Effect of thermal pasteurization and ultraviolet treatment on the quality parameters of not-from-concentrate apple juice from different varieties

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ABSTRACT

Consumers are increasingly seeking not-from-concentrate (NFC) juice as a convenient, less expensive alternative to fresh juice. This study evaluated the effect of thermal pasteurization (TP) and ultraviolet (UV) treatment of NFC apple juice on microbial growth and changes in the quality parameters. Both TP and UV decreased the total number of bacteria, mould and yeast and achieved the requirement of commercial sterilization in the NFC juice. The soluble solids, total sugars and phenolics in the juice were not significantly affected by both UV and TP. The shelf life of NFC samples treated with UV and TP was extended for 3 and 5 weeks longer than freshly squeezed juices, respectively. However, the samples treated with UV could effectively avoid the increasing of turbidity and improve quality characteristics. UV is a suitable alternative to TP during processing, because UV effectively retains fresh-like properties.

Efecto de la pasteurización térmica y el tratamiento ultravioleta en los parámetros de calidad del jugo (zumo) de manzana no de concentrado de diferentes variedades

RESUMEN

Cada vez más los consumidores buscan el jugo no de concentrado (NFC) como una alternativa conveniente y menos costosa al jugo fresco. Este estudio evaluó el efecto provocado por la pasteurización térmica (TP) y el tratamiento ultravioleta (UV) en el crecimiento microbiano y los parámetros de calidad al ser aplicados al jugo de manzana NFC. Tanto la TP como el UV disminuyeron el número total de bacterias, moho y levadura, logrando el requisito de esterilización comercial en el jugo NFC. Los rayos UV y la TP no afectaron significativamente los contenidos de sólidos solubles, azúcares totales y fenólicos en el jugo. La vida útil de las muestras de NFC tratadas con UV y TP se extendió por 3 y 5 semanas más que la de los jugos recién exprimidos, respectivamente. Por otra parte, se comprobó que las muestras tratadas con UV podrían evitar efectivamente el aumento de la turbidez, mejorando las características de calidad. El UV es una alternativa adecuada a la TP durante el procesamiento, ya que conserva eficazmente las propiedades del jugo fresco.

Introduction

Consumers are becoming increasingly aware of the nutritional value and health benefits of juice (Beaulieu & Obando-Ulloa, 2017). Fruit juice processed by squeezing the fresh fruit and preserved without being concentrated is labeled as “not-from-concentrate (NFC) juice” (Grimi, Mammouni, & Lebovka, 2011). NFC juices are very popular because of retaining the majority of nutrients and aroma of the fresh-like fruits. NFC juice sales were expected to increase 5.3% annually from 2012 to 2016, and NFC juice sales were expected to increase 4% annually in Europe over the same time period (Rohan, 2013). However, the majority of juice in the Chinese market is the concentrated juice (FC), mixed with water, sugar, acid, essence and preservatives, and the juices processed from concentrated have significantly reduced the nutritional and aroma compounds (Beaulieu & Stein-Chisholm, 2016). The higher price and shorter shelf life of NFC juice lead to slow development in the Chinese market.

The crucial processing of NFC juice is the microbial reduction step in order to ensure safety of the product. In recent years, the demands from the consumers for fresh-like properties, minimally processing has been rapidly increasing (Khandpur & Gogate, 2016). The heat-treatment is the frequently-used approach, due to inactive the enzymes and microorganisms to expand the shelf life of juices in the industrial production. However, the heat-treatment may have adverse effects on the sensory and functional attributes of juices (Noci et al., 2008). Currently, the non-thermal treatments have received more attention for inactivating spoilage and pathogenic microorganisms and could minimize the quality loss in terms of sensory and nutritional properties (Mertens & Knorr, 1992). The non-thermal treatments include pulse or radio frequency electric fields (PEF/RFEF), ultraviolet irradiation (UV), ultrasound (US), pulsed light (PL), high pressure processing (HP), ionizing radiation, dense phase carbon dioxide (DPCO₂) and ozone (Mohan & Benchamaporn, 2007).

UV treatment is a promising approach due to the reduction of food-borne microbial contamination of juices. The Food and Drug Administration (2000) has approved UV as a non-thermal method treatment for fruit juice. UV affected the DNA of microbial cells based on the formation of thymine dimer and destruction of microbial cells (Koutchma, 2009).
addition, the UV sterilization may retain the majority of nutritional attributes and does not generate the by-products that may adversely affect sensory properties of juices (Guerrero-Bertran & Barbosa-Canovas, 2004).

The efficiency of UV sterilization depends on the flow rate of the fluid, moisture content, amount of solid particles, fluid thickness, the exposure time, age of lamps used, the type and initial density of microorganisms and et al (Begum, Hocking-Ailsa, & Miskelly, 2009; Guerrero-Betran & Barbosa-Canovas, 2005). UV sterilization of apple, orange, pineapple juice has been reported (Keyser, Muller, Cillers, Nel, & Gouws, 2008; Shamsudin, Adzahan, Yee, & Mansor, 2014). However, research on the effect of UV on the sensory and nutritional properties of the NFC fruit juice is still scarce and requires the further investigation. The solid particles of the NFC juice are larger than that of the FC juice, resulted in the different efficiency of UV (Beaulieu & Obando-Ulloa, 2017). Therefore, the object of this study is to evaluate the influence of conventional thermal pasteurization (TP) and UV on the physical and chemical properties of the NFC apple juices and to establish the optimum sterilization approach to expand the shelf life and retain the most of fresh-like nutrients and sensory properties.

Materials and methods

Chemicals

Phenolphthalein, sodium hydrate, citric acid, potassium ferrocyanide, methylene blue, methyl red, cupric sulfate and potassium sodium tartrate were purchased from Sigma-Aldrich (China). Plate count agar and Bengal red Agar medium were from Beijing Auberstar Biotechnology Co., Ltd.

NFC apple juices preparation

Fresh Fuji, Red Delicious and Granny Smith apples were purchased from the local supermarket (Xi’an, Shaanxi province, China) and stored at 4 ± 1°C. The apples were removed 1/2–3/4 diameter disc of peel, and extracted the juices with the household juicer (HU-780WN, Hurom, Korea). 4% (w/v) ascorbic acid solution was used to protect the color during the juice extraction processing. The juices were filtered using the mesh filters and processed by the following treatments, respectively: (1) TP (98°C for 30 s); (2) UV (254 nm, 1 h, 6 W). Then the juice samples were stored at 4 ± 1°C for further analysis.

UV treatment conditions

The filtered apple juices were treated with a SB-12/T5 laboratory unit (Xi’an, Shaanxi province, China) that was similar with UV used by Koutchma, Keller, Chirtel, and Parisl (2004) and Shamsudin, Ling, Adzahan, and Daud (2013). The UV irradiation consist two low pressure lamps that emit the UV light at 254 nm. The apple juices were pumped and flowed through the UV laboratory unit at a flow rate of 2.12 L/min. For the repetitive, the ultraviolet irradiated juices run through two cycles and were transferred aseptically to sterile bottles for further analysis.

Physicochemical properties

Total soluble solids (TSS)
The total soluble solid (TSS) of the apple juices was evaluated with the digital refractometer (Atago PAL-1, Japan) and expressed as DBrix. Triplicates of the treatment were conducted and the results were averaged.

Color measure

The color of apple juices was determined with a colorimeter (NS800, 3nh Inc., China) and the CIE L*, a* and b* color system. After the calibration, 5 mL juice was placed into a plastic dish and repeated thrice for every treatment. The L*, a*, b*, Chroma* (C*) and hue angle (h°) values correspond to lightness, greenness (−a*) or redness (+a*), blueness (−b*) or yellowness (+b*), chroma, and hue angle, respectively. Chroma and hue angle were calculated with a* and b*values (Bernalte, Sabio, Hernandez, & Gervasin, 2003).

\[
C^* = \left( (a^*)^2 + (b^*)^2 \right)^{1/2}
\]

\[
h^\circ = \arctan(b^*/a^*)
\]

Turbidity analysis

The turbidity of juices was evaluated with the turbidimeter (ET76910, Lovibond Inc., Germany) and expressed with Nephelos Turbidity units (NTU). The standard solutions of the range 0.02, 20.0, 200 and 800 NTU were used to the standard curve. As the turbidity of samples was higher than the range of standard curve, the samples were diluted 10-fold with the distilled water. The turbidity repeated thrice for every treatment. The value was calculated with the following equation (Canitez, 2002):

\[
\text{Turbidity}(\text{NTU}) = \frac{S^* (B + C)}{C}
\]

Where S* = turbidity found in the diluted sample, B = volume of dilution water (mL), and C = volume of juice sample juice used for dilution (mL).

Determination of titratable acidity, pH and total sugars

The digital pH meter (FE20 Plus, Mettler-Toledo Inc., China) was used to measure the pH of juices under continuously stirring with a magnetic stirrer at 25 ± 0.5°C. Titratable acidity was determined by the method of AOAC 1999. 942.15. The sample was diluted 20-fold with the deionized water. Then, the solution was titrated with 0.1 mol/L of NaOH to the end point (pH 8.2 ± 0.1) with continuously stirring by a magnetic stirrer. The results were expressed as g/L with reference to malic acid. Total sugars content was determined by the Fehling reagent (Falguera, Pagan, & Ibarz, 2011). The results were expressed as g/100mL.

Phenolic content analysis

The phenolic compounds of juices were determined with the Thermo Ultimate 3000 HPLC system (Thermo Electron Co. USA) equipped with a UV-Vis detector (Brea, CA, USA) and the separation was performed with the Synchrois C18 column (250 × 4.6 mm I.D., 5 μm, USA). The sample of apple juice was prepared and the HPLC analysis was carried out according to the method of Sun et al. (2017) and Marszalek et al. (2018) with some slight modifications. 20 mL of apple
juice mixed with 40 mL of methanol: water (80:20, v/v) and ultrasonicated for 10 min at 25°C. The supernatant solvent was evaporated using a RE-3000E rotary vacuum evaporator (China) at 45°C. And then, the residue was dissolved in the 5 mL solution of methanol: water (80:20, v/v) and filtered through the 0.45 µm filter. The extraction was filtered with a 0.22 µm membrane filter and 20 µL of every filtrate was injected. The mobile phase included the solvent A (30% acetonitrile and 70% methanol) and solvent B (1% trifluoroacetic acid and 5% methanol). The elution gradient of the solvents was as follows: 100% of solvent A for 3 min; 100–60% of solvent B for 16 min; 60% of solvent B for 11 min; 60–100% of solvent B for 1 min; 100% of solvent B for 10 min. The flow rate was 0.95 mL/min. All HPLC grade standards were dissolved in the methanol. The concentrations of standard solutions ranged from 5 to 50 mg/L for the calibration curve. During the run, the total time was 41 min and the wavelength was 280 nm. Every extraction was evaluated in triplicate and the results were expressed as µg/mL.

**Microbial assay**

The bacteria counts were determined with a Plate Count Agar and the yeast and mould counts with Rose Bengal Agar (Standard Methods Agar) (Shamsudin et al., 2014). For the bacteria counts, 0.1 ml sample from every serial dilution (10⁻¹ to 10⁻⁸) and untreated juice were enumerated on Plate Count Agar with pour plated method and incubated for 2 days at 35 ± 2°C. While, yeast and moulds were enumerated on the Rose Bengal agar using pour plating technique and the incubation was performed for 5 days at 25 ± 2°C (Tran & Farid, 2004). Triplicates of the treatment were conducted and every sample was done duplicate measurements of the bacteria, yeast and mould counts. The results are expressed as CFU/mL.

**Shelf life study**

The NFC apple juices with fresh squeezed, UV and TP were aseptically filled into the sterile glass bottles. The bottles were tightly capped with minimum amount of headspace volume and stored at 4 ± 1°C for five weeks (Santhirasesaram, Razali, George, & Somasundram, 2015). The samples were determined at each week for the bacteria, yeast and mould count.

**Statistical analysis**

The results reported are the average values. The Turkey’s honest significant difference (HSD) test with 95% confidence level was used to calculate the difference between the average values. The distinction between the content of NFC apple juice samples was determined using one-way analysis of variance (ANOVA) with Minitab 18 statistical software (MINITAB Inc., State College, PA, USA). Principle component analysis (PCA) was analyzed the relationships among the properties of NFC apple juices with STAT-ITCF statistical software (ITCF, Bordeaux, France).

**Results and discussion**

**Microbial inactivation**

Most fruit juices are opaque because of the high suspended solids and the UV treatment was usually used for the water treatment. The bacteria, yeast and mould counts in the NFC juice samples with freshly squeezed and treated was shown in Table 1. After TP, the bacteria, yeast and mould counts were reduced to the below detection limits in all the tested samples. While UV treatment reduced about 99% of the bacteria counts and 90% of the yeast and mould counts in all studied NFC juice samples, which showed that the colonies of yeast and mould were more resistant to the UV treatment compared to the TP treatment (Stother, 1999). This result was in agreement with the findings of Tran and Farid for the orange juice with UV treatment (Tran & Farid, 2004). Miller, Jeffrey, Mitchell, and Elasri (1999) stated that the deactivation of UV maybe due to the crosslinking between neighboring pyrimidine nucleosides of the DNA strand, which caused to the disruption of DNA chain and inhibited the replication of DNA. Although the colonies of bacteria, yeast and mould were detected in all the tested NFC apple juices with the UV treatment, the microbial load of the plate count and the total yeast and mould counts in the fruit juices were below 4 and 3 log CFU/mL, respectively, which were within the range of commercial sterilization. Therefore, both the TP and UV irradiation could obtain the requirements of commercial sterilization for the NFC apple juice.

**Color measurement**

The color parameters are very important attributes that affect the consumer’s preference for juices (Khandpur & Gogate, 2016). The influence of TP and UV treatments on the color parameters of samples were shown in the Table 2. The value of L* decreased significantly after TP compared to the freshly squeezed juices, which could be attributed to the high temperatures in the processing. The color changing of UV treatment was lower as compared to the samples treated with TP. The value of L* slightly increased in the Red Delicious and Granny smith NFC apple samples after UV treatment, which was consistent with other reports in the clarified apple, peach and lemon juices with UV irradiation (Ibarz, Pagan, Panades, & Garza, 2005). The reports stated the increasing brightness may be related to the decrease of the soluble solid content during processing. The a* parameter decreased in the UV treated samples compared to the control samples, which means that the

| Cultivars       | Treatments | Total plate count (log CFU/mL) | Yeast count (log CFU/ mL) | Mould count (log CFU/mL) |
|-----------------|------------|-------------------------------|--------------------------|-------------------------|
| Fuji            | Control    | 2.76                          | 1.70                     | 1.65                    |
|                 | TP         | ND                            | ND                       | ND                      |
|                 | UV         | 0.60                          | 0.66                     | 0.78                    |
| Red Delicious   | Control    | 3.41                          | 1.66                     | 1.64                    |
|                 | TP         | ND                            | ND                       | ND                      |
|                 | UV         | 1.00                          | 0.76                     | 0.40                    |
| Granny Smith    | Control    | 3.52                          | 1.66                     | 1.60                    |
|                 | TP         | ND                            | ND                       | ND                      |
|                 | UV         | 0.90                          | 0.51                     | 0.61                    |

Values are expressed as mean values ± standard deviations (n = 6). ND: no detected; CFU: colony-forming unit. Control: freshly squeezed; TP: thermal pasteurization; UV: ultraviolet treatment.
Table 2. Effect of TP and UV on the color parameters of three varieties of NFC apple juices.

| Cultivars       | Treatments | L*          | a*          | b*          | Chroma       | h°          |
|-----------------|------------|-------------|-------------|-------------|--------------|-------------|
| Fuji            | Control    | 24.71 ± 0.19a | −2.12 ± 0.02b | 5.88 ± 0.22a | 6.25 ± 0.22a | 109.86 ± 0.49b |
| TP              |            | 23.00 ± 0.03b | −1.92 ± 0.10a | 1.65 ± 0.18b | 2.56 ± 0.14b | 139.91 ± 3.30a |
| UV              |            | 24.52 ± 0.06a | −2.37 ± 0.08c | 5.52 ± 0.04a | 6.00 ± 0.07a | 113.18 ± 0.57b |
| Red Delicious   | Control    | 27.79 ± 0.04a | −2.48 ± 0.06b | 0.71 ± 0.06a | 2.58 ± 0.07b | 163.89 ± 1.08c |
| TP              |            | 25.02 ± 0.10b | −2.02 ± 0.05a | −1.10 ± 0.16c | 3.33 ± 0.03a | 204.83 ± 2.79a |
| UV              |            | 28.48 ± 0.08a | −2.61 ± 0.15b | 0.45 ± 0.07b | 2.55 ± 0.04b | 169.96 ± 1.41b |
| Granny Smith    | Control    | 24.25 ± 0.09b | −2.12 ± 0.02a | 1.17 ± 0.10a | 2.42 ± 0.07b | 151.29 ± 1.86c |
| TP              |            | 20.27 ± 0.12a | −2.07 ± 0.02a | −1.12 ± 0.13c | 2.74 ± 0.07a | 204.23 ± 2.44a |
| UV              |            | 26.07 ± 0.18a | −2.50 ± 0.06b | 0.79 ± 0.17b | 2.22 ± 0.07c | 159.21 ± 4.28b |

Values are expressed as mean values ± standard deviations (n = 9).

One-way balance ANOVA was performed for each variety of NFC apple juice.

Mean values with different small letters are significant in columns (p < 0.05).

Control: freshly squeezed; TP: thermal pasteurization; UV: ultraviolet treatment.

The pH in the TP samples showed a higher value than that in the UV varieties of NFC apple juices are shown in Table 3. The pH in the control samples. Our results were consistent with the research of Muller, Noack, Greiner, Stahl, and Posten (2014). Therefore, compared with TP, the UV could effectively avoid the changing of turbidity and retained the fresh-like quality of NFC apple juices.

Table 3. Effect of TP and UV on the physical and chemical properties of NFC apple juices from three varieties.

| Cultivars       | Treatments | TSS ('Brix) | Turbidity (NTU) | pH     | TAC (g/L) | TSC (g/100 mL) |
|-----------------|------------|-------------|-----------------|--------|-----------|---------------|
| Fuji            | Control    | 13.03 ± 0.37a | 2026.67 ± 45.09b | 3.90 ± 0.03a | 3.08 ± 0.08a | 21.59 ± 0.72a |
| TP              |            | 13.00 ± 0.45a | 3450 ± 55.49a   | 3.48 ± 0.02b | 2.60 ± 0.14b | 12.20 ± 0.16b |
| UV              |            | 13.10 ± 0.26a | 2053.33 ± 55.07b | 3.91 ± 0.03a | 3.24 ± 0.09a | 12.56 ± 0.35b |
| Red Delicious   | Control    | 12.93 ± 0.51a | 1796.67 ± 35.12b | 3.94 ± 0.05a | 3.33 ± 0.12a | 22.47 ± 0.16b |
| TP              |            | 12.97 ± 0.35a | 3233.33 ± 56.86a | 3.32 ± 0.03b | 3.19 ± 0.04b | 11.95 ± 0.21b |
| UV              |            | 12.93 ± 0.49a | 1850.00 ± 65.39b | 3.94 ± 0.08a | 3.69 ± 0.13a | 11.84 ± 0.17b |
| Granny Smith    | Control    | 11.83 ± 0.31a | 2066.67 ± 32.14b | 3.52 ± 0.04a | 6.74 ± 0.12a | 17.49 ± 0.15a |
| TP              |            | 12.00 ± 0.36a | 1916.67 ± 38.59a | 3.04 ± 0.03b | 5.32 ± 0.14b | 10.81 ± 0.12b |
| UV              |            | 11.83 ± 0.15a | 1213.33 ± 20.81b | 3.52 ± 0.08a | 6.99 ± 0.06a | 9.44 ± 0.08c |

Values are expressed as mean values ± standard deviations (n = 9).

One-way balance ANOVA was performed for each variety of NFC apple juice.

Control: recién exprimido; TP: pasteurización térmica; UV: tratamiento ultravioleta.

Table 3. Effect of TP and UV on the physical and chemical properties of NFC apple juices from three varieties.

| Cultivars       | Treatments | TSS ('Brix) | Turbidity (NTU) | pH     | TAC (g/L) | TSC (g/100 mL) |
|-----------------|------------|-------------|-----------------|--------|-----------|---------------|
| Fuji            | Control    | 13.03 ± 0.37a | 2026.67 ± 45.09b | 3.90 ± 0.03a | 3.08 ± 0.08a | 21.59 ± 0.72a |
| TP              |            | 13.00 ± 0.45a | 3450 ± 55.49a   | 3.48 ± 0.02b | 2.60 ± 0.14b | 12.20 ± 0.16b |
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| Granny Smith    | Control    | 11.83 ± 0.31a | 2066.67 ± 32.14b | 3.52 ± 0.04a | 6.74 ± 0.12a | 17.49 ± 0.15a |
| TP              |            | 12.00 ± 0.36a | 1916.67 ± 38.59a | 3.04 ± 0.03b | 5.32 ± 0.14b | 10.81 ± 0.12b |
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Values are expressed as mean values ± standard deviations (n = 9).

One-way balance ANOVA was performed for each variety of NFC apple juice.

Control: recién exprimido; TP: pasteurización térmica; UV: tratamiento ultravioleta.

The pH and total acidity

The effects of TP and UV on pH and total acidity in different varieties of NFC apple juices are shown in Table 3. The pH in the TP treated juice samples. Therefore, the results of color parameters indicated that the UV treatment induced slight changes compared to the conventional TP treatment.

Turbidity, soluble solid content and total sugar content

As shown in Table 3, the turbidity of juice samples increased remarkably (p < 0.05) after TP, which may be due to that the heating processing destroyed the cell wall and promoted the pectin, protein and other macromolecular substances out of the cell (Shamsudin et al., 2013). In contrast, no significant increase (p > 0.05) was observed between the turbidity of the UV treatment and control samples. Our results were consistent with the research of Muller, Noack, Greiner, Stahl, and Posten (2014). Therefore, compared with TP, the UV could effectively avoid the changing of turbidity and retained the fresh-like quality of NFC apple juices.
There was no significant difference (p > 0.05) between the soluble solid content of the controls and all treated samples, which agreed with the reports of Islam et al. (2016). However, the total sugars content of apple juices treated with TP and UV significantly decreased. Wolfrom, Kashimura, and Horton (2004) indicated that the reducing sugar of juices during processing partially converted to 5-hydroxymethyl furfural (5-HMF), resulting in the decrease of the total sugars content, and the combination with the organic acid formed the brown substance, which also explained the UV and TP affected on the color parameters of NFC apple juices.

Phenolic compounds

The content of individual phenolic compounds in the different varieties of NFC apple juices treated with UV and TP were presented in Table 4. 16 individual phenolic standards were used and the mixed standards were separated well with the UPLC (Figure 1(a)). The chromatograms of Fuji, Red Delicious and Granny Smith of NFC apple juices with different treatments were shown in Figure 1(b–d), respectively. The chlorogenic acid, epicatechin and procyanidin B2 were the abundant individual phenolic compounds present in the NFC apple juices. The content of chlorogenic acid (420.0 ± 3.5 μg/mL) of the Red Delicious apple control juices was the highest value compared with Fuji (169.7 ± 3.2 μg/mL) and Granny Smith control apple juices (52.0 ± 2.0 μg/mL) (Figure 2), but the procyanidin B2 was not detected in the control and treated Red Delicious apple juices. The minor changing of the individual phenolic content in all samples treated with UV were observed compared with TP, indicating that the UV treatment could maintain the most of phenolic compounds in the NFC apple juices. Conversely, the content of individual phenolic compounds in all NFC apple juice samples significantly decreased after the conventional TP treatment except gallic acid, procyanidin B2, 4-hydroxybenzoic acid, epicatechin gallate and hyperin which were not detected in Granny Smith and Red Delicious apple juices. Results indicated that the individual phenolic compounds of the NFC apple juices were more sensitive to TP compared with UV. Previous researches stated that the degradation of phenolic compounds was relevant to the photo-oxidation or photo-induced molecular rearrangement, which was affected by many factors such as pH, the presence of oxygen, wavelength of light, the content and structure of phytochemicals (Aramwit, Bang, & Srichana, 2010; Bordignon-Luiz, Gauche, Gris, & Falcao, 2007).

Shelf life

The changes of the microbial counts (total plate count, total of yeast and mould counts) in fresh squeezed and treated apple juices stored at temperature (4 ± 1°C) for 5 weeks are shown in Figure 3. The shelf life test was developed by the Institute of Food Science and Technology, IFST (1999). According to the IFST, the acceptable maximum microbial load of the plate count and the total yeast and mould counts in the fruit juices were about 4 and 3 log CFU/mL, respectively. The plate count (TPC) in the different varieties of freshly squeezed juices stored for 5 weeks increased from 2.76 to 5.2 log CFU/mL in Fuji apple juices, 3.41 to 5.8 log CFU/mL in Red Delicious apple juices and 3.52 to 6.54 log CFU/mL in Granny Smith apple juices, respectively. While, the total of yeast and mould counts (YMC) increased from 2.97 to 4.54 in log CFU/mL in Fuji apple juices, 2.94 to 4.19 log CFU/mL in Red Delicious apple juices and 2.93 to 4.63 log CFU/mL in Granny Smith apple juices after 5 weeks storage, respectively. The rate of microbial growth in the freshly squeezed samples was higher than the UV and TP samples during the storage period. The shelf life of all tested apple juices was 4 weeks for the UV treated samples, more than 5 weeks for the TP treated samples and 1week for the freshly squeezed samples according to the microbial load (TPC and YMC) limit of IFST (1999). Therefore, the shelf life of UV and TP treated NFC apple juices stored at 4 ± 1°C was extended for at least 3 weeks longer than the freshly squeezed juices. The results were similar with the previous research stated that the UV treatment was prolonged the shelf life of mango and orange juices (Pala & Toklucu, 2013; Tran & Farid, 2004). TPC and YMC of all TP treated samples were less than 1 log CFU/mL and 0.5 log CFU/mL, respectively, indicating that the shelf life of TP treated juices was more than 5 weeks.

Principal component analysis

The relationship between the properties of NFC apple juices treated with TP or UV was analyzed by the principle component analysis (PCA). Some variables of the properties of NFC apple juices were removed by the PCA to simply the data and identify the associations of new, uncorrelated variables (Alvarez & Canet, 2000).

The PCA results revealed that twenty-four principle components (F1–F24) explained the variance among the data, and the first two principle components accounted for 70.37% of the total variation (Figure 4(a,b)). The first component (F1) and the second component (F2) accounted for 47.42% and 22.95% of the total variance, respectively, indicating that the first two principal components included variables that differentiated all the tested cultivars. As shown in Figure 4(a), the color parameters (a*, b* and Chroma) and some individual phenolic properties (gallic acid, epigallocatechin, catechin, procyanidin B2, 4-hydroxybenzoic acid, epicatechin, epicatechin gallate, hyperin, ellagic acid, quercitin) had positive loadings for the first principal component. In the contrast, the lightness (L*) and hue angle (h°) had the negative loadings for the first principal component. The total soluble solids, pH and chlorogenic acid had positive loadings, while the total acidity and protocatechuic acid had negative loadings for the second principal component. The properties of NFC apple samples were similar directions, indicating the strong relationship between these attributes.

Three cultivars of NFC apple juices were scattered for the F1 and F2 in Figure 4(b). The PCA showed that the cultivars had greater influence on the properties of NFC apple juice samples than the sterilization method (TP or UV). F1 primarily discriminated the Fuji from the Red Delicious apple juices. The Fuji apple juices with freshly squeezed, TP and UV treatment exhibited the higher TSS, pH, TSC, b*, Chroma and the content of hyperin, catechin, rutin, epicatechin gallate and ellagic acid, while all the Red Delicious apple juice samples were associated with higher L*, turbidity and the content of chlorogenic acid and phloridzin. Regarding F2 for the tested samples, the Granny Smith apple juices with freshly squeezed, TP and UV treatment were associated with higher a*, TAC and the content of gallic acid, epicatechin, 4-hydroxybenzoic acid, procyanidin B2, epigallocatechin and protocatechuic acid. Additionally, the NFC apple juice samples...
### Table 4. The individual phenolics content in the fresh squeezed and treated NFC apple juices from different cultivars.

| Cultivars      | Individual phenolics | gallic acid | protocatechuic acid | epigallocatechin | catechin | procyanidin B2 | chlorogenic acid | 4-hydroxybenzoic acid | epicatechin | epicatechin gallate | rutin | hyperin | ellagic acid | quercitin | phloridzin |
|----------------|----------------------|-------------|---------------------|-----------------|----------|----------------|-----------------|---------------------|-------------|--------------------|-------|---------|-------------|-----------|------------|
| Fuji Control   | 10.3 ± 0.8a          | 4.2 ± 0.4a  | 26.9 ± 1.7a         | 12.3 ± 1.5a     | 68.0 ± 1.7a|                | 169.7 ± 3.2a    | 41.5 ± 1.7a         | 1.3 ± 0.1a  | 5.5 ± 0.4a         | 7.4 ± 0.6a| 8.0 ± 0.4a| 0.8 ± 0.1a   |
| Fuji TP        | 5.0 ± 0.4c           | 2.6 ± 0.4b  | 18.0 ± 1.6c         | 8.4 ± 0.4b      | 56.9 ± 1.4c| 136.4 ± 2.4c    | 8.2 ± 0.6b       | 29.9 ± 1.4b         | 0.7 ± 0.1b  | 2.0 ± 0.3b         | 1.7 ± 0.3c| 3.6 ± 0.4c| 0.6 ± 0.0b   |
| Fuji UV        | 8.3 ± 0.7b           | 3.8 ± 0.4a  | 22.6 ± 1.4b         | 11.0 ± 0.4a     | 61.1 ± 1.2b| 159.1 ± 7.2b    | 10.3 ± 0.6a      | 39.2 ± 1.6a         | 1.1 ± 0.2a  | 5.4 ± 0.4a         | 6.1 ± 0.4b| 6.7 ± 0.6b| 0.8 ± 0.1a   |
| Granny Smith Control | 5.4 ± 0.6a          | 9.0 ± 0.6a  | 27.0 ± 1.3a         | 11.6 ± 0.6a     | 55.1 ± 2.4a|                | 52.0 ± 2.0a      | 9.0 ± 0.6a         | 31.2 ± 2.0a| 5.1 ± 0.5a         | 6.2 ± 0.4a| 4.6 ± 0.4a| 1.5 ± 0.1a   |
| Granny Smith TP | 1.3 ± 0.3c           | 4.5 ± 0.5b  | 17.2 ± 1.4b         | 6.6 ± 1.0c      | 36.5 ± 2.8c| 26.4 ± 2.5c     | 3.0 ± 0.3b       | 21.9 ± 1.4b         | nd         | 3.4 ± 0.5b         | 1.4 ± 0.3c| 1.4 ± 0.1b| 0.8 ± 0.1b   |
| Granny Smith UV | 3.6 ± 0.4b           | 8.1 ± 0.7a  | 23.8 ± 2.2a         | 9.7 ± 0.8b      | 47.3 ± 3.4b| 46.7 ± 0.9b     | 8.2 ± 0.4a       | 30.1 ± 0.9a         | nd         | 4.9 ± 0.7a         | 5.3 ± 0.4b| 4.2 ± 0.4a| 1.4 ± 0.1a   |
| Red Delicious Control | nd          | 1.5 ± 0.1a  | 8.6 ± 0.6a          | 11.5 ± 0.6a     | nd        |                | 420.0 ± 3.5a     | nd                  | 16.2 ± 1.9a| 5.8 ± 0.4a         | 4.5 ± 0.5a| 6.8 ± 0.7a| 1.6 ± 0.1a   |
| Red Delicious TP | 0.8 ± 0.1c           | 6.3 ± 0.6b  | 7.5 ± 0.6b          | nd              | 36.25 ± 5.9c| 407.6 ± 4.8b    | nd              | 14.9 ± 1.3a         | nd         | 5.7 ± 0.4a         | 4.2 ± 0.5a| 5.8 ± 0.4a| 1.5 ± 0.1a   |

Values are expressed as mean values ± standard deviations (n = 9). One-way balance ANOVA was performed for each variety of NFC apple juice. Mean values with different small letters are significant in columns (p < 0.05). Control: freshly squeezed; TP: thermal pasteurization; UV: ultraviolet treatment. "nd" means not detected.
with higher color parameter values (L* and h°) exhibited higher content of gallic acid, epigallocatechin, catechin, procyanidin B2, epicatechin gallate, hyperin, ellagic acid and quercetin \((p < 0.01)\).

According to the PCA, the color parameters could be considered as an indicator of some of the functional properties of NFC apple juices, which may be useful in the processing. Moreover, some of the nutritional and functional
properties of NFC apple juices from the different cultivars demonstrated the potential efficacy to satisfy the functional needs of consumers and industry.

Conclusions

The effect of the conventional TP and UV treatment on the physical and chemical properties of NFC apple juices from different varieties was investigated. Both TP and UV could obtain the requirements of commercial sterility for the NFC apple juices. However, only minor changes of the individual phenolic content and color parameters ($L^*$, $a^*$, $b^*$ Chroma and Hue angle) in all tested samples with UV treatment were observed when compared to TP, indicating that UV could provide the better retention of phenolics and color properties. No significant differences ($p > 0.05$) in the soluble solid and total sugars content were observed between the fresh squeezed juices and all treatment samples. The shelf life of the samples treated with UV stored at

Figure 3. Changes in the microbial counts (total plate count, total yeast and mold count) of control, after TP and UV treatments applied to apple juices obtained from different varieties (a: Fuji; b: Red Delicious; c: Granny Smith) during storage at 4±1 °C.

The values are expressed as mean values ± standard deviations ($n = 6$). TPC: total plate count; YMC: total yeast and mold counts; Control: freshly squeezed; TP: thermal pasteurization; UV: ultraviolet treatment. The first and second dashed lines (-) indicate the limit of the total plate count and the total count of yeast and mold during the useful life, respectively.

Figura 3. Cambios en los recuentos microbianos (recuento total de placa, recuento total de levadura y moho) del control, después de tratamientos TP y UV aplicados a jugos de manzana obtenidos de diferentes variedades (a: Fuji; b: Red Delicious; c: Granny Smith) durante el almacenamiento a 4 ± 1 °C.

Los valores se expresan como valores medios ± desviaciones estándar ($n = 6$). TPC: recuento total de placas; YMC: conteos totales de levadura y moho; Control: recién exprimido; TP: pasteurización térmica; UV: tratamiento ultravioleta. La primera y segunda líneas discontinuas (-) indican el límite del recuento total de placas y el recuento total de levadura y moho durante la vida útil, respectivamente.
4 ± 1°C was extended for at least 3 weeks longer than the freshly squeezed juices, while the shelf life of TP juices was extended more than 5 weeks. Although the conventional TP was more effective in inactivating microbial growth and prolonging the shelf life of NFC apple juices, it also has detrimental effects on the fresh-like quality. Therefore, UV is a suitable alternative to the conventional TP to maintain the most of nutritional and functional properties during the NFC apple juices processing.

The PCA results demonstrated a significant relationship between the physical properties and nutritional properties in NFC apple juices from different cultivars. The color properties could be considered as the indicator of some nutritional and functional properties of NFC apple juices.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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