation, appear suitable for preserving quality attributes of the juice largely due to thermal effects associated with this technology.

Acknowledgement

The authors acknowledge the financial support of Flanders' Food under the HighQJuice project, including the stakeholders platform members involved.

Declarations of interest

The authors of the present work declare no conflict of interests.

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Supplementary

Supplementary 1. Microbiology results of cloudy apple juice after treatments and during refrigerated storage.

Treatment[1)]	Total aerobic psychrotrophic	Lactic acid bacteria	Yeast	Mold	Aerobic spore-forming bacteria
Week 0					
Control	$1.6*10^4$	$1.1*10^3$	<1	$1.5*10^3$	$9.5*10^3$
PEF1	$3.6*10^3$	<1	<1	4.5	9.1
PEF2	$8.8*10^2$	<1	<1	<1	<1
HPP1	8.2	<1	<1	<1	<1
HPP2	<1	<1	<1	<1	<1
TP1	2	<1	<1	<1	<1
TP2	<1	<1	<1	<1	<1
Week 15					
PEF1	$1.7*10^3$	<1	<1	$1.2*10^1$	1.3
PEF2	$1.4*10^2$	<1	<1	1.3	<1
HPP1	$1.6*10^1$	<1	<1	3	<1
HPP2	$1.3*10^1$	<1	<1	$1.6*10^2$	<1
TP1	<1	<1	<1	<1	<1
TP2	<1	<1	<1	<1	<1

[1)] Control is the untreated sample, the number 1 indicates low intensity and 2 represents high intensity.
Limit of detection = 10^3 colony forming unit/mL

Supplementary 2. Particle size of untreated and treated cloudy apple juice expressed as the size of which 10, 50 or 90 vol% of the particles have a smaller diameter (D[v,0.1], D[v,0.5] and D[v,0.9]).

Treatment[1)]	D[v,0.1] μm	D[v,0.5] μm	D[v,0.9] μm
Week 0			
Control	102.27 ± 0.38^{cd}	201.55 ± 0.61^{ae}	351.56 ± 2.38^{bc}
PEF1	102.45 ± 0.32^{cd}	196.90 ± 0.79^{b}	336.67 ± 3.08^{de}
PEF2	102.75 ± 0.20^{c}	197.81 ± 0.18^{bc}	338.10 ± 0.81^{de}
HPP1	105.06 ± 0.06^{b}	203.69 ± 0.13^{ad}	352.98 ± 0.88^{b}
HPP2	103.07 ± 0.58^{c}	199.89 ± 1.25^{ce}	343.34 ± 4.40^{cd}
TP1	109.00 ± 0.14^{a}	206.25 ± 0.10^{fg}	364.28 ± 0.39^{a}
TP2	110.05 ± 0.11^{a}	205.24 ± 0.16^{fg}	357.79 ± 0.51^{ab}
Week 3			
PEF1	102.21 ± 0.15^{cd}	197.39 ± 0.52^{bc}	335.11 ± 1.33^{de}
PEF2	101.04 ± 0.40^{d}	198.42 ± 0.48^{bc}	338.20 ± 1.13^{de}
HPP1	103.54 ± 0.38^{c}	198.35 ± 0.30^{bc}	329.69 ± 1.43^{e}
HPP2	103.42 ± 0.30^{c}	198.01 ± 0.23^{bc}	331.66 ± 1.05^{e}
TP1	109.77 ± 0.05^{a}	207.15 ± 0.05^{g}	365.71 ± 1.09^{a}
TP2	109.30 ± 0.05^{a}	203.92 ± 0.23^{adf}	353.21 ± 1.15^{b}

[1)] Control is the untreated sample, the number 1 indicates low intensity and 2 represents high intensity.
Values are means and standard errors of four determinations.
Values with the different letters within one column are significantly different ($p < 0.05$).

Supplementary 3. Sensory panel scores for cloudy apple juice sweet, sour and bitter attributes after PEF, HPP and thermal pasteurization treatments.

	PEF2	HPP1	HPP2	TP1	TP2
Sweet	6.29 ± 0.92^{b}	5.13 ± 1.10^{a}	5.75 ± 1.14^{ab}	5.07 ± 0.96^{a}	5.14 ± 0.99^{a}
Sour	4.98 ± 0.97^{a}	5.49 ± 1.17^{a}	4.64 ± 0.99^{a}	5.31 ± 0.98^{a}	4.72 ± 1.01^{a}
Bitter	2.47 ± 1.11^{a}	2.87 ± 1.28^{a}	3.03 ± 1.13^{a}	3.34 ± 1.22^{a}	3.06 ± 1.23^{a}

The number 1 indicates low intensity and 2 represents high intensity (see **Table 1**).
Values with the different letters within one column are significantly different ($p < 0.05$).

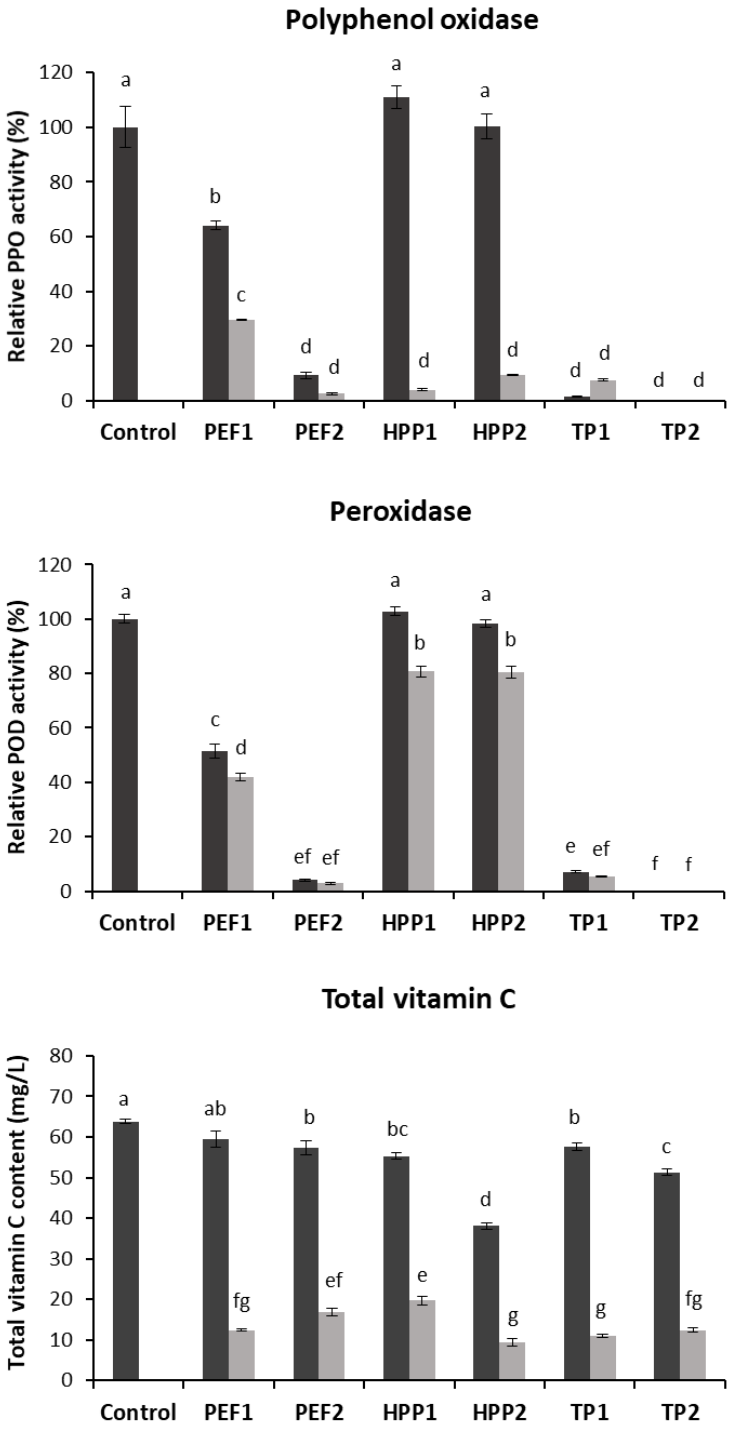
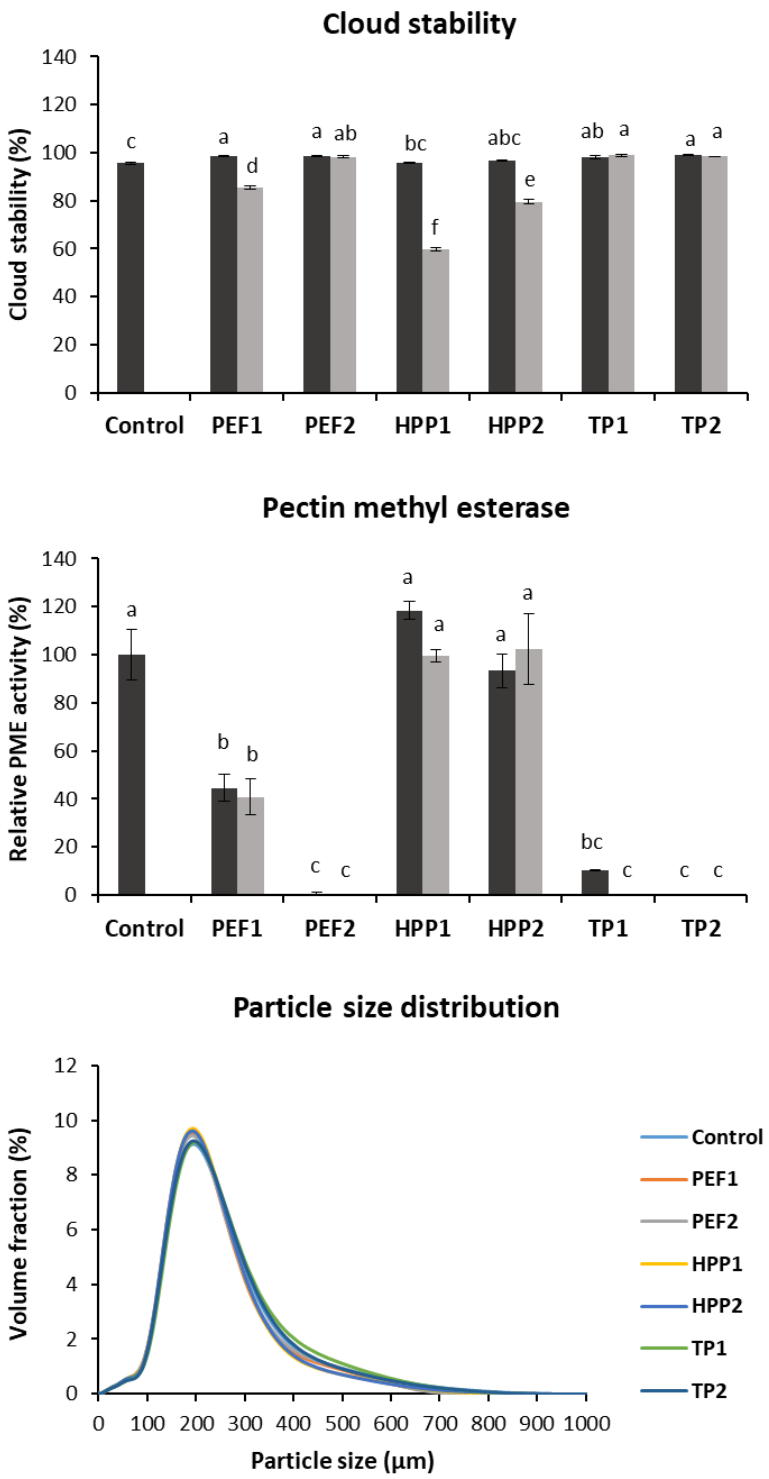
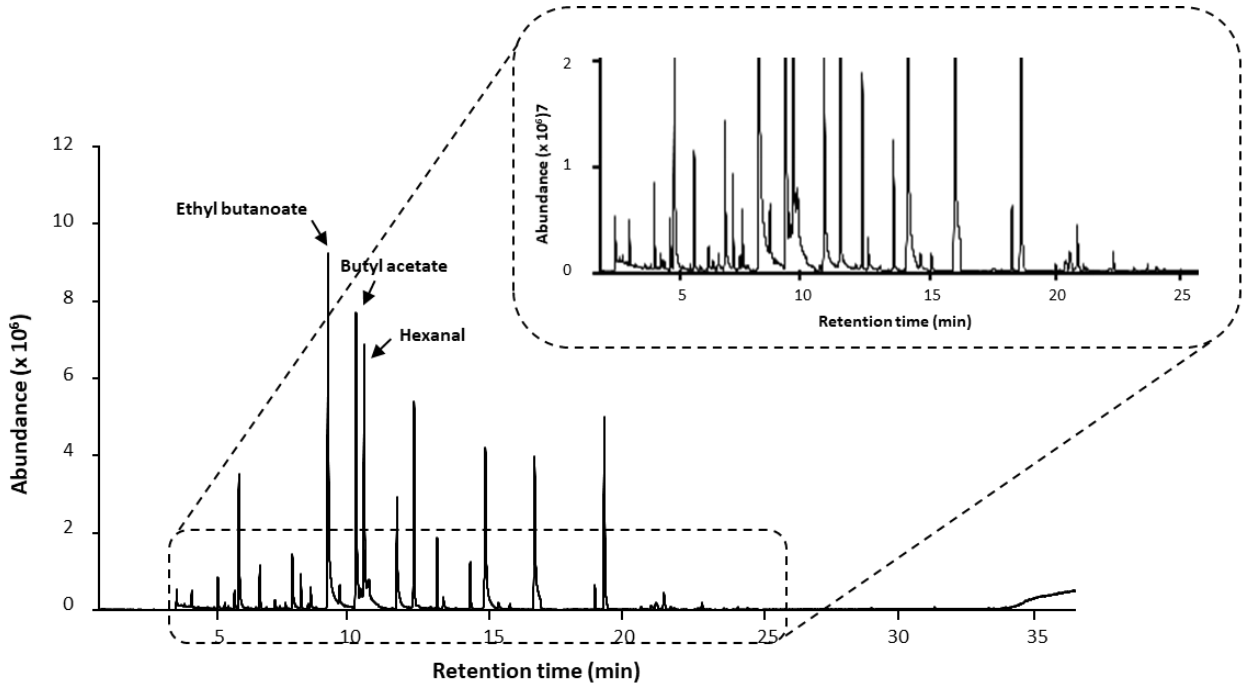


Figure 1. Residual PPO and POD activity, and total vitamin C content of untreated (control), PEF, HPP and thermally pasteurized cloudy apple juice before (■) and after storage for 3 weeks at 4 °C (■). The number 1 indicates low intensity and 2 represents high intensity (see **Table 1**). Significant differences ($p < 0.05$) are indicated with different letters. Error bars represent the standard error of measurements (n = 3).



959

960 **Figure 2**. Cloud stability, residual PME activity of untreated (control), PEF, HPP and thermal pasteurized cloudy apple
961 juice before (■) and after storage for 3 weeks at 4 °C (■). Volumetric particle size distribution of cloudy apple juice
962 after PEF, HPP and thermal pasteurization treatments. The number 1 indicates low intensity and 2 represents high
963 intensity (see **Table 1**). Significant differences ($p < 0.05$) are indicated with different letters. Error bars represent the
964 standard error of measurements (n = 3).



965
966 **Figure 3**. Total ion chromatogram of the headspace of untreated cloudy apple juice at the beginning of storage (week
967 0), obtained by headspace solid-phase microextraction GC–MS (HS-SPME–GC–MS) fingerprinting.

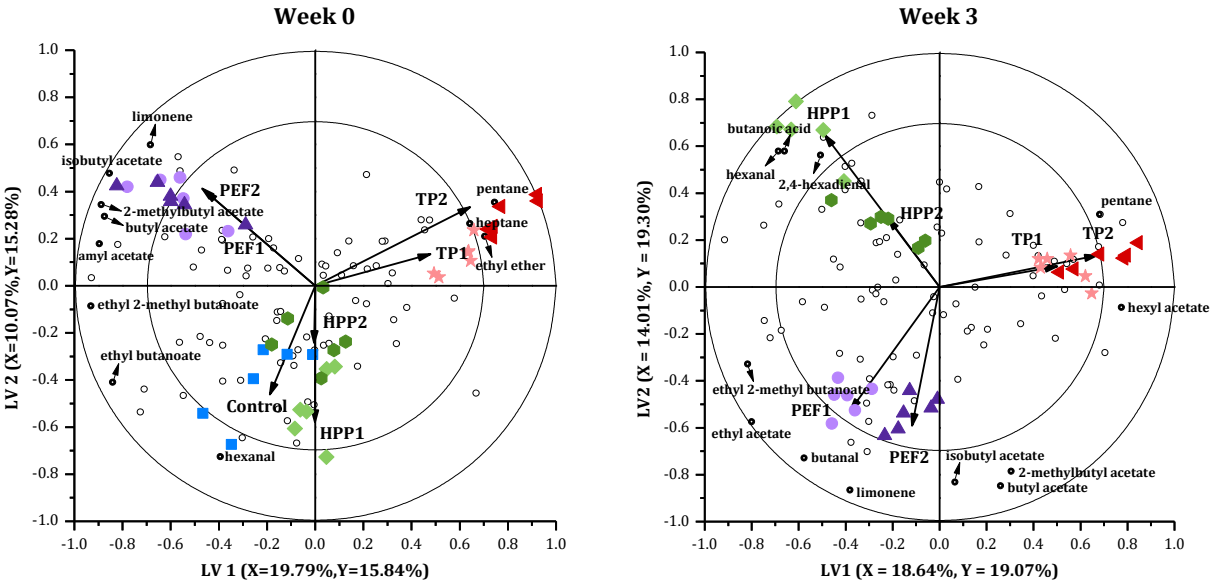


Figure 4. PLS-DA biplots describing comparison of treatment impact of PEF, HPP, and thermally-pasteurized (TP) cloudy apple juice compared to untreated (control) at storage week 0 and treatment impact of PEF, HPP and TP after 3 weeks of storage at 4 °C. The open circles represent the different volatiles, of which only the compounds selected through the VID procedure are named (**Table 4**). The correlation loadings for the categorical *Y*-variable are represented as vectors. The percentages of the variances in X and Y explained by each latent variable (LV1 and LV2) are indicated on the respective axes.

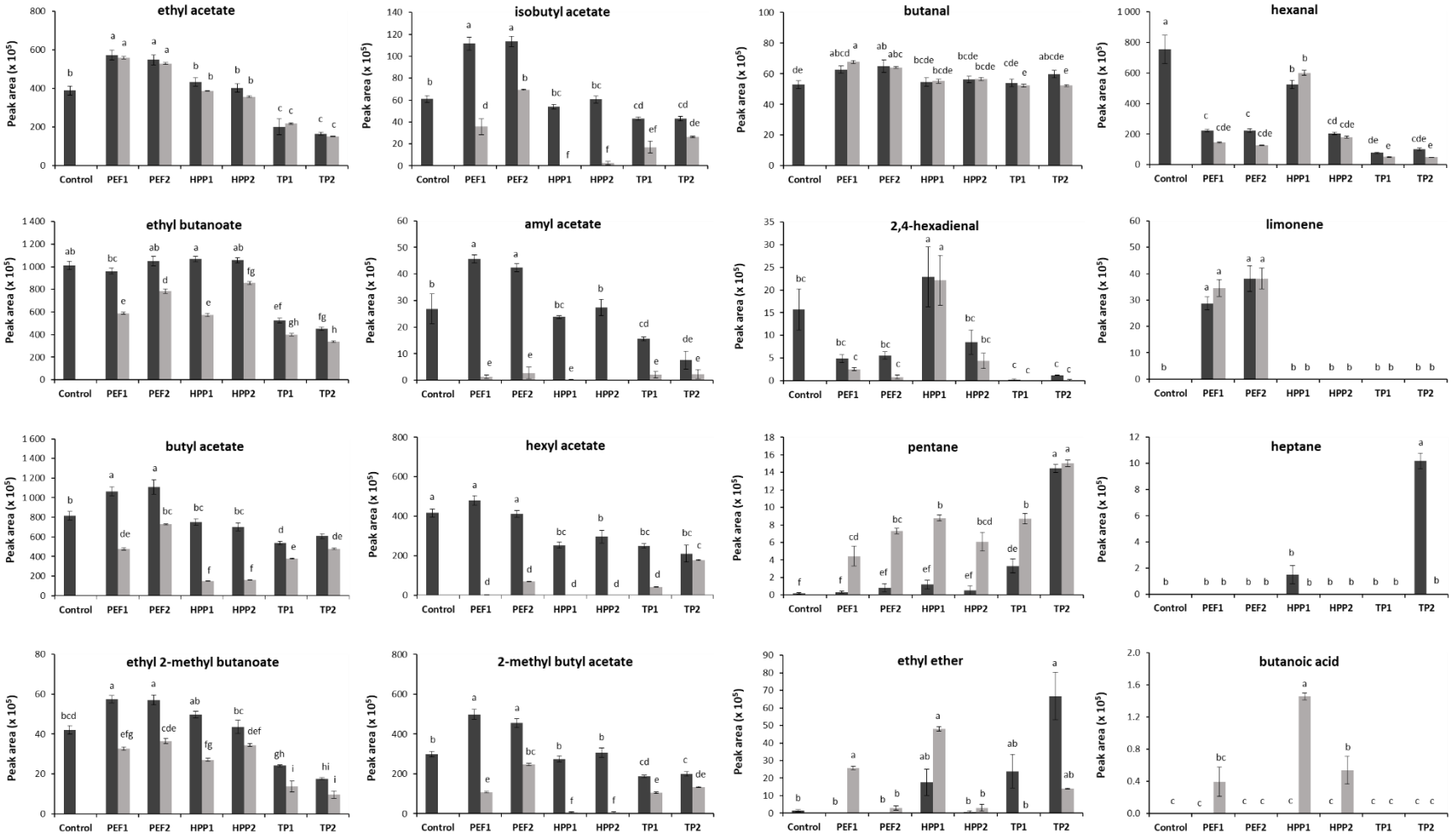


Figure 5. Discriminative headspace components for comparison of treatment impact, selected through the VID procedure (**Table 4**) before storage (■) and after storage for 3 weeks at 4 °C (■). The number 1 indicates low intensity and 2 represents high intensity (see **Table 1**). The Y-axis indicates the peak area x 10^5. Significant differences ($p < 0.05$) are indicated with different letters. Error bars represent the standard error of analysis (n = 6).

Table 1. Different processing conditions applied to cloudy apple juice production.

Treatment	Low intensity (1)	High intensity (2)
Control (Untreated)	-	-
Pulsed Electric Field (PEF)	Electric field strength 12.5 kV/cm Flow 27.6 L/h Energy input 76.4 kJ/L Frequency 62 Hz T_{inlet} 37.6 °C T_{outlet} 59.5 °C	Electric field strength 12.3 kV/cm Flow 24.5 L/h Energy input 132.5 kJ/L Frequency 94 Hz T_{inlet} 37.3 °C T_{outlet} 72.8-73.8 °C
High pressure processing (HPP)	Pressure 400 MPa Room temperature time 3 min	Pressure 600 MPa Room temperature time 3 min
Thermal pasteurization (TP)	Temperature 72 °C time 15 s	Temperature 85 °C time 30 s

Table 2. Color values of cloudy apple juice after treatments and during refrigerated storage.

Treatment[1)]	L^*	a^*	b^*	C^*_{ab}	H_{ab}
Week 0					
Control	50.62 ± 0.14^{h}	0.24 ± 0.15^{e}	32.36 ± 0.39^{b}	32.37 ± 0.39^{b}	89.21 ± 0.28^{a}
PEF1	57.43 ± 0.63^{cd}	2.65 ± 0.18^{c}	31.74 ± 0.81^{bc}	31.85 ± 0.82^{bc}	85.23 ± 0.21^{def}
PEF2	57.96 ± 0.43^{c}	3.00 ± 0.04^{c}	31.73 ± 0.75^{bc}	31.88 ± 0.75^{bc}	84.59 ± 0.16^{ef}
HPP1	52.91 ± 0.05^{g}	0.50 ± 0.04^{e}	34.90 ± 0.06^{a}	34.91 ± 0.06^{a}	89.19 ± 0.06^{a}
HPP2	52.72 ± 0.09^{g}	0.54 ± 0.09^{de}	34.93 ± 0.05^{a}	34.94 ± 0.22^{a}	89.11 ± 0.15^{ab}
TP1	59.62 ± 0.11^{b}	1.91 ± 0.01^{c}	32.75 ± 0.22^{b}	32.80 ± 0.22^{b}	86.67 ± 0.03^{cdef}
TP2	61.65 ± 0.11^{a}	1.68 ± 0.02^{cd}	30.51 ± 0.12^{c}	30.56 ± 0.12^{cde}	86.85 ± 0.04^{bcde}
Week 3					
PEF1	53.02 ± 0.09^{g}	7.78 ± 0.02^{a}	28.13 ± 0.18^{d}	29.18 ± 0.18^{d}	74.54 ± 0.06^{h}
PEF2	55.24 ± 0.11^{ef}	7.86 ± 0.01^{a}	28.07 ± 0.07^{de}	29.47 ± 0.07^{de}	74.35 ± 0.02^{h}
HPP1	56.12 ± 0.16^{de}	0.56 ± 0.01^{e}	29.04 ± 0.10^{d}	29.04 ± 0.10^{d}	88.90 ± 0.02^{abc}
HPP2	54.12 ± 0.13^{f}	0.78 ± 0.03^{de}	25.43 ± 0.19^{g}	25.44 ± 0.23^{g}	88.25 ± 0.07^{abcd}
TP1	56.64 ± 0.22^{cd}	5.68 ± 0.60^{b}	27.00 ± 0.17^{ef}	27.64 ± 0.17^{ef}	78.20 ± 1.23^{g}
TP2	60.54 ± 0.47^{b}	2.61 ± 0.45^{c}	26.77 ± 0.20^{f}	26.92 ± 0.22^{f}	84.44 ± 0.93^{f}

[1)] Control is the untreated sample, the number 1 indicates low intensity and 2 represents high intensity.
Values are means and standard errors of three determinations.
Values with the different letters within one column are significantly different ($p < 0.05$).

989 **Table 3**. Sugar and organic acid contents of cloudy apple juice after treatments and during refrigerated storage.
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Treatment[1)]	Sugars				Organic acids				
	TSS (°Brix)	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)	pH	TA (%)	Malic acid (g/L)	Quinic acid (g/L)	Citric acid (g/L)
Week 0									
Control	13.08 ± 0.04^{abcd}	33.03 ± 0.96^{a}	11.59 ± 0.23^{a}	7.94 ± 0.20^{ab}	3.32 ± 0.02^{e}	0.47 ± 0.03^{ab}	4.63 ± 0.07^{bcd}	0.41 ± 0.03^{abc}	0.14 ± 0.01^{a}
PEF1	12.78 ± 0.09^{f}	30.92 ± 1.47^{ab}	11.37 ± 0.90^{a}	7.96 ± 0.60^{ab}	3.45 ± 0.01^{abc}	0.45 ± 0.00^{ab}	4.76 ± 0.02^{abcd}	0.43 ± 0.03^{abc}	0.15 ± 0.01^{a}
PEF2	13.04 ± 0.00^{bcde}	30.73 ± 0.92^{ab}	11.33 ± 0.76^{a}	7.82 ± 0.47^{ab}	3.35 ± 0.02^{de}	0.47 ± 0.00^{ab}	4.84 ± 0.01^{abcd}	0.37 ± 0.01^{abc}	0.14 ± 0.01^{a}
HPP1	13.10 ± 0.01^{abc}	31.35 ± 1.53^{ab}	11.17 ± 0.88^{a}	7.64 ± 0.58^{abc}	3.40 ± 0.01^{cd}	0.46 ± 0.02^{ab}	4.60 ± 0.05^{cd}	0.39 ± 0.03^{abc}	0.15 ± 0.01^{a}
HPP2	13.07 ± 0.02^{abcd}	32.87 ± 0.38^{a}	11.60 ± 0.28^{a}	8.01 ± 0.19^{ab}	3.41 ± 0.01^{bcd}	0.41 ± 0.01^{b}	4.68 ± 0.02^{abcd}	0.43 ± 0.03^{abc}	0.16 ± 0.01^{a}
TP1	12.95 ± 0.02^{cdef}	30.88 ± 0.53^{ab}	11.47 ± 0.25^{a}	8.28 ± 0.19^{a}	3.45 ± 0.02^{abc}	0.49 ± 0.02^{ab}	5.02 ± 0.03^{abcd}	0.36 ± 0.04^{bc}	0.14 ± 0.01^{a}
TP2	12.92 ± 0.01^{def}	27.07 ± 0.85^{b}	10.51 ± 0.45^{a}	7.41 ± 0.31^{abcd}	3.47 ± 0.01^{ab}	0.50 ± 0.01^{a}	4.46 ± 0.17^{d}	0.31 ± 0.02^{c}	0.14 ± 0.01^{a}
Week 3									
PEF1	12.89 ± 0.04^{ef}	31.80 ± 1.22^{ab}	12.01 ± 0.79^{a}	5.92 ± 0.60^{cd}	3.42 ± 0.02^{bcd}	0.40 ± 0.01^{b}	5.21 ± 0.14^{ab}	0.54 ± 0.03^{ab}	0.13 ± 0.02^{a}
PEF2	13.09 ± 0.00^{abcd}	32.88 ± 0.71^{a}	12.29 ± 0.71^{a}	6.25 ± 0.30^{bcd}	3.46 ± 0.01^{abc}	0.46 ± 0.01^{ab}	5.22 ± 0.13^{a}	0.56 ± 0.01^{a}	0.13 ± 0.01^{a}
HPP1	13.22 ± 0.03^{a}	34.37 ± 0.40^{a}	12.62 ± 0.64^{a}	5.46 ± 0.19^{d}	3.40 ± 0.01^{bcd}	0.49 ± 0.01^{ab}	4.64 ± 0.27^{abcd}	0.45 ± 0.06^{abc}	0.15 ± 0.01^{a}
HPP2	13.16 ± 0.02^{ab}	33.66 ± 1.92^{a}	12.15 ± 0.73^{a}	5.43 ± 0.22^{d}	3.51 ± 0.02^{a}	0.48 ± 0.02^{ab}	4.89 ± 0.10^{abcd}	0.42 ± 0.05^{abc}	0.15 ± 0.01^{a}
TP1	12.96 ± 0.02^{cde}	31.15 ± 1.43^{ab}	11.87 ± 0.73^{a}	5.96 ± 0.25^{cd}	3.45 ± 0.01^{abc}	0.49 ± 0.01^{ab}	5.18 ± 0.13^{abc}	0.42 ± 0.03^{abc}	0.15 ± 0.01^{a}
TP2	13.05 ± 0.02^{bcde}	29.44 ± 0.92^{ab}	10.67 ± 0.65^{a}	7.10 ± 0.45^{abc}	3.41 ± 0.00^{bcd}	0.48 ± 0.00^{a}	5.13 ± 0.05^{abc}	0.41 ± 0.06^{a}	0.16 ± 0.00^{a}

991 [1)] Control is the untreated sample, the number 1 indicates low intensity and 2 represents high intensity (see **Table 1**).
992 Values are means and standard errors of three determinations.
993 Values with the different letters within one column are significantly different ($p < 0.05$).
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Table 4. Discriminant headspace components selected in cloudy apple juice for all treatments (control, PEF, HPP and thermal) based on VID procedure at week 0 and week 3 of shelf-life. The compounds are listed in decreasing order of absolute VID value, where a positive VID value indicates a higher concentration of a compound for that class compared to others and negative value lower concentration compared to other classes. The retention index (RI) and chemical group are listed for proof of identity. The compounds in italics have been reported in literature as key aroma volatiles in apple juice.

Treatment[1)]	VID[2)]	Identity	RI	Chemical group
Week 0				
Control	0.84	*hexanal*	1043	Aldehyde
PEF1	0.90	*2-methylbutyl acetate*	1095	Ester
	0.88	*isobutyl acetate*	959	Ester
	0.81	*amyl acetate*	1168	Ester
PEF2	0.88	*isobutyl acetate*	959	Ester
	0.88	limonene	1201	Terpene (hydrocarbon)
	0.83	*butyl acetate*	1031	Ester
HPP1	n.d.			
HPP2	n.d.			
TP1	n.d.			
TP2	0.94	pentane	557	Hydrocarbon
	0.90	heptane	702	Hydrocarbon
	0.84	ethyl ether	620	Ether
	–0.81	*ethyl butanoate*	986	Ester
	–0.82	*ethyl 2-methylbutanoate*	1005	Ester
Week 3				
PEF1	0.84	butanal	840	Aldehyde
PEF2	0.88	*isobutyl acetate*	959	Ester
	0.81	*2-methylbutyl acetate*	1095	Ester
	0.81	limonene	1200	Terpene (hydrocarbon)
	0.80	*butyl acetate*	1030	Ester
HPP1	0.96	*hexanal*	1042	Aldehyde
	0.90	butanoic acid	1626	Carboxylic acid
	0.84	2,4-hexadienal	1412	Aldehyde
HPP2	n.d.			
TP1	n.d.			
TP2	0.90	*hexyl acetate*	1277	Ester
	0.84	pentane	557	Hydrocarbon
	–0.81	*ethyl acetate*	846	Ester
	–0.83	*ethyl 2-methylbutanoate*	1005	Ester

[1)] Control is the untreated sample, the number 1 indicates low intensity and 2 represents high intensity.
[2)] n.d. means not detected by the VID procedure.