Changes in the physicochemical properties, aromas and polyphenols of not from concentrate (NFC) apple juice during production

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ABSTRACT
The effects of unit operations on the quality of not from concentrate (NFC) apple juice were investigated in this study. After the main processing steps (juicing, pre-pasteurization, homogenization and pasteurization), juice samples were collected and characterized by total soluble solid (TSS), titratable acidity (TA), pH, turbidity, non-enzymatic browning index (NEBI), color quality, aroma and phenolic constituents. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied for analyzing juice aromas and polyphenols. The results showed that homogenization only affected (increased) the juice turbidity, and only the TSS content was not affected by the unit operations. The TA, pH, aroma component and polyphenol content of the NFC apple juice were significantly decreased, while the turbidity, NEBI and color values were significantly increased by pre-pasteurization. Pasteurization had less effect on these properties than pre-pasteurization. Therefore, those were the thermal treatments that mainly affected the juice quality during NFC apple juice production.

Cambios en las propiedades fisicoquímicas, aromas y polifenoles durante la producción de jugo de manzana no procedente de concentrado (NFC)

RESUMEN
El presente estudio investigó los efectos detectados en las unidades de producción de jugo de manzana no procedente de concentrado (NFC). Después de realizarse los principales pasos del proceso (extracción, prepasteurización, homogeneización y pasteurización), se recogieron muestras que fueron caracterizadas a partir de la presencia de sólidos solubles totales (TSS), la acidez valorable (TA), el pH, la turbidez, el índice de oscurecimiento no enzimático (NEBI), la calidad de color, el aroma y los componentes fenólicos. Para valorar el aroma del jugo y la presencia de polifenoles se llevó a cabo un análisis de componentes principales (PCA) y un análisis de cluster jerárquico (HCA). Los resultados confirmaron que la homogeneización solo afectó la turbidez del jugo, aumentándola, siendo el contenido de TSS el único aspecto no afectado por la unidad de producción. La TA, el pH, el componente de aroma y el contenido fenólico del jugo de manzana NFC se redujeron significativamente, mientras, debido a la pasteurización, se registraron aumentos en la turbidez, el NEBI y los valores de color. La pasteurización tuvo menos efecto en estas propiedades que la prepasteurización. Por lo tanto, se concluye que, durante la producción de jugo de manzana NFC, la calidad es afectada principalmente por los tratamientos térmicos.

1. Introduction
Apple (Malus pumila Mill.) is one of the most widely cultivated tree fruits around the world and the main contributor of nutritional phytochemicals in human diet (Wang & Lu, 2014; Yang, Yang, Guo, Jiao, & Zhao, 2013). Apple can be consumed as fresh-eat fruit, as well as be processed into juice, fermented apple cider and vinegar (Bondonno, Bondonno, Ward, Hodgson, & Croft, 2017), among which apple juice is the most common and popular drink (Dhillon, Kaur, & Brar, 2013).

Because consumers are increasingly concerned about the nutrition and originality of food, not from concentrate (NFC) fruit juice that possesses natural fresh appearance, textural properties, flavor and nutrition has become more and more popular (Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011). Apple juice is well known to be rich in polyphenols (Lee, Seo, Rhee, & Kim, 2016; Persic, Mikulik-Petkovsek, Slatnar, & Veberic, 2017), showing the potential health effects on vascular function, lipids metabolism, inflammation, hyperglycaemia, anti-oxidation and anticancer (Bondonno et al., 2017; Ceymann, Arrigoni, Schärer, Bozzi Nising, & Hurrell, 2012). Currently, thermal processing remains the most widely used sterilization technology in food industry, which ensures the microbiological safety of products (Rawson, Patras, Tiwari, Noci, Koutchma, & Brunton, 2011). As a kind of perishable fruit product, juice is still commonly processed by applying thermal treatment to provide the products with better preservation properties (Tiwari, O’Donnell, & Cullen, 2009; Wibowo, Grauwet, Gedefa, Hendrickx, & Van Loey, 2015).

During NFC apple juice industrial production, mainly seven unit operations are involved, including washing and grading, milling, juicing, pre-pasteurization, homogenization, pasteurization, and hot filling and cooling. Among the operations, thermal treatments include pre-pasteurization and pasteurization, which are usually performed at 90°C for 30 s and 92–95°C for 25–60 s, respectively (Markowski, Baron, Le Quére’, & Plocharski, 2015; Taştan & Baysal, 2017). The thermal treatment and
homogenization are usually applied to extend shelf life and improve quality of fruit juice (He, Tao, Zeng, Zhang, Tao, Qin, & Chen et al., 2016). It has been reported that both the unit operations affected fresh juice quality to some extent (A佐efefa, Quesada, Pérez, Vaillant, & Michel, 2015; Aguilar-Rosas, Ballinas-Casarrubias, Nevezzo-Moorillon, Martin-Belloso, & Ortega-Rivas, 2007; He et al., 2016; Rawson et al., 2011; Santhirasegaram, Razali, George, & Somasundram, 2015). However, the effects of the unit operations on NFC apple juice quality should further shed light on exploring the potential way to improve the juice sensory and nutritional properties. To do this, NFC apple juice processing steps were simplified in our pilot-scale plant to four main unit operations, including juicing, pre-pasteurization, homogenization and pasteurization, as shown in Figure 1. Then, the effects of the main unit operations on the juice quality were investigated in terms of total soluble solid (TSS), titratable acidity (TA), pH, turbidity, non-enzymatic browning index (NEBI), color quality, aroma component and polyphenol composition. In addition, the aroma and polyphenol properties of apple juice were further analyzed by principal component analysis (PCA) and hierarchical cluster analysis (HCA) to provide a supportive concept of the effects of unit operations on the two indexes.

2. Materials and methods

2.1 Apple juice production and juice sampling

Fresh Fuji apples (Malus domestica Borkh. cv. Red Fuji) were purchased from Xi’an Guoyou Association (Xi’an, China) and transported to the pilot-scale factory in Shaanxi Normal University (Xi’an, China). Juice samples were collected in three independent and replicate productions. Because the polyphenoloxidase induced enzymatic browning makes apple juice browning, it was necessary to perform pre-pasteurization to inactivate enzymatic activity. Thus, as shown in Figure 1, the main unit operations in NFC juice processing included juicing (J), pre-pasteurization (P1), homogenization (HM) and pasteurization (P2). Additionally, the milling process (cold pulping) in this study removed the apple peel and seed, only delivering flesh pulp to the subsequent unit operation. Samples from the four main unit operations in the production were collected for three different processing batches. The bottled juices were cooled to room temperature (25 ± 0.5°C) by water bath and stored at 4°C for further analysis. Both the sample preparations and treatments were conducted in triplicates.

2.2 Determination of physicochemical properties

The TSS was measured using a refractometer (PAL-1, Atago, Japan) at 25 ± 0.5°C and the result was expressed as °Brix. The TA was determined using acid-base titration. Diluted juice sample was titrated with standardized 0.1 M NaOH solution to the end point with phenolphthalein (pH = 8.1 ± 0.1). The TA was expressed as g/L with malic acid as equivalent.

The pH of apple juice was measured using a digital pH meter (FE20 Plus, Mettler-Toledo, China) at 25 ± 0.5°C. During the measurement, juice sample was continuously stirred with a magnetic stirrer.

The turbidity of juice sample was determined using a turbidimeter (ET76910, Lovibond, Germany). The juice was diluted 10-fold with distilled water. Then, the diluted sample was filled into the test vials to measure the turbidity and the process was repeated three times for every treatment. The real turbidity of apple juice was calculated using the following equation:

\[ T_r = T_d \times f \]  

where \( T_r \) is real turbidity, \( T_d \) is turbidity of the diluted juice and \( f \) is dilution factor. The turbidity was expressed in standard nephelometric turbidity units (NTU).

The NEBI was determined according to the method reported by Cohen, Birk, Mannheim, and Saguy (1998) with some modifications. 3 mL of juice sample was mixed with 3 mL of 95% ethanol and the mixture was centrifuged at 7800 × g for 10 min. Next, the absorbance of the supernatant was measured at 420 nm. The absorbance of distilled water was applied as a blank.

The color of juice was measured using a colorimeter (NS800, 3°nh, China) at room temperature. The CIE \( L^*, a^*, b^* \) color system was applied in color measurement. After white and black calibration, 5 mL of juice sample was placed to a plastic dish (\( D = 3.5 \) mm) for the measurement. The \( L^* \), \( a^* \) and \( b^* \) correspond to lightness, greenness (-\( a^* \)) or redness (+ \( a^* \)), blueness (-\( b^* \)) or yellowness (+ \( b^* \)), were measured, respectively. Color difference (\( \Delta E \)) compared to the initial juice (after juicing), was calculated using the following equation.

\[ \Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \]
2.3 Aroma determination

Electronic nose (e-nose) determination has high sensitivity in non-destructive determination, real-time detection and online monitoring of volatile compounds, and has good correlation with data coming from human sensory panels for specific applications (Peris & Escuder-Gilabert, 2009). The e-nose sensors are able to detect the chemical groups in the headspace, which are released by solid or liquid samples in test tube. The aroma of apple juice samples was measured using an e-nose (PEN3, Airsense Analytics GmbH, Germany) containing 10 metal-oxide semiconductor chemical sensors, which are shown in Table 1. Aromas of all four groups of juice samples (J, P1, HM and P2) and 10 replicates for each group were detected. Prior to measurement, the gas path of e-nose was cleaned by cleaning gas (ambient air filtered through activated charcoal) for 20 min to normalize the sensor signals. Then, 10 mL of apple juice was transferred into a 50 mL glass vial with a plastic septum in the screw cap. The sample was equilibrated for 5 min to develop the headspace volatiles before analysis. During the measurement, the headspace volatiles were absorbed by a Luer-lock needle connected to a Teflon tubing (3 mm) at a flow rate of 300 mL/min (Wang et al., 2016). After each test, the sample gas path was cleaned by cleaning gas for 300 s to ensure the sensor signals to return to the base line. The response of sensor was expressed as the ratio of conductance G/G0 (G0 and G are the conductances of sensor before and after being exposed to the gas samples, respectively). The data were collected per second, and the measurement lasted for 60 s, which was long enough to stabilize the sensor signals. Then, 10 mL of apple juice was transferred into a 50 mL glass vial with a plastic septum in the screw cap. The sample was equilibrated for 5 min to develop the headspace volatiles before analysis. During the measurement, the headspace volatiles were absorbed by a Luer-lock needle connected to a Teflon tubing (3 mm) at a flow rate of 300 mL/min (Wang et al., 2016). After each test, the sample gas path was cleaned by cleaning gas for 300 s to ensure the sensor signals to return to the base line. The response of sensor was expressed as the ratio of conductance G龚 G龚 (G龚和G龚是传感器前后的导电率) and after being exposed to the gas samples, respectively). The data were collected per second, and the measurement lasted for 60 s, which was long enough to stabilize the sensor signal. The e-nose measurement was performed at room temperature.

2.4 Polyphenols determination

3 mL of apple juice was mixed with 6 mL 80% methanol (v/v) in the Teflon tube and sonicated at 25°C for 15 min. Then, the tubes were centrifuged with 9000 x g for 10 min at 4°C. The supernatant was collected and filtered through a 0.22 µm Teflon membrane filter. The determination of the individual polyphenols in juice samples was performed using ultra performance liquid chromatography (UPLC) analysis according to our previous reported method (Sun, Chen, Niu, Yang, & Guo et al., 2017). Separation was performed on a Thermo® syncronis C18 column (250 x 4.6 mm, 5 µm, I.D., USA).

Chromatographic analyses were carried out on a Thermo® Ultimate 3000 UPLC system (Thermo Electron Co. USA). Elution with solvent A (30% acetonitrile and 70% methanol) and solvent B (1% trifluoroacetic acid and 5% methanol) in a step gradient was carried out as follows: 0–3 min, 100% B at a flow rate of 0.95 mL/min; 3–19.05 min, 100–60% B at a flow rate of 0.95 mL/min; 19.05–30.1 min, 60% B from a flow rate of 0.95 mL/min to a flow rate of 1 mL/min; 30.1–30.2 min, 60–100% B from a flow rate of 1 mL/min to a flow rate of 0.95 mL/min; 30.2–41 min, 100% B at a flow rate of 0.95 mL/min. During the run, the detection wavelength was 280 nm and the injection volume was 4 µL.

2.5 Statistical analysis

All analyses in this study were conducted in triplicate. Data obtained were subjected to statistical analysis using SPSS 18.0 software (SPSS, USA) and were expressed as means ± standard deviations. Results were submitted to analysis of variance (ANOVA) with significance level of p < 0.05, evaluating by Duncan’s test. For the aroma and polyphenolic quality, PCA and HCA were performed to analyze the juices sampled at different processing stages.

3. Results and discussion

3.1 Effect on physicochemical properties

NFC apple juice was collected and sampled at different stages during simplified pilot scale production (Figure 1) and four groups of juice samples were obtained (J, P1, HM and P2). The physicochemical properties of juice samples including TSS, TA, pH, turbidity, NEBI and color quality (L*, a*, b* and ΔE) are shown in Table 2.

For fruit juice, TSS contains a small part of dissolved nutrients but mainly sugar components including fructose, glucose, sucrose, etc., (Kelebek, Selli, Canbas, & Cabaroglu, 2009; Wu, Gao, Zhao, Liao, Chen, Wang, & Hu 2007). No significant (p < 0.05) changes were found for TSS of the four samples, indicating that the unit operations applied in the production hardly affected the content of TSS of NFC apple juice. The TA of apple mainly indicates the content of organic acids including tartaric, quinic, malic, shikimic, citric and succinic acids, among which the malic acid is the predominant one (Wu et al., 2007). In the current study, with the processing performed, the TA of juice was decreased significantly (p < 0.05) from 3.06 g/L (juicing) to 2.72 g/L (pre-pasteurization), and then to 2.39 g/L (pasteurization), while the homogenization had no effect on TA. It is worth noting that the significant changes of TA in this study were only observed for heat treatments (pre-pasteurization and pasteurization), which could be due to the evaporation of organic acids during thermal treatments (Charles-Rodriguez, Nevárez-Moorillón, Zhang, & Ortega-Rivas, 2007).

During the processing, the pH value was decreased from 3.87 (juicing) to 3.52 (pre-pasteurization), which could be due to the evaporation of organic acids during thermal treatments (Charles-Rodriguez, Nevárez-Moorillón, Zhang, & Ortega-Rivas, 2007).

Table 1. Aromas of all four groups of juice samples (J, P1, HM and P2) and 10 replicates for each group were detected. Prior to measurement, the gas path of e-nose was cleaned by cleaning gas (ambient air filtered through activated charcoal) for 20 min to normalize the sensor signals. Then, 10 mL of apple juice was transferred into a 50 mL glass vial with a plastic septum in the screw cap. The sample was equilibrated for 5 min to develop the headspace volatiles before analysis. During the measurement, the headspace volatiles were absorbed by a Luer-lock needle connected to a Teflon tubing (3 mm) at a flow rate of 300 mL/min (Wang et al., 2016). After each test, the sample gas path was cleaned by cleaning gas for 300 s to ensure the sensor signals to return to the base line. The response of sensor was expressed as the ratio of conductance G龚 G龚 (G龚和G龚是传感器前后的导电率) and after being exposed to the gas samples, respectively). The data were collected per second, and the measurement lasted for 60 s, which was long enough to stabilize the sensor signal. The e-nose measurement was performed at room temperature.

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During the processing, the pH value was decreased from 3.87 (juicing) to 3.52 (pre-pasteurization), but was increased significantly (p < 0.05) to 3.64 by the pasteurization. Fruit juice is one of the most common natural buffer systems containing a considerable amount of weak acids, which provide juice with a resistance to pH change. With the thermal treatment performed, the buffer system is destroyed and the conjugated base in juice loses the neutralizing ability, making more H+ released and resulting in the lower

Table 1. Sensors used in PEN3 e-nose and the main applications.

| Number | Name     | Relative volatile compounds                   | Reference |
|--------|----------|-----------------------------------------------|-----------|
| S1     | W1C      | Aromatic compounds                            | Toluene, 10 mg/L |
| S2     | WSS      | Polar and nitrogen oxides                     | NO<sub>2</sub>, 1 mg/L |
| S3     | WSC      | Aromatic compounds, ketones and aldehydes     | Benzene, 10 mg/L |
| S4     | W6S      | Hydrogen                                       | H<sub>2</sub>, 100 mg/L |
| S5     | W5C      | Low-polarity aromatic compounds and alkanes    | Propane, 1 mg/L |
| S6     | W1S      | Broad-methane                                  | CH<sub>3</sub>H, 100 mg/L |
| S7     | W1W      | Sulfur organic compounds and terpenes          | H<sub>2</sub>S, 1 mg/L |
| S8     | W2S      | Broad alcohols, ketones and partially aromatic compounds | CO, 100 mg/L |
| S9     | W2W      | Sulfur and aromatic compounds                  | H<sub>2</sub>S, 1 mg/L |
| S10    | W3S      | Methane-aliphatic compounds                    | CH<sub>4</sub>, 100 mg/L |
Table 2. Physicochemical properties of NFC apple juice during juice production.

|                | J         | P1        | HM        | P2        |
|----------------|-----------|-----------|-----------|-----------|
| TSS (Brix)     | 13.10 ± 0.16a | 13.00 ± 0.24a | 13.13 ± 0.29a | 13.23 ± 0.17a |
| TA (g/L)       | 3.06 ± 0.14a | 2.72 ± 0.07b | 2.82 ± 0.12ab | 2.39 ± 0.18c |
| pH             | 3.87 ± 0.05a | 3.52 ± 0.04c | 3.56 ± 0.04bc | 3.64 ± 0.04b |
| Turbidity      | 2057 ± 202c | 3503 ± 113b | 3803 ± 152ab | 3910 ± 126a |
| NEBI (NTU)     | 1.15 ± 0.03c | 1.31 ± 0.04b | 1.28 ± 0.05b | 1.85 ± 0.08a |
| L*             | 27.51 ± 0.13c | 35.50 ± 0.14ab | 36.10 ± 0.15a | 36.47 ± 0.18a |
| a*             | −3.36 ± 0.03a | −3.93 ± 0.02c | −3.95 ± 0.03c | −3.78 ± 0.04b |
| b*             | 7.62 ± 0.16c | 10.08 ± 0.35c | 11.05 ± 0.07b | 12.12 ± 0.04a |
| ΔE             | −3.23 ± 0.02c | 8.39 ± 0.21c | 9.21 ± 0.22c | 9.99 ± 0.14a |

All the data were expressed as the means ± standard deviations. Values followed by different letters within the same row are significantly different (Duncan’s test, p < 0.05). J: Juicing; P1: Pre-pasteurization; HM: Homogenization; P2: Pasteurization.

Table 2. Propiedades fisicoquímicas del jugo de manzana NFC durante la producción de jugo.

|                | J         | P1        | HM        | P2        |
|----------------|-----------|-----------|-----------|-----------|
| TSS (Brix)     | 13.10 ± 0.16a | 13.00 ± 0.24a | 13.13 ± 0.29a | 13.23 ± 0.17a |
| TA (g/L)       | 3.06 ± 0.14a | 2.72 ± 0.07b | 2.82 ± 0.12ab | 2.39 ± 0.18c |
| pH             | 3.87 ± 0.05a | 3.52 ± 0.04c | 3.56 ± 0.04bc | 3.64 ± 0.04b |
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| L*             | 27.51 ± 0.13c | 35.50 ± 0.14ab | 36.10 ± 0.15a | 36.47 ± 0.18a |
| a*             | −3.36 ± 0.03a | −3.93 ± 0.02c | −3.95 ± 0.03c | −3.78 ± 0.04b |
| b*             | 7.62 ± 0.16c | 10.08 ± 0.35c | 11.05 ± 0.07b | 12.12 ± 0.04a |
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All the data were expressed as the means ± standard deviations. Values followed by different letters within the same row are significantly different (Duncan’s test, p < 0.05). J: Juicing; P1: Pre-pasteurization; HM: Homogenización; P2: Pasteurización.

Thus, the pH value of NFC juice was decreased significantly (p < 0.05) by the pre-pasteurization due to the destruction of buffer system. On the other hand, the increased pH by the pasteurization might be attributed by the decrease of TA (Table 2) caused by the evaporation of organic acids as discussed above. For fruit juices, turbidity represents the level of the cloudiness or haziness caused by the dispersed matter that is mainly formed by the cellular tissues comminuted during fruit processing (Benitez & Lozano, 2007). As shown in Table 2, the turbidity of juice samples was increased gradually along with the processing performed. Compared to the juicing processing, the turbidity was increased by 70.3%, 84.9% and 90.1% by pre-pasteurization, homogenization and pasteurization, respectively. The increase in the turbidity after the thermal treatments (pre-pasteurization and pasteurization) could be attributed to the increased concentration of colloidal pectin in juice because thermal pasteurization greatly ruptures cell structure, which allows pectin to leak out (Santhirasegaram et al., 2015). In NFC juice production, homogenization has been suggested as a manner to stabilize various cloudy substances. Although there was no significant difference in apple juice turbidity between pre-pasteurization and homogenization, the slight increase in turbidity by homogenization was observed (3503 to 3803 NTU). This could be due to the fact that during juice processing, homogenization breaks particles or droplets into micron sizes to create a stable dispersion or emulsion, providing juice with higher turbidity (Silva et al., 2010). Moreover, turbidity is often used to reflect juice stability (Lv, Kong, Mou, & Fu, 2017). Thus, the increased turbidity of juice indicated that the juice stability was enhanced by the unit operations. NFC considered an important quality index of food products, is highly related to Maillard reaction that causes color change, off-flavor and nutrient loss (Caminiti et al., 2011). In the present study, significant (p < 0.05) increase in the NEBI of juice sample was observed for the thermal treatment (pre-pasteurization and pasteurization), while the homogenization had less effects on juice browning (Table 2). Although thermal processing is still the most widely applied sterilization processing technology in food industry, which ensures microbiological safety of products (Rawson et al., 2011), it has been also widely reported that thermal treatment causes non-enzyme browning severely (Damasceno, Fernandes, Magalhães, & Brito, 2008; Vaikousi, Koutsoumanis, & Billaderis, 2008). Because of the Maillard reaction enhanced by the thermal pasteurization, the browning compounds were formed and subsequently darkened the juice color, making the NEBI increased with apple juice production performed (Santhirasegaram et al., 2015).

The color quality of apple juices sampled at different processing steps were characterized by CIE L*, a*, b* and ΔE (Table 2). The L* value was increased significantly (p < 0.05) by pre-pasteurization, while homogenization and pasteurization almost kept the value stable. The elevated L* value in the first heat treatment (pre-pasteurization) may be due to the fact that the heat strongly suppresses the browning of apple juice (Lee, Lusk, Mirosa, & Oey, 2016). Regarding the browning, both enzymatic and non-enzymatic browning darken the juice color, among which enzymatic browning can be prevented by inactivating the related enzymes. It has been reported that high-temperature and short-time treatment at 80°C could inactivate polyphenol oxidase, while pectinesterase activity could not be inactivated completely even at 90°C (Krapfenbauer, Kinner, Gössinger, Schönlechner, & Berghofer, 2006). The pre-pasteurization (98°C, 30 s) in the current study is considered to be efficient for enzyme inactivation, suppressing enzymatic browning during juice production. The increased L* value after thermal treatment was also observed in previous studies (Davidov-Pardo, Gumus, & McClements, 2016; Krapfenbauer et al., 2006), providing the juice with brighter color and more transparent appearance. Lutein is the main free carotenoid existing in most apple cultivars (Delgado-Pelayo, Gallardo-Guerrero, & Hornero-Méndez, 2014). There are about 15μg/100 g Lutein trans, 18μg/100 g β-Carotene trans and 6μg/100 g β-Carotene cis in apple (Perry, Rasmussen, & Johnson, 2009), providing apple juice with yellow color that is highly related with b* value. The increased b* value by pre-pasteurization could be due to the fact that lutein is relatively stable in high temperature (Davidov-Pardo et al., 2016) and the thermal treatment greatly ruptures cell structure, making more lutein dissolved into the juice system. Besides, the increased b* value indicated that the juice color became darker with more yellow components, which might be caused by Maillard reaction during the processing as well (Santhirasegaram et al., 2015), and that result was in accordance with that of NEBI.

3.2. Aroma quality analysis

3.2.1 Electronic nose analysis

Figure 2 shows the typical response signals of e-nose sensors to the apple juices sampled at different processing steps. The response signal was expressed as G/G0. Here, G0 and G represented the resistance of the sensor in the zero gas and sample gas, respectively. The response signals of 10 sensors were stabilized after 50 s (Figure 2). It was found that the response signals of sensor 2 (S2, the following abbreviations are the same), S6, S7 and S8 were increased to different degrees for all the NFC juices. Among all the sensors of initial juice (after juicing processing), the signals of S7 and S2 were intensively increased and remained at a relatively high level (Figure 2a), indicating that terpenes, sulphur organic compounds and NOx mainly contributed to the aroma of apple juice (Wang et al., 2016). It is worth noting that, for the juice samples before pasteurization (J, P1 and
HM) (Figure 2(a-c)), response peaks of S2 and S7 were observed. However, for the juice after pasteurization, no response peak was detected and the response signals of S2 and S7 reached at a relatively lower level, suggesting that the content of terpenes, sulphur organic compounds and NOX in the juice after pasteurization were significantly decreased.

The average stable response signals of 10 sensors during 55–57 s were formed the radar chart (Figure 2(e)). Although the radar chart of different juice samples had shown a similar shape, the response strengths of S2, S6, S7 and S8 for the different juice samples were different. The concentration and threshold values of volatile compounds should be taken into account for studying the contribution of volatile compounds to the flavor perception. As shown in Figure 2(e), the response values of S2 and S7 of juices after pre-pasteurization and homogenization were higher than that of juice after juicing, indicating that the volatile components, including terpenes, sulphur organic compounds and NOX were enhanced by the pre-pasteurization, while homogenization kept the aroma components stable. On the other hand, the response values of S2 and S7 of juice after pasteurization were decreased significantly, even lower than that of the initial juice (after juicing) (Figure 2(e)), indicating that pasteurization reduced the aroma components in juices. The aroma enhancement after pre-pasteurization might be attributed to the volatile substances released from ruptured flesh cells; whereas, the aroma loss could be due to the fact...
that subsequent thermal treatment causes distinct loss of the major juice aroma component (Steinhaus, Bogen, & Schieberle, 2006). Thus, the results demonstrated that the number of thermal treatment might affect the consumer perception to the apple juice aroma. However, to state the reaction mechanism of changes of juice aroma during the production, more information of physicochemical changes occurring in the sensors of e-nose needs to be investigated (Smyth & Cozzolino, 2013).

3.2.2 Aroma analysis based on PCA and HCA

To further analyze the effect of the certain unit operation in juice processing on aroma quality, response signals of 10 sensors were analyzed by PCA analysis (40 samples × 10 sensors). The loading variables of PCA in the first two principal components (PCs) are shown in Figure 3(a). The first two PCs explained 79.1% of the total variance. PC1 that accounted for 62.8% of the total variance was positively correlated to S2, S6, S7 and S8 and negatively correlated to S1, S3 and S5. PC2 that accounted for 16.3% of the total variance was mainly positively correlated to S10. The two-dimensional score plot of PCA according to the aroma quality of juices is shown in Figure 3(b). A clear separation was observed and four juice samples were grouped into three isolate regions, in which the overlapping area was found for the juices after pre-pasteurization and homogenization. Since samples lying close together in the coordinate system defined by PC1 and PC2 are considered to be characterized by a certain similar property, the juices after pre-pasteurization and homogenization possessed a similar aroma quality. This is in accordance with the similar result of radar chart analysis for the two juice samples (Figure 2(e)). Besides, the classification of the four juices by PCA is consistent with the difference in aroma properties characterized by radar chart analysis.

As shown in Figure 3(b), both the initial juice (after juicing) and the juice after pasteurization were negatively located in the PC1 direction (PC1: positively correlated to S2, S6, S7 and S8), while the juice after pre-pasteurization was positively located in the PC1 direction. This classification confirmed the finding that pre-pasteurization could enhance the aroma quality, while subsequent pasteurization was able to reduce the main aroma components of NFC apple juice. In addition, homogenization kept the aroma quality untouched.

As a technique attempting to separate data into specific groups based on similarity or distance among observations (Huang, Guo, Qiu, & Chen, 2007), HCA could combine the closest data more flexibly, ignoring the category of sample. Compared to PCA, HCA dendrogram provided a deeper

![Figure 3. Loading variable plot (a), score plot (b) and heatmap associated with HCA (c) based on the aroma component of the juices.](image-url)

**Figure 3.** Diagrama de las variables de carga (a); Diagrama de datos (b); y mapa de calor asociado con HCA (c), basados en el componente de aroma de los jugos.
3.3. Polyphenols analysis

3.3.1 UPLC analysis

Apart from the traditional nutrients in apples like sugar, fibers, minerals and vitamins, apple polyphenols have been increasingly studied for their bioactivity and health-benefit effects (Bao et al., 2013; Tu, Chen, & Ho, 2017). To determine the composition of polyphenols of the studied juices, 16 individual phenolic standards were used and the mixed standards were separated well using UPLC (Figure 4(a)). The chromatograms of juices sampled after different unit operations are shown in Figure 4(b). In the current study, 13 individual polyphenols of juices were identified and quantified, and the contents of individual phenolics are shown in Table 3. Among the 13 determined polyphenols, the predominant ones in the juices were procyanidin B2, chlorogenic acid and epicatechin. Besides, the identified individual polyphenols summarized in Table 3 belong to three classes: epigallocatechin, catechin, procyanidin B2, epicatechin and epicatechin gallate belong to procyanidins. Protocatechuic acid, chlorogenic acid, 4-hydroxybenzoic acid and ellagic acid belong to phenolic acids, and rutin, hyperin, quercetin and phlorizin belong to flavonoids. Previous studies have reported that procyanidins are the main polyphenols in fresh apple fruits, and phenolic acids are the second abundant ones (Renard et al., 2011). However, in the apple juices, the content of phenolic acids was higher than that of procyanidins in this study, which could be attributed to the higher solubility of phenolic acids, making these compounds as the main polyphenols in apple juices (Oszmianski & Wojdylo, 2006; Van der Sluis, Dekker, Skrede, & Jongen, 2002). Besides, apple peel and seed contain much more polyphenols than apple flesh; in addition, flavonoids are mainly included in apple peel in most varieties (Francini & Sebastiani, 2013). Because apple peel and seed were removed before juicing in the current study, the lower content of flavonoids in the tested juices was due to the removal of peel and seed.

Quantitative UPLC data indicated that all the individual polyphenols were dramatically decreased with juice processing performed. It was found that both the heat treatments (pre-pasteurization and pasteurization) decreased the individual polyphenol contents, while homogenization hardly affected that (Table 3). Compared to the initial juice (after juicing), the total polyphenol content was decreased by 21.06%, 21.41% and 41.92% after pre-pasteurization, homogenization and pasteurization, respectively. Procyanidin and

Table 3. Individual phenolic contents (mg/L) of NFC apple juice during juice production.

| J | P1 | HM | P2 |
|---|----|----|----|
| Protocatechuic acid | 4.24 ± 0.37a | 2.58 ± 0.42bc | 2.69 ± 0.54b | 1.68 ± 0.64c |
| Epigallocatechin | 26.98 ± 1.73a | 17.95 ± 1.62b | 17.15 ± 1.79b | 11.12 ± 2.42c |
| Catechin | 12.25 ± 1.45a | 8.36 ± 0.40b | 7.94 ± 0.55b | 5.12 ± 0.37c |
| Procyanidin B2 | 68.02 ± 1.75a | 55.91 ± 1.45b | 54.35 ± 4.28b | 43.19 ± 2.97c |
| Chlorogenic acid | 161.25 ± 7.24a | 136.19 ± 9.84b | 139.54 ± 7.34b | 112.64 ± 10.02c |
| 4-hydroxybenzoic acid | 11.34 ± 0.97a | 8.20 ± 0.63b | 8.08 ± 0.78b | 3.36 ± 0.47c |
| Epicatechin | 41.51 ± 1.70a | 29.85 ± 1.35b | 28.57 ± 2.49b | 18.28 ± 2.01c |
| Epicatechin gallate | 1.25 ± 0.14a | 0.71 ± 0.08b | 0.63 ± 0.11b | 0.37 ± 0.06c |
| Rutin | 5.52 ± 0.41a | 4.12 ± 0.23b | 3.86 ± 0.34b | 2.01 ± 0.27c |
| Hyperin | 2.58 ± 0.28a | 1.02 ± 0.12b | 1.08 ± 0.15b | 0.35 ± 0.06c |
| Ellagic acid | 7.44 ± 0.62a | 5.78 ± 0.33b | 5.35 ± 0.32b | 1.65 ± 0.27c |
| Quercetin | 8.04 ± 0.92a | 5.85 ± 0.46b | 6.06 ± 0.36b | 3.62 ± 0.41c |
| Phlorizin | 0.82 ± 0.06a | 0.67 ± 0.05b | 0.65 ± 0.06b | 0.43 ± 0.02c |
| Procyanidin | 149.91 ± 5.05a | 122.78 ± 4.19b | 108.65 ± 3.52b | 78.08 ± 2.44c |
| Phenolic acids | 184.26 ± 7.61a | 152.75 ± 9.09b | 155.68 ± 6.94b | 119.44 ± 10.16c |
| Flavonoids | 16.96 ± 0.86a | 11.67 ± 0.53b | 11.65 ± 0.35b | 6.40 ± 0.19c |
| Total polyphenols (mg/L) | 351.14 ± 10.89a | 277.19 ± 12.56b | 275.98 ± 6.95b | 203.93 ± 12.41c |

All the data were expressed as the means ± standard deviations. Values followed by different letters within the same row are significantly different (Duncan’s test, p < 0.05). J: Juicing; P1: Pre-pasteurization; HM: Homogenization; P2: Pasteurization.
Todos los datos se expresan como medias ± desviaciones estándares. Los valores en los que figuran distintas letras en la misma fila son significativamente diferentes (prueba de Duncan, p < 0.05). J: Extracción; P1: Prepasteurización; HM: Homogenización; P2: Pasteurización.
phenolic acid are heat-labile compounds; therefore, they were thermally degraded by both the pre-pasteurization and pasteurization. Flavonoids are considered to be stable during processing because of their glycosylated flavonol structure (Capanoglu, de Vos, Hall, Boyacioglu, & Beekwilder, 2013). However, in our study, the contents of flavonoids in NFC apple juices were significantly decreased by 31.2% and 62.3% after pre-pasteurization and pasteurization processing, respectively. This indicates that the heat treatment during NFC apple juice processing indeed destroyed polyphenol components.

3.3.2 Polyphenols analysis based on PCA and HCA

Similar to the analysis of aroma quality, both PCA and HCA were applied to analyze the UPLC result. The score plot (Figure 4(c)) of phenolic constituents in apple juices showed that the first two principal components accounted for 91.8% and 2.8% of the total variance, respectively, covered the original data summarized in Table 3 well. Data scattered close together were characterized by a high similarity in polyphenol composition. In the plot of the scores on the spaces defined by PC1 and PC2, a clear separation of the apple juices according to different phenolic contents was obtained. The initial juice (after juicing) and the juice after pasteurization were located in a respective isolated region, while the juices after pre-pasteurization and homogenization were located in a same group. This indicates that both the heat treatments (pre-pasteurization and pasteurization) affected the polyphenol profile of NFC juice, while homogenization did not change that significantly.

To further verify the results of PCA, the UPLC result was analyzed by HCA method and presented using a heatmap combined with the dendrogram of HCA (Figure 4(d)). In accordance with the result of PCA, four juice samples were grouped into three clusters. According to the heatmap and content/color rule, procyanidin B2 and chlorogenic acid were the predominant polyphenols existed in apple juices in the current study, while the contents of other phenols were shown at a relatively low level. Characterized by the color change, it is clear that with the juice processing performed, the contents of determined polyphenols decreased.
individual polyphenols were decreased to different degrees. Both PCA and HCA of UPLC analysis illustrated that it is not the heat treatment, but homogenization that affected the phenolic quality of juice during NFC juice production.

4. Conclusion
Compared with fresh squeezed apple juice, quality changes of NFC apple juice such as basic physicochemical property decline, aroma component loss and polyphenol content reduce are more inevitable during industrial production. Therefore, the effects of the main unit operations including pre-pasteurization, homogenization and pasteurization during NFC apple juice production on the juice quality were investigated in the current study. It was found that pre-pasteurization decreased the TA, pH, aroma component and polyphenol content of the NFC juice, and increased the turbidity, NEBI and color values of the juice. Although pasteurization had such the influences on the juice as well, the effects were less than pre-pasteurization. In addition, homogenization had no significant effects on the juice quality, except for the turbidity. The TSS content was kept unchanged by all the unit operations. Therefore, the optimization of processing techniques to improve NFC apple juice quality may focus on the modification of pre-pasteurization conditions during NFC juice production.

Abbreviations
NFC: not from concentrate; TSS: total soluble solids; TA: titratable acidity; NEBI: non-enzymatic browning index; UPLC: ultra performance liquid chromatography; PCA: principal component analysis; HCA: hierarchical cluster analysis.

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