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Defining the stability interfaces of apple juice: Implications on the optimisation and design of High Hydrostatic Pressure treatment

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ABSTRACT

In this article an experimental approach is applied to determine the impact of High Hydrostatic Pressure (HHP) processing (350 to 550 MPa at 20 °C and for 1 to 25 min of holding time) on the survival of *Issatchenkia orientalis* and the spoilage of apple juice (with 300 ppm added ascorbic acid) during different storage conditions, i.e., 4 to 12 °C and 0 to 36 days of storage. Probabilistic modelling approaches based on logistic regression models were developed in order to describe quantitatively the spoilage/no spoilage and survival/death interfaces. For a microbially stable processed apple juice treated at 400 MPa, 10 °C and a holding time of 15 min the degradation kinetics of vitamin C were described quantitatively during subsequent storage at 4, 8, 12 °C. The rate of vitamin losses were highly reduced after the first 13 days of storage. The stability of the apple juice with respect to browning and cloudiness was evaluated by studying qualitatively the activity of polyphenol oxidase (PPO), and pectin methyl esterase (PME) enzymes at combined treatments of HHP and temperature (10 to 50 °C, HHP at 750 MPa and holding time from 1 to 25 min). The highest achieved reduction of PPO and PME was 51.47% and PME 81.44%, respectively.
Industrial relevance: This paper demonstrates an approach based on quantitative probabilistic and qualitative studies for defining the stability interfaces of apple juice. Its applicability contributes on the design and optimisation of High Hydrostatic Pressure treatments.

1. Introduction

During the life cycle of a product (from harvesting to consumption) the food is exposed to many adverse factors. Processing is the step of the food chain that mainly affects its physical and bio-chemical characteristics and determines the safety and shelf life of the product. Therefore, the food industry is devoting considerable resources and expertise to the production of wholesome and safe products. In the last century, numerous alternative or complementary preservation technologies to classical thermal processing were developed (refer to Morris, Brody, & Wicker, 2007). The goal of these processes is minimal processing in order to preserve foods' nutritional value while diminishing any pathogenic, spoilage risk and/or any quality deterioration. Among these processes, High Hydrostatic Pressure (HHP) has been widely commercialised (in Europe, Japan and the U.S.A.) and in 2001, the European Commission authorised the placing on the market of pasteurised fruit-based preparations produced using high-pressure pasteurisation (2001/424/EC, 2001). Fruit juices are normally processed at 400 MPa or greater for a few minutes at 20 °C or less. This can significantly reduce numbers of yeasts, moulds and lactic acid bacteria, which have been implicated in spoilage of fruit juices, and so extend shelf life for up to 30 days (Patterson, 2005).

Although, *Issatchenkia orientalis* represents less than 3% of the total population in apple juice, it seems to be one of the most frequently isolated yeast species in fruit concentrates (Deak & Beuchat, 1993; Sancho, Gimenez-Jurado, Malfeito-Ferreira, & Loureiro, 2000). *I. orientalis* NCYC 795 was the most pressure-resistant of a number of yeasts isolated from apple juice (Boyd, 2000). If *I. orientalis* NCYC 795 is considered a target spoilage yeast organism for designing a HHP processing regime then apple juice products of extended shelf life can be produced. Patterson (2005) noted that a high pressure treatment may not always completely inactivate micro-organisms. Therefore, survival studies and spoilage studies of the food products during different storage conditions following HHP treatment is also of high interest for evaluating the shelf life of these products.

The main quality characteristics of fruit juice products are the colour, the presence or absence of cloudiness and the nutritional value. The nutritional parameters of importance for fruit juices are the anthocyanin pigments in red and purple juices and the content of vitamin C (Kouniaki, Kajda, & Zabetakis, 2004). The vitamin C content of apple juice is naturally low of the order 0.05 mg/g (Kirk & Tressler,

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1941), but it is current industrial practice to add ascorbic acid (e.g., 300 ppm) to inhibit enzymatic browning of the juice while providing at the same time an additional source of vitamin C (Tortoe, Orchard, & Beezer, 2007). HHP has limited effect on covalent bonds and hence vitamin content of fruit and vegetables have been shown to be only slightly affected by HHP processing (Butz et al., 2003; Oey, Van der Plancken, Van Loey, & Hendrickx, 2008).

There are about five causes of browning in processed and/or stored peeled or sliced fruits and vegetables: enzymatic browning of the phenols, ascorbic acid oxidation, caramelisation, formation of brown-red polymers by oxidized lipids and Maillard reaction (Tortoe et al., 2007; Vaikousi, Koutsoumanis, & Biliaderis, 2008). Nevertheless, enzymatic browning which is caused by the action of polyphenol oxidase (PPO), is the major problem for apple juice (Gui et al., 2007). In particular, PPO oxidises the *o*-diphenols to *o*-quinones, which quickly polymerise to produce brown pigments (melanin). Another enzyme responsible for the quality characteristics of apple products is the pectin methyl esterase (PME). PME activity can lead to precipitation of pectin in fruit juices with subsequent loss of desirable cloud. PME de-esterifies the methyl group of pectin (methyl ester of polygalacturonic acid) and converts it into low-methoxy pectin or pectic acid (Krapfenbauer, Kinner, Gossinger, Schonlechner, & Berghofer, 2006). The enzyme is also responsible for undesirable gel formation in fruit juice concentrates (Ludikhuyze, Van Loey, Indrawatti, Denys, & Hendrickx, 2002).

This study (which was presented at the 14th World Congress of Food Science & Technology (Valdramidis et al., 2008) aimed to further characterise and interpret the bio-chemical responses of apple juice. In this approach quality indices of PPO, PME, and vitamin C were considered next to the pre-defined apple juice spoilage constraint of *I. orientalis* for a given number of design variables that included the level of pressure/treatment time, and/or level of temperature during HHP processing, and included storage time/storage temperature during post-processing. Design variables were chosen depending on the processing efficiency to inactivate the spoilage target microorganism and the enzymatic quality indices. The final aim was to define the stability interfaces of apple juice that contribute to the optimisation and design of HHP treatments. For that purpose a probabilistic modelling approach was developed focusing on the spoilage/no spoilage interface of apple juice as well as the survival/death interface of *I. orientalis* and a qualitative study was carried out for the other quality indices, i.e., PME, PPO activities due to the process performance limitations. The vitamin C kinetics during storage of a microbial stable apple juice were also quantified to evaluate degradation of the vitamin.

2. Materials and methods

2.1. Apple juice

Cloudy apple juice of Jonagold, Cox's Pippin, Bramley and Idared varieties in the ratio of 30/30/10/30 produced after pressing in a commercial juice plant in Portadown, Northern Ireland, was obtained. The juice did not undergo any (thermal) treatment and it had 11°Brix, pH of 3.3 and water activity (α_w) of 0.977 ± 0.006 at 25 °C (Rotronic Hygrolab (Crawley, U.K.)). At time of production 300 ppm ascorbic acid was added in the factory to inhibit browning reactions before further treatment was applied. The juice was vacuum packed in bags on the same day of production and held at −20 °C until used for the study. The same batch of apple juice has been used for all the experiments.

2.2. Microbial studies

2.2.1. Apple juice irradiation for microbial analysis

Irradiation of apple juice was performed in order to produce a sterile juice product. The sterile apple juice has then been used throughout all the microbial study. The treatment was carried out at 0 °C using a ^{60}Cobalt Gamma-beam 650 facility (Nordon International Inc., Kanata, Ontario, Canada) to an averaged dose rate of 8 kGy which was sufficient to eliminate any vegetative cells in the apple juice as confirmed by microbial analysis. Actual dosage received by the apple juice was assessed using Gammachrome YR (PMMA) dosimeters (United Kingdom Atomic Energy Authority, Harwell, England, UK), and the dose-induced absorbance at 530 nm was measured spectrophotometrically. The corresponding doses were obtained from a calibration graph provided by the National Physical Laboratory, Teddington, England, UK.

2.2.2. I. orientalis identification

The yeast isolate was identified by partial sequencing of the 26S RNA gene and sequencing of the internal transcribed spacer region using primer pairs ITS1/ITS4 and NL1/NL4 respectively (Arias, Burns, Friedrich, Goodrich, & Parish, 2002). Yeast DNA sequences showed 97–100% homology with sequences for *I. orientalis* in the FASTA database (European Bioinformatics Institute). The next closest homologies for the entire sequences were 80% or less for Pichia and Candida species which give a good identification of the isolate as *I. orientalis*.

2.2.3. I. orientalis experimental data

Isolates were maintained on malt extract agar (MEA) (Oxoid) slopes at 4 °C. A 1 µl loopful from the slope was used to inoculate 10 ml of malt extract broth (MEB) (Oxoid) and incubated for 48 h at 22 °C. A 10^{-2} dilution was prepared in Maximum Recovery Diluent (MRD) (Oxoid) and 2 ml used to inoculate 100 ml of MEB (Oxoid) and incubated for 48 h at 22 °C. After this second incubation approximately 10^6 cfu/ml were present in the MEB. The cells were harvested by centrifugation (2900 ×*g*, 15 min) and the pellet washed in 100 ml phosphate buffered saline (PBS). The cells were again centrifuged, the supernatant removed and the pellet resuspended in a 50 ml volume of irradiated thawed apple juice. The 50 ml volume of suspension was transferred back to the 350 ml of irradiated thawed apple juice and thoroughly mixed. The viable cell count in the apple juice after inoculation was in average 6.65 log(cfu/ml) ± 0.20. Two duplicate polyethylene pouches were filled with about 130 ml of juice and heat sealed excluding all air bubbles. Each pouch was placed within a second polyethylene pouch and heat sealed again. Samples were exposed to High Hydrostatic Pressure. Unpressurised samples were used as the controls. The pressurised samples were then transferred in small sterile containers aseptically and stored at different storage conditions for a period of 36 days (see Experimental design, Section 2.5). Each sample was plated onto MEA after it was serially diluted in 9 ml maximum recovery diluent (MRD, Oxoid code CM733. Oxoid, Basingstoke, UK) and incubated for 48 h at 22 °C.

2.3. Quality studies

All the quality studies were performed for non-irradiated apple juice of the same batch.

2.3.1. Ascorbic acid assay

The assay was based on the methods developed by Graham and Annette (1992). A stock solution of ascorbic acid (AA) (200 µg/ml) was prepared by dissolving 20 mg AA (BDH, Poole, UK) in 100 ml 62.5 mM metaphosphoric acid (BDH, Poole, UK). This was stored at refrigeration temperature and used to prepare a 20 µg/ml daily working standard to serve as an external standard (ESTD) for quantification purposes. Samples of apple juice were prepared for analysis by weighing 10 g of juice into a volumetric flask and making it up to 50 ml with 62.5 mM metaphosphoric acid. L-AA concentration was determined by injecting 10 µg/l of ESTD solutions or sample extracts into the HPLC system comprising a Hewlett-Packard 1090 M liquid chromatograph with Integral photodiode array detector, autosampler and autoinjector and a Hewlett-Packard 9000 series 320 datastation