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Effect of UV-C and UV-B Treatment on Polyphenol Oxidase Activity and Shelf Life of Apple and Grape Juice

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Abstract

In order to minimize quality losses due to enzymatic browning and spoilage reactions during the storage, the effect of a flow through UV-C and UV-B technology on the activity of polyphenol oxidase (PPO) as well as on the shelf life of apple and grape juices was investigated. The absorption of soluble compounds led to smaller effects of UV-C energy on PPO activity in juice than in buffer. Moreover, the pumping and the flow conditions in the coiled tube reactor had additional effects on the activity of the enzymes studied. In contrast, no effect of UV-B energy on PPO activity could be detected at the applied doses. An up to 2 $\log_{10}$ reduction of total aerobic plate count as well as yeasts and molds was reached at a dose of 100.47 kJ L^{-1} leading to an extended shelf life of the UV-C treated juice. The high reduction of PPO activity at this dose prevented further browning of apple juice during the refrigerated storage.

Key words: apple juice, grape juice, polyphenol oxidase, shelf life, UV-B, UV-C

1 Introduction

Between harvesting and consumption, the activity of inherent microorganisms and enzymes may change the quality of food. For enhancing shelf life, food products have to be processed in order to prevent microbiological and enzymatic spoilage reactions. Here, heat processing is most commonly used, having adverse effects on sensory and nutritional qualities (Henry, 1997; Manas & Pagan, 2005; Raso & Barbosa-Canovas, 2003). In this context, the consumer demand for fresh-like and minimal processed food products increased and therefore recent research is devoted to the application of non-thermal technologies for improving food safety while simultaneously minimizing the loss of quality (Gould, 2000; Henry, 1997; Noci, Riener, Walkling-Ribeiro, Cronin, Morgan, & Lyng, 2008; Raso & Barbosa-Cánovas, 2003).

The application of UV-C light is an emerging technology for the pasteurization of juices (Koutchma, Forney, & Moraru, 2009). UV-C light at wavelength about 254 nm is effective against microorganisms by inhibiting the DNA replication without using chemicals or producing byproducts (Keyser, Müller, Cilliers, Nel, & Gouws, 2008; Koutchma, et al., 2009). Since many years, the UV-C treatment has been used successfully for the disinfection of air, surfaces and drinking water (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Bolton, 2010; Hoyer, 2007; Koutchma, et al., 2009; Shama, 1999). However, the lack of penetration due to the presence of solutes and particles in the juice reduces the effectiveness of UV-C energy (Koutchma, et al., 2009; Shama, 1999). To overcome this limitation, an appropriate process technology is required in order to inactivate as many harmful microorganisms as possible (Müller, Stahl, Graef, Franz, & Huch, 2011). In 2000, the US Food and Drug Administration (FDA) approved the UV-C treatment as a suitable method for the pasteurization of fruit juices in the case of an obtained minimum 5 $\log_{10}$ reduction of pathogens at turbulent flow conditions (United States Food and Drug Administration (USFDA), 2000).

In addition to microbial spoilage, reactions facilitated by inherent enzymes such as polyphenol oxidase can also adversely affect shelf life and consumer acceptance (Tomás-Barberán & Espín, 2001). Polyphenol oxidase (PPO) is a copper containing enzyme, which occurs in many fruits (V. Falguera, Pagan, Garza, Garvin, & Ibarz, 2012). The enzyme catalyzes the oxidation of various phenolic substrates, whose polymerization leads to the formation of undesirable brown pigments and therefore has to be inactivated by processing to enhance the shelf life of the juice (Chisari, Barbagallo, Spagna, & Artés, 2011; V. Falguera, et al., 2012; Mason, 1955; Tomás-Barberán, et al., 2001). While the inactivation of natural occurring and inoculated microorganisms in fruit juices is well investigated (Forney, Pierson, & Ye, 2004; Franz, Specht, Cho, Graef, & Stahl, 2009; Fredericks, du Toit, & Krügel, 2011; Koutchma, et al., 2009; Lu, Li, Liu, Cui, Yao, & Zhang, 2010; Tran & Farid, 2004) little is known about the effect of UV treatment on enzymes in fruit juices.

In our study, sodium acetate (NaAc) buffer inoculated with PPO as well as apple and grape juice were conducted to an UV-C (0 - 100.48 kJ L^{-1}) and UV-B (0 - 71.51 kJ L^{-1}) flow through treatment in order to investigate the effect on PPO activity. Physical and physiochemical properties of the juices were determined before and after processing. In order to obtain information about the microbial and enzymatic stability of the UV treated juice, untreated and UV-C treated apple and grape juices were stored under refrigerated conditions at 4 °C for 18 days. The microbial count and the color were measured each 3 days during the storage.

2 Materials and Methods

2.1 The Coiled Tube Reactor

The coiled tube reactor MRIUV2010 was developed by the Max Rubner-Institut (Karlsruhe, Germany). The main component is a module which consists of a FEP envelope (UV-C transmittance of 66 ± 1 %) with an inner diameter of 3.7 mm (Adtech Polymer Engineering Ldt., Stroud, UK) which is wound around a UV lamp. As UV source a 36 W low pressure mercury lamp with maximum peak radiation at 253.7 nm (UVN 30, uv technik Speziallampen GmbH, Wümbach, Germany; illuminated length = 76.5 cm) as well as a 18.3 W UV-B lamp (TL 20W/12 RS SLV, Phillips, Hamburg, Germany; illuminated length = 60.4 cm) with maximum emission between 290 and 315 nm were used in this study. Because of the greater length of the UV-C lamp a PVC covering was inserted between lamp and coiled tube in order to obtain a constant irradiated volume. The liquids can be pumped through the device at flow rates between 10 and 40 L h^{-1} by a peristaltic pump (Pumpdrive Pd 5206, Heidolph, Schwabach, Germany). In this study, a flow rate of 30 L h^{-1} was applied. At this flow rate, the applied Reynolds numbers amount to 2,719 for buffer as well as to 1,002 and 1,015 for apple and grape juice, respectively.

The electrical energy input per liter (D_{el} in J L^{-1}) of treated liquid for the coiled tube reactor was calculated as the electrical energy of the lamp (W) per flow rate (L s^{-1}) (Müller, et al., 2011). For the UV-C experiments, where a PVC covering was inserted between lamp and coiled tube, the additional factor of 0.93 (ratio of irradiated volume to volume of the coiled tube) was considered for the dose calculation. Therefore, the electrical energy per cycle was 4.019 kJ L^{-1} and 2.043 kJ L^{-1} for UV-C and UV-B treatment, respectively.

2.2 Preparation of Samples

2.2.1 Enzyme Solution and Juices

According to the preparation instructions of the supplier, polyphenol oxidase (Tyrosinase from mushroom, Sigma Aldrich, Saint Luis, Missouri, USA) was dissolved at a concentration of 2 mg mL^{-1} in 50 mM potassium phosphate buffer, pH 6.5. The stock solution retains its activity for several days at 4 - 8 °C. For the experiments, the stock solution was diluted 1:1000 with 200 mM sodium acetate (NaAc) buffer (pH 5).

Before juice extraction, apples (cultivar Gala, Germany/Italy; Sep/Oct. 2013) and grapes (cultivar Sublima, seedless, Italy and cultivar Thompson, seedless, Greece; Sep/Oct. 2013) were thoroughly washed in warm water. Apples were cutted in small pieces and the juice was extracted with a commercially available twin gear juicer (Green Star GS-1000, Green Star, Anaheim, California, USA). The apple and grape juices were filtered through a sieve with a mesh width of 315 µm to avoid blockage in the coiled tube reactor. The juices were freshly produced for each experiment.

2.2.2 UV Treatment

To investigate the impact of UV processing on the PPO activity and the physical and physiochemical properties of the juice, enzyme solution as well as the freshly produced apple and grape juice were UV-C and UV-B treated at 30 L h^{-1} in cycles, respectively. For UV-C treatment, samples of PPO solution were collected after the 3^{th} (12.06 kJ L^{-1}), 6^{th} (24.11 kJ L^{-1}), 9^{th} (36.17 kJ L^{-1}), 12^{th} (48.23 kJ L^{-1}) and 15^{th} (60.29 kJ L^{-1}) cycle. The juices were UV-C treated for 25 cycles (100.48 kJ L^{-1}) and samples were taken after each 5^{th} (20.1 kJ L^{-1}) cycle. However, samples of enzyme solution were collected after the 5^{th} (10.22 kJ L^{-1}), 10^{th} (20.43 kJ L^{-1}), 15^{th} (30.65 kJ L^{-1}), 20^{th} (40.86 kJ L^{-1}) and 25^{th} (51.10 kJ L^{-1}) of UV-B treatment, respectively. And the samples of UV-B treated apple and grape juice were taken after each 7^{th} cycle, which leads to an electrical energy of 14.30 kJ L^{-1}, 28.60 kJ L^{-1}, 42.9 kJ L^{-1}, 57.2 kJ L^{-1} and 71.51 kJ L^{-1}, respectively. In addition, as control all liquids were pumped through the reactor at the same flow rate in

the lamp off mode and samples were taken after cycles corresponding to the UV treatment. As further control, samples of the PPO solution and the juices, respectively, were incubated at room temperature for 3 h (test duration). All experiments were conducted in triplicate.

2.2.3 Assay of Enzyme Activity

For the determination of PPO activity in PPO solution and apple juice the reaction mixture consisted of 200 mM pyrocatechol (Sigma-Aldrich, Saint Luis Missouri, USA) in 200 mM NaAc buffer (pH 5). To determine the PPO activity in grape juice a reaction mixture of 100 mM pyrocatechol in 200 mM NaAc buffer was used. The solids of the juices were centrifuged (Biofuge B, Heraeus Sepatech, Osterode, Germany) at 6,153 x *g* for 5 min and the supernatant was used for the activity assay. Enzymatic reactions were started by addition of 0.25 mL of the sample to 1.25 mL reaction mixture. PPO activity was determined spectrophotometrically (UV/Vis-Spectrometer, UV.2, UNICAM, Kassel, Germany) by measuring the increase in absorbance at 410 nm at 30 °C. The initial rate was calculated from the slope of the absorbance–time curve (Ümit Ünal, Sener, & Sen, 2007). One unit of enzyme activity was defined as the change of 0.01 in the absorbance value per minute under assay conditions.

2.2.4 Determination of the Physical and Physiochemical Properties of the Juices

The physical and physiochemical properties of the UV processed apple and grape juices as well as of the untreated juices were determined at 20 °C. The viscosity was determined using a viscosimeter (Rheostress RS150, Gebr. HAAKE GmbH, Karlsruhe, Germany) with double slit cylinder system (DG41, Gebr. HAAKE GmbH, Karlsruhe, Germany) in a controlled shear stress mode at 20 °C. Shear stress was increased by ramping from 0 to 400 mPa. The zero shear viscosity was determined by calculation of the mean between the values of 300 and 400 mPa. The absorption coefficients (α) were

determined at λ = 254 nm from the slope of a linear plot of liquid absorbance *A* (UV/Vis-Spectrometer, UV.2, UNICAM, Kassel, Germany) versus path length *l* of quartz demountable cuvettes (106-QS, Hellma GmbH & Co. KG, Müllheim, Germany). Turbidity was measured using a nephelometer (Turbiquant 3000 IR, Merck, Darmstadt, Germany) and the corresponding glass cuvettes at a wavelength of 860 nm according to EN ISO 7027. Density of tested juices was measured with pycnometers (KS, VWR, Darmstadt, Germany). The pH values of the apple and grape juices were measured using the pH meter inoLab pH 720 (WTW, Weilheim, Germany). The color of the apple and grape juice was measured using the chroma meter CR-300 (Minolta GmbH, Chu-Ku, Japan) in the L*a*b* color space at constant lighting conditions. The instrument was standardized using a white ceramic plate. All values of physical and physiochemical properties of processed and unprocessed juices were checked for significant differences using a t-test (Sigma Plot for Windows 12.3, Systat Software GmbH, Erkrath, Germany) ($p < 0.05$).

2.2.5 Shelf Life Studies

To evaluate the effect of ultraviolet processing on microbial growth and color stability of apple and grape juice during refrigerated storage, shelf life studies were performed in triplicate. Immediately after processing, UV-C (100.5 kJ L^{-1}) and UV-B (71.51 kJ L^{-1}) treated apple and grape juice as well as untreated juice were aseptically transferred in sterile glass flasks closed with a fermentation lock and stored at 4 °C. Every 3 days, samples were aseptically withdrawn from the stored samples for microbial enumeration and the determination of L*a*b* values. The shelf life studies were conducted over a storage period of 18 days.

In order to enumerate the total aerobic microbial count and the viable count of yeasts and molds, the juice samples were ten-fold serially diluted in Quarter-strength Ringer's

solution (QSRS) and plated out in duplicate on standard I agar (Merck, Darmstadt, Germany) for total aerobic plate count (TAPC) as well as on malt glucose agar (Merck, Darmstadt, Germany) containing a mix of antibiotics (20 µg mL^{-1} chloramphenicol, 50 µg mL^{-1} erythromycin, 25 µg mL^{-1} vancomycin, 100 µg mL^{-1} penicillin, 250 µg mL^{-1} streptomycin) for yeasts and molds (Y & M). Plates were incubated at 30 °C for 48 h and 25 °C for 72 h, respectively. Colonies were enumerated and results were expressed as the logarithm of colony forming units (cfu) per mL.

3. Results and Discussion

3.1 The Effect of UV-C and UV-B treatment on PPO activity in Buffer, Apple and Grape Juice

The evolution of PPO activity in buffer, apple and grape juices with increasing UV-C treatment is shown in figure 1a. The highest reduction in PPO activity was observed in NaAc buffer. Here, the activity of PPO was reduced to 4.8 ± 2.6 % after 6 cycles (24.11 kJ L^{-1}) and total loss of activity was obtained at an UV-C dose of 60.29 kJ L^{-1} (15 cycles). However, at a dose of 100.48 kJ L^{-1} (25 cycles) a residual PPO activity of 15.8 ± 7.0 % and 60.9 ± 14.2 % was determined in apple and grape juice, respectively. Compared to the control (lamp off mode, figure 1b) a reduction of PPO activity of more than 20 % and 40 % was observed in apple and grape juice, respectively, results from the impact of UV-C energy (100.48 kJ L^{-1}). For the flow through UV-C treatment of these liquids an additional effect on PPO activity was detected due to the pumping and flow conditions in the reactor (figure 1b). After 15 cycles in the lamp off mode, PPO activity was reduced to 41.7 ± 10.1 % in NaAc buffer and a value of PPO in apple juice of 44.3 ± 9.1 % was measured after 25 cycles. In contrast, an increase of PPO activity was detected in grape juice. Here, a value of 140.9 ± 19.2 % was determined after 25

cycles in the lamp off mode. Furthermore, a loss in PPO activity to 64.5 ± 24.2 % (NaAc buffer), 76.7 ± 12.1 % (apple juice) and 86.2 ± 8.7 % (grape juice) was determined for the duration of the experiment, respectively (figure 1c).

In this study, an increasing impact of UV-C on PPO activity corresponding to the decrease of absorption coefficient (α) at 254 nm was observed. The highest effect of UV-C on PPO activity was reached in buffer ($\alpha_{254nm} \approx 0$), followed by grape juice ($\alpha_{254nm} = 43.4 \pm 5.1$) and apple juice ($\alpha_{254nm} = 52.4 \pm 4.0$) and can be explained by the attenuation of UV-C energy due to the absorption of soluble compounds in apple and grape juice as well as by the lower mixing efficiency in juice compared to buffer ($Re_{buffer} = 2719$, $Re_{AJ} = 1002$, $Re_{GJ} = 1015$). The UV-C surface treatments conducted by Manzocco et al. lead to the inactivation of PPO in the applied range of irradiance and exposure time in aqueous solution and apple juice (Manzocco, Quarta, & Dri, 2009). Here again, a higher irradiance and exposure time was needed for the reduction of PPO in apple juice (21.9 W m^{-2} for almost 100 min) than in aqueous solution (13.8 W m^{-2} for about 75 min).

A further conclusion of the determined UV-C sensitivities in buffer as well as in apple and grape juice could be, that different PPO enzymes (different origin) might have a different intrinsic tolerance to UV-C. The increased value of PPO activity in grape juice due to the pumping and the flow conditions can be an indication for an additional influencing factor on the enzyme inhibitor. This has to be verified in further investigations.

In previous studies by Müller et al., no effect of UV-C treatment in a Dean-Vortex reactor on PPO activity could be observed. Though, the electrical energy input was higher than in our current study (20 cycles x 12.656 kJ L^{-1} = 253.12 kJ L^{-1}), the lower mixing efficiency (Re =167) and the higher absorption coefficient of the used apple

juice (α_{254nm} = 61.0 ± 2.2 cm^{-1}) may be responsible for the different results. Based on HPLC-gel permeation analysis the UV-C inactivation of PPO occurred as a consequence of protein aggregation other than those obtained by thermal inactivation (Manzocco, et al., 2009). Ultraviolet and visible light can modify protein structures by photo-oxidation and therefore affect the enzyme activity (Manzocco, et al., 2009). The absorption of radiation by the protein structure can cause direct photo-oxidation, while indirect photo-oxidation is mediated by singlet oxygen (Davies & Truscott, 2001; Manzocco, et al., 2009).

Figure 2a shows the kinetics of PPO activity in buffer, apple and grape juice after UV-B treatment. At a dose of 51.10 kJ L^{-1} (25 cycles) a high reduction of PPO activity in buffer with a remained value of 12.10 ± 6.72 % was obtained. However, the main part of the reduction of PPO activity is due to the mechanical stress of flow conditions and pumping as well as the time instability of the PPO in buffer (figure 2b and 2c). Similar results were obtained for PPO activity in apple and grape juice. Here, no differences could be detected between UV-B treatment and the lamp off mode.

In contrast, Falguera et al. obtained a reduction of PPO activity in phosphate buffer as well as in apple and grape juice of different varieties conducted by a surface treatment of UV-Vis light (250-740 nm) with a 400 W lamp (Victor Falguera, Garza, Pagan, Garvin, & Ibarz, 2013; V. Falguera, et al., 2012; V. Falguera, Pagán, & Ibarz, 2011).

After 35 min of UV-Vis treatment, PPO was completely inactivated in phosphate buffer (V. Falguera, et al., 2012). As expected, with increasing concentration of melanin in the buffer a slower inactivation of PPO was observed due to the absorption of the radiant energy by the pigments (V. Falguera, et al., 2012). A higher exposure time of 100 min was needed for the inactivation of PPO in apple juice derived from several varieties (V. Falguera, et al., 2011).

Whereas, after 140 min the residual activity of PPO in grape juices derived from pink grapes was only about 50 % of the initial activity and therefore higher than the PPO from white grape varieties (Victor Falguera, et al., 2013). However, the used energy (400 W for 100-140 min) by Falguera et al. was much higher than in our study (18.3 W for about 10 min (35 cycles)) and explains the different results. In addition, Manzocco et al. (Manzocco, et al., 2009) observed an effect of visible light on the PPO activity in buffer. However, the required exposure time was much higher for visible light than for UV-C light and indicates higher effects on the enzyme activity with decreasing wavelength. This result can be deducted from the fact that the radiation energy rises with increasing wavelength.

3.2 Effect of UV-C and UV-B treatment on physiochemical properties of apple and grape juice

With the exception of viscosity and color, the values of the physical properties of apple and grape juice were largely unchanged by UV-C and UV-B treatment (table 1 and 2). A decrease in viscosity was detected in UV-C and UV-B treated apple and grape juice as a result of the shear stress of pumping. In addition, an effect of the treatment on the color of both juices was observed. Here, a reduction of the L* (lightness) values was measured in apple and grape juice and simultaneously the a* (redness-greenness) and b* (yellowness-blueness) values of both juices increased. Therefore, the apple and grape juices became darker, more red and more yellow. These results indicate browning reactions especially at the beginning of the experiment, whereat the high amount of active PPO catalyze the oxidation of the phenolic substances. In addition, the high amount of oxygen in the juice as a result of the pumping and the cyclic treatment (data not shown) stimulated the browning reaction in apple juice (table 3). Here a significant difference was observed in L*a*b* values after 35 cycles at the lamp off mode. The

changes in the color of the juice is also seen in the absorption coefficient (α_{254nm}), which increased by the UV-C and UV-B treatment. Monazacco et al. also reported an increased browning in apple juice by UV-C light exposure (Manzocco, et al., 2009). Despite the inactivation of PPO, direct photo-oxidation of its phenolic compounds browned the apple juice (Manzocco, et al., 2009). In contrast, Falguera et al. reported a photodestruction of pigments in apple and grape juice by UV-Vis light (Victor Falguera, et al., 2013; V. Falguera, et al., 2011). However, in one grape variety the results indicate the destruction of initial pigments and simultaneously the formation of new ones during the UV-Vis light treatment (Victor Falguera, et al., 2013). Transferred these findings to our results, the browning reactions predominated the photobleaching by UV flow through treatment.

3.3 Effect of UV-C and UV-B treatment on microbial growth during refrigerated storage of apple and grape juice

Figure 3 illustrates the evolution of microbial growth in untreated and UV-C treated apple juice during storage at 4 °C. The UV-C treatment (100. 47 kJ L^{-1}) reduced the total aerobic plate count (TAPC) only about 0.5 $\log_{10}$(cfu mL^{-1}). Whereas, a reduction of yeasts and molds (Y & M) from 3.10 ± 0.20 $\log_{10}$(cfu mL^{-1}) to 1.54 ± 0.18 $\log_{10}$(cfu mL^{-1}) was observed at the same dose. During the refrigerated storage the microbial count constantly increases and TAPC of untreated apples juice exceeds the amount of 10^7 cfu mL^{-1} on day 15, followed by TAPC of UV-C treated apple juice on day 18. However, at the end of storage a value of 4.85 ± 0.42 $\log_{10}$(cfu mL^{-1}) and 5.4 ± 0.24 $\log_{10}$(cfu mL^{-1}) for Y & M was detected in untreated and UV-C treated apple juice, respectively.

In contrast to apple juice, more than a 2 $\log_{10}$ reduction of TAPC and Y & M was reached in grape juice at a dose of 100.47 kJ L^{-1} (figure 4). With increase of TAPC and

Y & M, the difference of 2 $\log_{10}$(cfu mL^{-1}) in microbial count between untreated and UV-C treated grape juice was attained during the 18 days of storage at 4 °C. At the end of storage the amount of Y & M in untreated grape juice exceeded 10^7 cfu mL^{-1} on day 18. At the same day a value of 5.27 ± 0.47 $\log_{10}$(cfu mL^{-1}) was measured for Y & M in UV-C treated grape juice. At day 18, in untreated and UV-C treated grape juice the amount of TAPC was 6.16 ± 0.14 $\log_{10}$(cfu mL^{-1}) and 4.7 ± 0.19 $\log_{10}$(cfu mL^{-1}), respectively. In the study of Tandon et al., a reasonable reduction of microbial counts in apple cider was reached by UV-C treatment using a Cider Sure model 3500 UV machine (Tandon, Worobo, Churey, & Padilla-Zakour, 2003). Subsequent investigation of the evolution of microbial growth during storage at 7 °C showed an extended shelf life of UV-C treated apple cider compared to untreated juice (Tandon, et al., 2003).

Concerning grape no information about shelf life of UV-C treated juice was available in literature.

No effect of UV-B treatment on microbial count in apple and grape juice was observed at the applied dose (figure 5). Here, it can be assumed that the microbial growth of apple and grape juice behaves like untreated juice during the storage period and no subsequent storage was conducted after UV-B treatment.

3.4 Effect of UV-C treatment on the evolution of color during refrigerated storage of apple and grape juice

L*a*b* values of untreated and UV-C treated apple and grape juice were measured each 3 days of refrigerated storage and results are shown in table 4. In untreated apple juice, a decrease of L* values with significant differences from day 9 was detected, which means that the juice becomes darker during storage at 4 °C. In contrast, a* (redness-greenness) and b* (yellowness-blueness) values significantly increase from day 3 during the 18 days of storage. However, no significant difference in L*a*b* values was

observed in UV-C treated apple juice during the shelf life studies for 18 days. The changes in the color of untreated apple juice were caused by browning reactions catalyzed by the active PPO. Whereas, in UV-C treated apple juice the most part of PPO was inactivated and no further browning of the juice could be observed during storage.

No significant difference in L* values of untreated grape juice was observed (table 5). Whereas, increasing a* and b* values were detected from day 6 and 3 of storage, respectively. Besides, differences in L*a*b* values were observed in UV-C treated grape juice. Therefore, a sufficient quantity of PPO remained active after UV-C treatment to cause browning in the grape juice during the refrigerated storage.

Manzocco et al. investigated the evolution of color apple slices during refrigerated storage at 4 °C. They observed a delayed appearance of brown pigments when apple slices were previously exposed to UV-C light (Manzocco, et al., 2009).

4 Conclusions

The highest effect of UV-C on PPO activity was reached in buffer, followed by grape and apple juice, and this decreasing order can be attributed to the attenuation of UV-C energy due to the absorption by soluble compounds in the juices or by the different sensitivities in enzyme structure resulting from their different origin. Here, further investigations are needed. For the flow through UV-C treatment of these liquids, an additional effect on PPO activity due to the pumping and flow conditions in the reactor was detected. Thus, a reduction of PPO activity in NaAc buffer and apple juice, as well as an increase of PPO activity in grape juice, could be observed. Moreover, a loss in activity was determined for PPO in NaAc buffer and apple juice for the duration of the experiment. In contrast, PPO in grape juice retained its stability over this period. This prompts further research on stability of enzymes from different origins and in different

food matrices. For UV-B treatment, the main part of the reduction of PPO activity was caused by the mechanical stress of flow conditions and pumping, as well as the instability over time of the PPO in buffer. No differences in PPO activity could be detected between UV-B treatment and the lamp off mode in both apple and grape juices. With the exception of viscosity and color, the values of the physical and physiochemical properties of apple and grape juice were largely unchanged by UV-C and UV-B treatments. A decrease in viscosity was detected in UV-C and UV-B treated apple and grape juice, which can be attributed to the shear stress of the liquid caused by the pumping. Based on the high amount of active PPO at the beginning as well as on the high amount of oxygen in the juice, browning reactions were stimulated and apple and grape juice became darker, more red and more yellow by the UV-C and UV-B treatment.

In apple juice, a reduction of 0.5 $\log_{10}$(cfu mL^{-1}) in TAPC and of 1.5 $\log_{10}$(cfu mL^{-1}) in Y & M counts were detected after UV-C treatment (100.47 kJ L^{-1}), and a faster spoilage of untreated apple juice was observed during the refrigerated storage. At the same dose, more than a 2 $\log_{10}$ reduction in both TAPC and Y & M counts could be reached in grape juice. During the 18 days of storage, the difference in microbial counts between untreated and UV-C treated grape juices was attained.

Regarding the L*a*b* values, untreated apple juice becomes browner during the storage and indicates the action of PPO. In contrast, in UV-C treated apple juice, the greatest part of PPO was inactivated and no further browning of the juice could be observed during the storage period. However, differences in L*a*b* values were observed in UV-C treated grape juice. Therefore, a sufficient quantity of PPO remained active after UV-C treatment to cause browning in the grape juice during the refrigerated storage.

No effect of UV-B treatment could be observed on microbial count in apple and grape juice at the applied dose. Thus, it can be assumed that the microbial growth in apple and grape juice would be similar to that in untreated juice during the storage period.

The results of this study show that the UV-C treatment is able to inactivate PPO and suppress browning reactions in juices during cold storage. Therefore, UV-C treatment could be an emerging technology to prevent microbial and enzymatic spoilage and enhance the shelf life of food products. However, the UV-C energy required for microbial and enzymatic inactivation depends on the food product and on the UV-C sensitivity of its inherent microorganisms and enzymes. For this reason, further studies are required to determine the required UV-C input for particular food products to optimize the process.

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Figure Captions

Figure 1 Effects of a) UV-C treatment b) mechanical stress due to pumping through the reactor c) test duration on PPO activity

Figure 2 Effects of a) UV-B treatment b) mechanical stress due to pumping through the reactor c) test duration on PPO activity

Figure 3 Microbial count of untreated and UV-C treated (100.47 kJ L^{-1}) apple juice during storage at 4 °C

Figure 4 Microbial count of untreated and UV-C treated (100.47 kJ L^{-1}) grape juice during storage at 4 °C

Figure 5 Impact of UV-B treatment (71.51 kJ L^{-1}) on microbial count

Tables

Table 1 physical and physiochemical properties of untreated and UV-C treated (100.47 kJ L^{-1}) apple and grape juice

Table 2 physical and physiochemical properties of untreated and UV-B treated (71.51 kJ L^{-1}) apple and grape juice

Table 3 L*a*b* values of untreated apple and grape juice as well as the L*a*b* values of control samples (35 cycles without lamp)

Table 4 L*a*b* values of untreated and UV-C (100.47 kJ L^{-1}) treated apple juice during storage time

Table 5 L*a*b* values of untreated and UV-C (100.47 kJ L^{-1}) treated grape juice during storage time

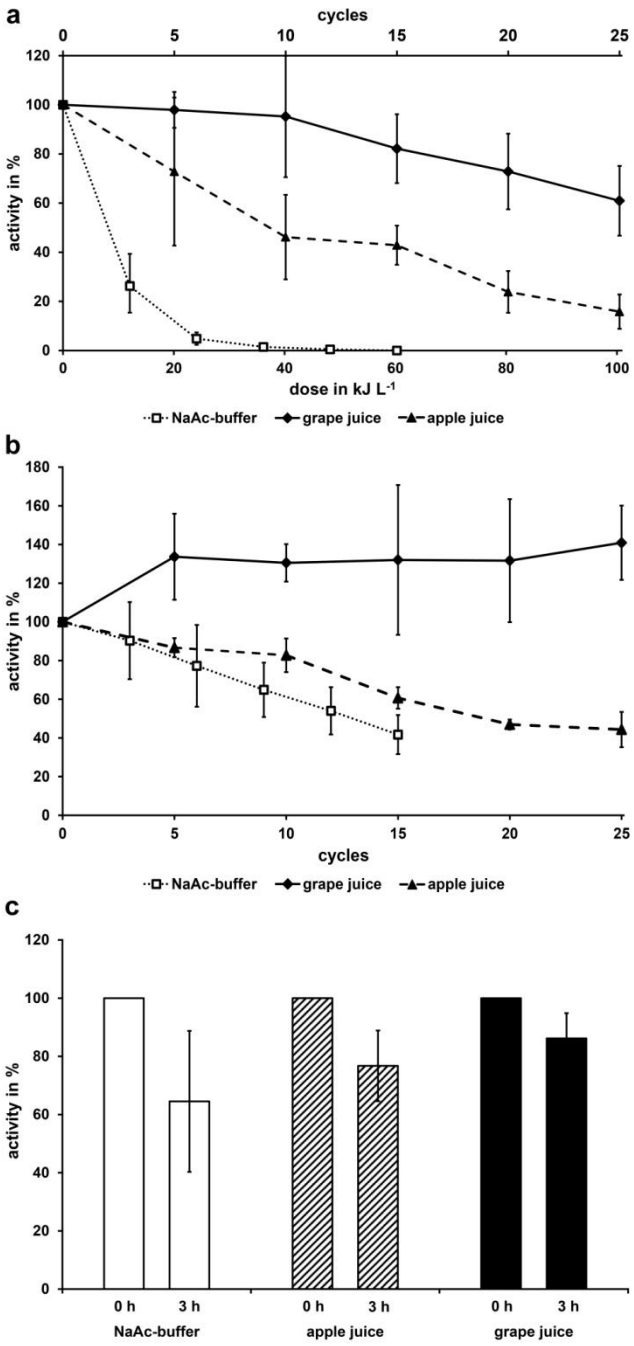

Figure 1

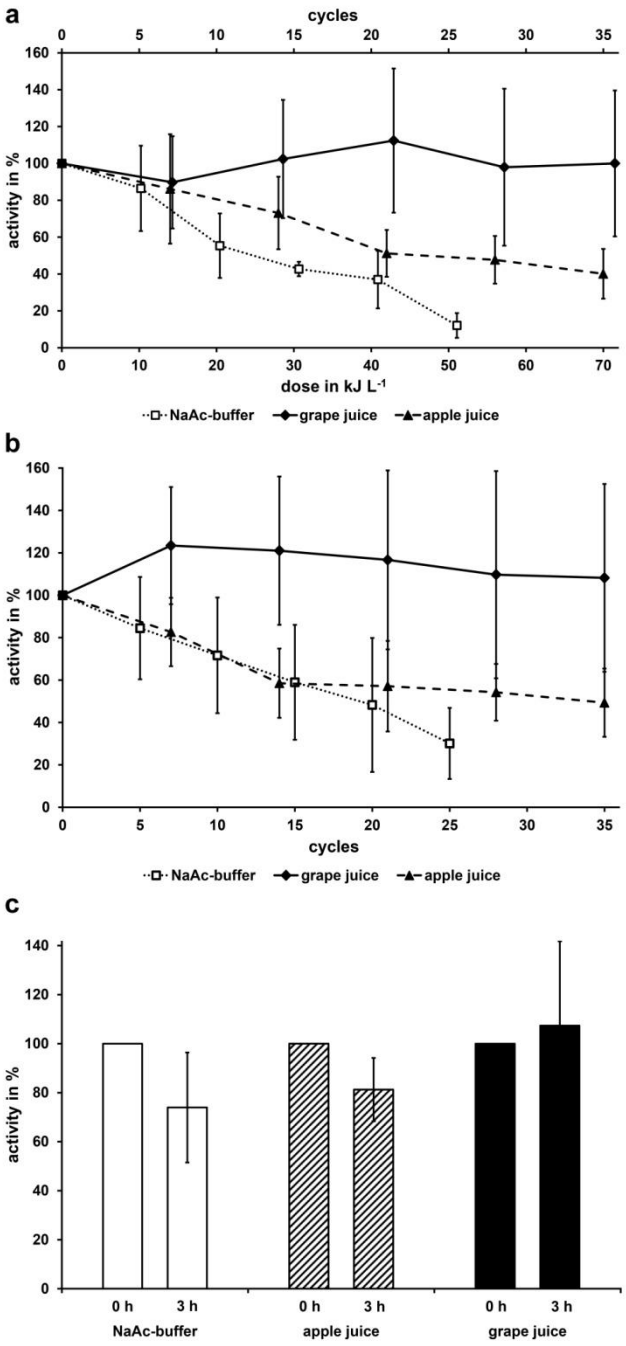

Figure 2

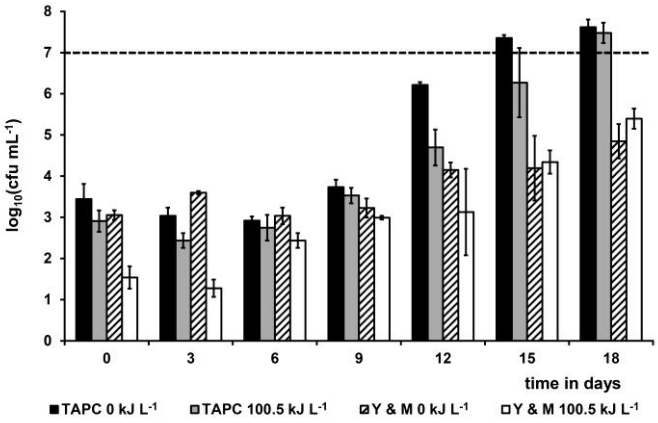

Figure 3

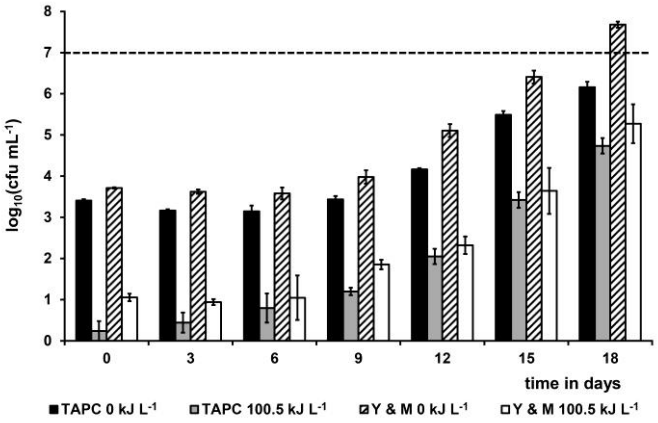

Figure 4

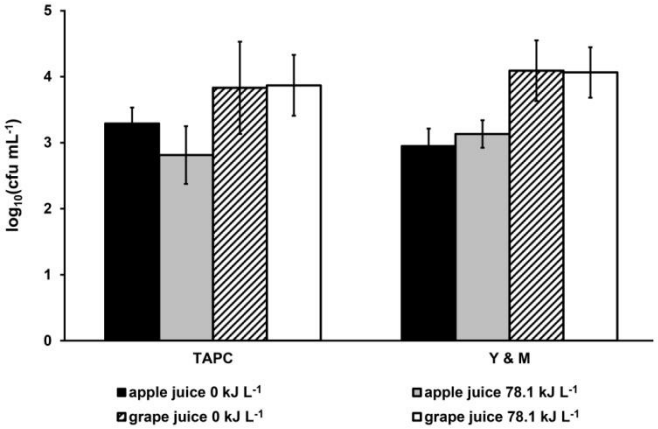

Figure 5

Table 1

	AJ untreated	AJ UV-C	GJ untreated	GJ UV-C
Viscosity (mPa s)	3.01 ± 0.61	2.66 ± 0.12[x]	3.10 ± 0.38	2.99 ± 0.18[x]
α_{254nm} (cm^{-1})	52.4 ± 4.0	56.9 ± 2.9	43.4 ± 5.1	49.6 ± 5.9[x]
Turbidity (NTU)	>11,000	>11,000	5853 ± 1577	6647 ± 702
Density (g cm^{-3})	1.053 ± 0.001	1.052 ± 0.002	1.073 ± 0.002	1.074 ± 0.001
pH value	3.80 ± 0.02	3.82 ± 0.03	3.67 ± 0.04	3.68 ± 0.04
L* value	28.7 ± 0.9	26.4 ± 1.1[x]	32.3 ± 0.7	31.7 ±0.4[x]
a* value	-0.1 ± 0.3	2.6 ± 0.6[x]	-2.6 ± 0.1	-1.3 ± 0.2[x]
b* value	19.0 ± 0.6	19.2 ± 4.9[x]	7.5 ±0.7	8.9 ± 2.0[x]

Table 2

	AJ untreated	AJ UV-B	GJ untreated	GJ UV-B
Viscosity (mPa s)	3.01 ± 0.41	2.74 ± 0.10[x]	3.04 ± 0.52	2.80 ± 0.35[x]
α_{254nm} (cm^{-1})	40.3 ± 1.6	51.7 ± 2.8[x]	44.6 ± 4.4	53.3 ± 1.3[x]
Turbidity (NTU)	>11,000	>11,000	6416 ± 1265	6709 ± 749
Density (g cm^{-3})	1.052 ± 0.002	1.054 ± 0.001	1.076 ± 0.013	1.077 ± 0.014
pH value	3.62 ± 0.04	3.63 ±0.03	3.86 ± 0.23	3.84 ± 0.23
L* value	30.5 ± 0.8	28.6 ± 1.7	30.9 ± 2.1	30.7 ± 1.0
a* value	1.0 ± 1.0	3.3 ± 0.8[x]	-3.8 ± 0.8	-2.5 ±0.5
b* value	18.6 ± 1.7	21.1 ±2.7	10.9 ± 1.9	11.9 ± 2.7

Table 3

	Apple juice			Grape juice		
	L*	**a***	**b***	**L***	**a***	**b***
fresh	30.5 ± 0.8	1.0 ± 1.0	18.6 ± 1.7	30.9 ± 2.1	-3.8 ± 0.8	10.9 ± 1.9
Control	25.7 ± 2.5[x]	4.0 ± 0.9[x]	23.2 ± 2.0[x]	30.4 ± 0.6	-3.8 ± 0.3	10.1 ± 1.6

Table 4

AJ	untreated			UV-C treated		
days	**L***	**a***	**b***	**L***	**a***	**b***
0	28.7 ± 0.9	0.1 ± 0.1	19.0 ± 0.6	26.4 ± 1.1	2.6 ± 0.6	19.2 ± 4.9
3	27.8 ± 0.3	0.9 ± 0.3[x]	20.5 ± 0.2[x]	26.4 ± 0.4	2.5 ± 0.3	22.3 ± 0.2
6	27.4 ± 0.3	1.7 ± 0.2[x]	21.1 ± 0.1[x]	26.5 ± 0.8	2.6 ± 0.1	19.9 ± 4.0
9	26.5 ± 0.6[x]	2.4 ± 0.4[x]	20.6 ± 1.9[x]	26.7 ± 0.4	2.6 ± 0.4	19.3 ± 3.5
12	26.0 ± 0.3[x]	3.3 ± 0.1[x]	20.9 ± 2.9[x]	27.4 ± 0.1	2.7 ± 0.1	23.5 ± 0.3

Table 5

GJ	untreated			UV-C treated		
days	**L***	**a***	**b***	**L***	**a***	**b***
0	32.3 ± 0.7	-2.7 ± 0.2	7.5 ± 0.7	31.7 ± 0.4	-1.3 ± 0.2	8.9 ± 2.0
3	30.7 ± 1.1	-2.2 ± 0.2	9.5 ± 0.6[x]	31.1 ± 0.1	-0.6 ± 0.1[x]	11.0 ± 0.2
6	30.0 ± 2.6	-0.9 ± 0.6[x]	9.4 ± 1.3[x]	31.3 ± 0.4	-0.2 ± 0.1[x]	11.8 ± 0.4
9	33.0 ± 1.7	-1.4 ± 0.1[x]	12.1 ± 0.3[x]	32.5 ± 0.1[x]	0.0 ± 0.1[x]	13.1 ± 0.3[x]
12	31.6 ± 1.4	-0.9 ± 0.3[x]	12.6 ± 0.5[x]	32.0 ± 0.1	0.3 ± 0.1[x]	13.3 ± 0.4[x]

Highlights

PPO activity can be affected by UV-C energy and mechanical stress

No effect of UV-B energy on PPO activity could be detected at the applied doses

UV-C treatment enhanced the microbial shelf life of apple and grape juice

The UV-C inactivation of PPO in apple juice prevented further browning during storage