Innovative Food Science and Emerging Technologies 26 (2014) 498–504

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

journal homepage: www.elsevier.com/locate/ifset

Effect of UV-C and UV-B treatment on polyphenol oxidase activity and shelf life of apple and grape juices

Alexandra Müller [a,*], Laura Noack [a], Ralf Greiner [a], Mario R. Stahl [a], Clemens Posten [b]

[a] Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Food Technology and Bioprocess Engineering, Haid-und-Neu-Str. 9, 76131 Karlsruhe, Germany
[b] Karlsruhe Institute of Technology (KIT), Institute of Engineering in Life Sciences, Department of Bioprocess Engineering, Fritz-Haber-Weg 2, 76131 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 15 April 2014
Accepted 28 May 2014
Available online 5 June 2014

Editor Proof Receive Date 4 July 2014

Keywords:
Apple juice
Grape juice
Polyphenol oxidase
Shelf life
UV-B
UV-C

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.

Industrial relevance: Since enzymatic reactions can lead to quality losses during storage, the inhibition of enzyme activity is almost as important as the microbial inactivation in order to prevent spoilage reactions. As shown by the results of this study, browning reactions in juices may be minimized by the UV-C inactivation of polyphenol oxidase providing a product of extended shelf life.

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 et al., 2008; Raso & Barbosa-Canovas, 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). For 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 et al., 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 (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; Falguera et al., 2012; Mason, 1955; Tomás-Barberán & Espín, 2001). While the inactivation of natural occurring and inoculated microorganisms in fruit juices is well investigated (Forney, Pierson, & Ye, 2004; Franz, Specht,

* Corresponding author at: Federal Research Institute of Nutrition and Food, Department of Food Technology and Bioprocess Engineering, Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany. Tel.: +49 721 6625 363; fax: +49 721 6625 303.
E-mail address: alexandra.mueller@mri.bund.de (A. Müller).

http://dx.doi.org/10.1016/j.ifset.2014.05.014
1466-8564/

Cho, Graef, & Stahl, 2009; Fredericks, du Toit, & Krügel, 2011; Koutchma et al., 2009; Lu et al., 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 juices were conducted to a 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 Ltd., Stroud, UK) which is wound around a UV lamp. As a 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 20 W/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 2719 for buffer as well as to 1002 and 1015 for apple and grape juices, 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 Louis, 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 at 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 cut 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 juices 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 3rd (12.06 kJ L^{-1}), 6th (24.11 kJ L^{-1}), 9th (36.17 kJ L^{-1}), 12th (48.23 kJ L^{-1}) and 15th (60.29 kJ L^{-1}) cycles. The juices were UV-C treated for 25 cycles (100.48 kJ L^{-1}) and samples were taken after each 5th (20.1 kJ L^{-1}) cycle. However, samples of enzyme solution were collected after the 5th (10.22 kJ L^{-1}), 10th (20.43 kJ L^{-1}), 15th (30.65 kJ L^{-1}), 20th (40.86 kJ L^{-1}) and 25th (51.10 kJ L^{-1}) of UV-B treatment, respectively. The samples of UV-B treated apple and grape juices were taken after each 7th 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 Louis 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 6153 ×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 et al., 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 juices was measured using the chroma meter CR-300 (Minolta GmbH, Chu-Ku, Japan) in the L*, a*, and b* color spaces 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) ($p < 0.05$).