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UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean vortex technology

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ABSTRACT

A novel UV-C irradiation device in laboratory scale was tested for its potential to inactivate bacteria in naturally cloudy apple juice. In this device, liquid flows through a helically wound tubing wrapped around a quartz glass tube containing a 9 W UV lamp with an irradiation intensity of 60 W/m^2 at 254 nm. The equipment was capable of reducing numbers of inoculated *Escherichia coli* and *Lactobacillus brevis* from an initial concentration of approximately 10^6 CFU/ml or 10^4 CFU/ml to below detectable limits in commercial naturally cloudy apple juice at a flow rate of 2 l/h, and to well below 1×10^2 also at higher flow rates of 4 and 8 l/h. Numbers of *Saccharomyces cerevisiae* could be reduced from an initial level of ca. 1×10^4–1×10^2 CFU/ml or less at flow rates of 2 and 4 l/h. Although *E. coli* could be effectively inactivated also in self-extracted, as well as industrially processed apple juice, contaminating yeast and lactic acid bacteria were not completely eliminated.

1. Introduction

The antimicrobial effect of ultraviolet (UV) light has been known since a long time, and it can be used in food technology for disinfection of water, surfaces of fresh produce or shell eggs surfaces, and liquids such as fruit juice, apple cider or milk (Basaran, Quintero-Ramas, Moake, Churey, & Worobo, 2004; Duffy, Churey, Worobo, & Schaffner, 2000; Hadjock, Mittal, & Warriner, 2008; Matak et al., 2005). The UV disinfection thus aims to either reduce the total microbial load to extend product shelf life, or to inactivate specific pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* serovars, *Staphylococcus aureus*, *Mycobacterium avium* spp. *paratuberculosis*, foodborne viruses such as calici viruses, or oocysts of protozoa (Altic, Rowe, & Grant, 2007; Duffy et al., 2000; Hanes et al., 2002; Krishnamurthy, Demirci, & Irudayaraj, 2007; Matak et al., 2005; Morita et al., 2002; Rodriguez-Romo & Yousef, 2005; Wright, Sumner, Hackney, Pierson, & Zoecklein, 2000). The principal inactivation effect of UV irradiation is the formation of photoproducts in the DNA. Of these, the most important is the pyrimidine dimer formed between adjacent pyrimidine molecules on the same strand of DNA, which can interrupt both DNA transcription and translation.

UV light ranging from 200 to 315 nm can be used to inactivate microorganisms, as absorption maxima of DNA components and secondary reactants (e.g., oxygen to ozone) fall within the same range (approximately 260 nm) (Anonymous, 1994). Mercury lamps can be used as UV-C sources because of their electromagnetic emission at about 254 nm. The absorption depends on the wavelength and concentration of the absorbing substance, according to the law of Lambert–Beer. As particle concentration and the concentration of dissolved compounds, and therefore the depth of penetration in juices is relatively small (absorption of 90% within the first millimetres), the efficiency of the process is highly dependent on process engineering. To minimize absorption, quartz glass and a special conduction of the liquid flow is being used in most applications (Bucher, 1999). Some applications work with thin-films (Anonymous, 1994) or capillaries (Matak et al., 2005), similar to the application described in this study, which utilises a teflon tube that is helically coiled around a mercury lamp, which itself is contained in a quartz glass tube (Schmidt & Kauling, 2007). This liquid flow leads to secondary vortices, known as 'Dean vortices' that allow radial mixing of the fluid even in a laminar flow field. Therefore, even in cloudy juices with low UV-penetration depths, all fluid elements can be treated. Thus the process using this technology aims to obtain a homogenous UV-treatment of cloudy solutions, with a minimum of energy losses and product changes (taste, ingredients) and a concurrent maximum in reduction of viable microorganisms.

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In the USA, the Food and Drug Administration has reacted to an increase in outbreaks of pathogens linked with fruit juices with regulations for all juice processing, requiring that juice manufacturers either obtain a minimum 5-log reduction of the pertinent pathogen in the finished juice, or provide a warning label on the bottle (Anonymous, 2000; Basaran, et al., 2004). Particularly apple juice products have received increasing attention, as they were implicated in a disease outbreak caused by *E. coli* O157:H7 in the early 1980's in Canada (Steele, Murphy, Arbus, & Rance, 1982), and the frequency of outbreaks have increased over the last decade (Basaran et al., 2004). Fresh apple cider is an unfermented, fresh and short-life juice extract from apples that has not been clarified or heat-treated. Refrigeration and chemical preservatives are the main techniques applied to prolong shelf life of apple cider in North America. Unclarified apple juice is also a favourite drink in Europe, particularly in Germany, where consumption is undoubtedly the highest among European countries. In Europe, unclarified, naturally cloudy apple juice is usually heat-treated to eliminate vegetative microorganisms, and to inactivate enzymes which cause oxidative browning and clarification. Heating cider products in North America is believed to generate off-flavours (in the case of excessive heating) and generally non-thermal processing techniques are thought to represent an alternative method for juice processors to produce a minimally processed food with few and minor quality changes. This was also the aim of our study, where a novel laboratory scale UV-C processing equipment produced by Bayer Technology Services, initially developed for application in the pharmaceutical field, was tested also for its ability to decrease inoculated spoilage and pathogenic microorganisms in either heat-treated and naturally cloudy, or in freshly extracted and unclarified apple juice.

2. Materials and methods

2.1. UV light inactivation equipment.

In this study, the patented UV-C reactor UVivatec® Lab was provided by the manufacturer, Bayer Technology Services GmbH (BTS) (Fig. 1). The essential part of the reactor is a helically wound Teflon® tubing (PTFE) wrapped around a quartz glass tube containing an UV lamp. The 9 Watt UV-C low-pressure mercury lamp (254 nm) has an energy density of 60 W/m^2 and a durability of >1000 h. Flow rates of liquids through the reactor can be adjusted from 2 to 20 l/h by a peristaltic pump (Schmidt & Kauling, 2007). The dose exposure at the microorganism's surface depends on the flow rate, irradiation intensity, turbidity, and flow field. As flow field modelling was not the focus of the present investigation, flow rate was used as point of reference to identify the efficacy of the process, as this is an important performance indicator for the juice industry.

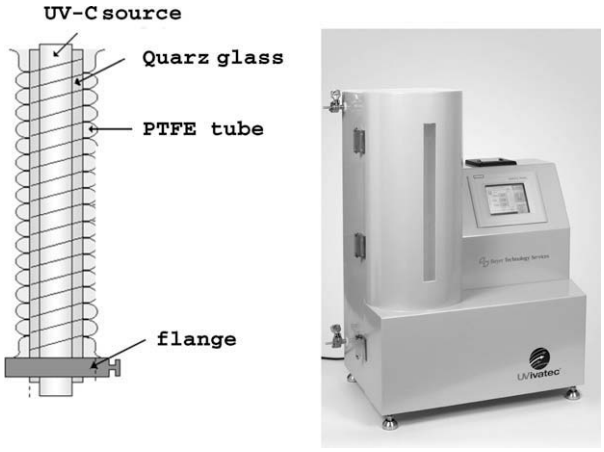

Fig. 1. Principle of the UVivatec technology and photograph of the UVivatec UV-C inactivation device (with kind permission of BTS).

The juice passes through the coiled tubing (cross-sectional area: 6.28 mm^2 and volume: 24 ml) which is wrapped around the UV lamp in a helical motion with a laminar flow rate. There is a pressure sensor after the pump at the inlet of the reactor and a temperature sensor at the outlet of the reactor for safety reasons. For the dose calculation, absorption of the fluid was measured by a spectrometer (Unicam UV/VIS UV-2). Dose calculations could be done based on the assumption of ideal fluid mixing by the Dean vortices (Poggel, Wübben, Brod, Jenne, & Schmidt 2008). The electrical energy input was between 1.9 W h/l (at 16 l/h) and 14.3 W h/l (at 2 l/h).

2.2. Microorganisms and culture media

The bacteria used in this study include *E. coli* DH5α, which was used as a non-pathogenic representative (surrogate) of pathogenic *E. coli* strains, as to gain information on the response of this bacterial species to UV light. *Lactobacillus brevis* LMG 11438 was used as a typical representative of the lactic acid bacteria spoilage microbiota of apple juice, while *Saccharomyces cerevisiae* DSM 70478 was used as typical spoilage yeast. *E. coli* and *S. cerevisiae* were routinely grown in Standard One broth (VWR, Darmstadt, Germany) at 37 °C under aerobic conditions and shaking at 150 rpm for 18 h, while the *L. brevis* strain was grown in de Man, Rogosa and Sharpe (MRS) broth (VWR) under anaerobic and stationary conditions, also for 18 h. All cultures were subcultured twice before use in UV-C inactivation experiments. For UV inactivation of microorganisms either (a) naturally cloudy apple juice, not from concentrate, bottled and pasteurised (tradename 'Jope', hereafter referred to as 'bottled apple juice') or (b) freshly squeezed, self-extracted apple juice from 'Braeburn' apples or (c) freshly squeezed, naturally cloudy apple juice from Jonagold apples obtained from a local processor and transported to our laboratory on ice (approximately 1 h), were used. For determination of microbial counts, the treated juices were diluted and plated out (see below) on either de Man, Rogosa and Sharpe (MRS) agar (Merck, Bruchsal, Germany) for determining the lactic acid bacterial count, Standard One (St-1) Agar (Merck) for determining the total mesophilic count, Violet Red Bile Dextrose agar (VRBD) for *Enterobacteriaceae* count (Difco, Heidelberg, Germany) or Potato Dextrose agar (PDA) adjusted to pH 3.9 with tartaric acid to determine the yeast and mould counts.

2.3. UV inactivation of microorganisms in apple juice

After culturing, microorganisms were centrifuged at 7500 rpm for 5 min and washed with quarter-strength Ringer's solution (VWR, Bruchsal, Germany) and serially diluted in a decimal dilution series in quarter-strength Ringer's solution. The last dilutions, to obtain viable counts of either 1×10^4 CFU/ml or 1×10^6 CFU/ml, were done in bottled apple juice or in self-produced apple juice from 'Braeburn' apples. For preparation of Braeburn apple juice, Braeburn apples were bought in July 2008, stored at 2 °C in the controlled atmosphere (CA) storage facility at the Max Rubner-Institut in Karlsruhe and washed, thereafter the juice was extracted using a centrifuge (Saftomat) from Rotor AG, Uetendorf, Switzerland.

In a pilot study, the microorganisms survival in bottled, naturally cloudy apple juice (Trade name 'Jope') was determined by inoculating them to varying levels in the apple juice, and determining the viable count after one hour. For all organisms tested, the apple juice itself with its pH of 3.5 did not reduce the viable cell