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Impact of different hyperbaric storage conditions on microbial, physicochemical and enzymatic parameters of watermelon juice

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ABSTRACT

Hyperbaric storage (HS) of raw watermelon juice, up to 10 days at 50, 75, and 100 MPa at variable/uncontrolled room temperature (18–23 °C, RT) was studied and compared with storage at atmospheric pressure (AP) under refrigeration (4 °C, RF) and RT, being evaluated microbiological (endogenous and inoculated), physicochemical parameters, and enzymatic activities.

Ten days of storage at 50 MPa resulted in a microbial growth evolution similar to RF, while at 75/100 MPa were observed microbial load reductions on endogenous and inoculated microorganisms (*Escherichia coli* and *Listeria innocua*, whose counts were reduced to below the detection limit of 1.00 log CFU/mL), resulting in a shelf-life extension compared to RF.

The physicochemical parameters remained stable at 75 MPa when compared to the initial raw juice, except for browning degree that increased 1.72-fold, whilst at 100 MPa were observed higher colour variations, attributed to a lycopene content decrease (25%), as well as reductions on peroxidase residual activity (16.8%) after 10 days, while both polyphenol oxidase and pectin methylesterase residual activities were similar to RF.

These outcomes hint HS as a reliable alternative to RF as a new food preservation methodology, allowing energy savings and shelf-life extension of food products. This is the first paper studying the effect of HS on inoculated microorganisms and on a broad number of physicochemical parameters and on endogenous enzymatic activities, for a preservation length surpassing the shelf-life by RF.

1. Introduction

Refrigeration (RF) is responsible for 35–50% of the total energy consumption in super and hypermarkets, contributing approximately 1% on the total CO_2 emissions worldwide (James & James, 2010), being also the third major source of CO_2 emissions in food industry (with 490 megatons of CO_2 released to the atmosphere in 2008) (Gilbert, 2012). Thus, food preservation methodologies capable of reducing the carbon footprint, while allowing energy savings and without compromising food safety and quality, are of great interest.

Hyperbaric storage (HS) is raising increasing interest as a new food preservation procedure, capable to preserve food products under pressure (between 25 and 220 MPa) at room temperature (RT) (Fernandes et al., 2015; Ko & Hsu, 2006; Segovia-Bravo, Guignon, Bermejo-Prada, Sanz, & Otero, 2012). Some studies pointed out HS as an alternative to RF when performed at RT, since it does not require temperature control (Fidalgo et al., 2014; Moreira, Duarte, et al., 2015; Pinto et al., 2016; Santos et al., 2015), being only needed energy for the compression and decompression phases, differently than RF. In fact, Bermejo-Prada, Colmant, Otero, and Guignon (2017) estimated that the energetic costs to store 800 kg of strawberry juice under pressure (25 MPa) over 15 days was 0.002$, against 0.034$ for RF. HS has another major advantage over RF, since it can inactivate the endogenous microflora, particularly at 100 MPa or higher, additionally to the microbial growth slowdown as occurs for RF (Fidalgo et al., 2014; Pinto et al., 2016; Santos et al., 2015).

To assess HS feasibility, *viz.* its impact on the microbial and physicochemical parameters at and above RT, several studies were performed in the last three years with different food products, namely on strawberry juice (Bermejo-Prada, Segovia-Bravo, Guignon, & Otero, 2015; Bermejo-Prada, Vega, Pérez-Mateos, & Otero, 2015; Segovia-Bravo, Guignon, Bermejo-Prada, Sanz and Otero, 2012), watermelon juice (Fidalgo et al., 2014; Pinto et al., 2016; Santos et al., 2015), melon juice (Queirós et al., 2014), sliced cooked ham (Fernandes et al., 2015), *requeijão* (whey cheese) (Duarte et al., 2014), carrot soup (Moreira, Fernandes, et al., 2015), two ready-to-eat meals (Moreira, Duarte, et al.,

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2015), and raw bovine meat (Freitas et al., 2016). Cape hake loins were also preserved under pressure (50 MPa) but combined with RF (5 °C) over 7 days, allowing a shelf-life extension when compared to conventional AP/RF (Otero, Pérez-Mateos, & López-Caballero, 2017). The outcomes of these studies proved the feasibility of HS as a preservation methodology with potential to substitute RF.

More recently, two studies with highly perishable foods reported that HS/RT could extend the shelf-life compared to RF: for up to at least 7 and 10 days for raw watermelon juice at 100 MPa (Pinto et al., 2016) and raw bovine meat at 50 MPa (Freitas et al., 2016), respectively.

Thus, the aim of this work was to study HS feasibility for shelf-life extension of raw watermelon juice, at naturally variable/uncontrolled RT (18–23 °C) compared to RF. Raw watermelon juice was selected since it is a highly perishable food product (high water activity (a_w > 0.95) and pH-value close to neutral (5.20–5.60) (Bridges-& Mattice, 1939)). Three pressure levels were tested (50, 75, and 100 MPa) and microbial analyses: total aerobic mesophiles (TAM), total aerobic psychrophiles (TAP), *Enterobacteriaceae* (ENT), and yeasts and moulds (YM) and physicochemical parameters: pH, titratable acidity, total soluble solids (°brix), browning degree, cloudiness, colour, total phenolic compounds and lycopene content were studied. Due to the scarcity of data in the literature regarding the effect of HS on endogenous enzymes (the only data available are for strawberry juice (Bermejo-Prada, Segovia-Bravo, et al., 2015; Bermejo-Prada & Otero, 2016)), enzymatic activities of polyphenol oxidase (PPO), peroxidase (POD), and pectin methylesterase (PME) were also determined along storage. Additionally, HS effect on two specific microorganisms, *L. innocua* (ATCC 33090) and *E. coli* (ATCC 25992), as surrogates for pathogens (respectively for *L. monocytogenes* and pathogenic *E. coli*), was also studied for the first time (as the authors are aware), by inoculating these microorganisms on juice samples, to give a first insight of the possible HS effects on pathogenic microorganisms. A comparison between the juice stored at AP/RT (0.1 MPa/18–23 °C) and AP/RF (0.1 MPa/4 °C) conditions was established for all the performed analyses. As far as the authors are aware, this is the first work in literature, where such a broad range of microbial behaviour, physicochemical, and enzymatic activities are studied, for a storage period that surpass the shelf-life achievable by RF.

2. Materials and methods

2.1. Reagents and solutions

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, butylated hydroxytoluene (BHT), 2,2′-azinobis(3-ethylbenzthiazolin-6-sulfonate) (ABTS), and catechol were obtained from Sigma-Aldrich (Seelze, Germany). Sodium hydroxide was purchased from Fluka (St. Louis, Missouri), acetic acid was purchased from ChemLab (Zedelgem, Belgium), sodium acetate from Panreac (Barcelona, Spain), citric acid from Acros Organic (New Jersey, USA), sodium citrate from VWR-International (Carnaxide, Portugal), and citrus pectin was purchased from Riedel-de haen (Hanover, Germany). Plate count agar (PCA), violet red bile dextrose agar (VRBDA), rose-bengal chloramphenicol agar (RBCA), coliform count agar (CCA), *Listeria* identification agar base (PALCAM) (with a selective supplement PALCAM FD061), ringer tablets and ethanol (United States Pharmacopeia-grade, USP-grade) were acquired from Merck (Darmstadt, Germany).

2.2. Watermelon juice samples

Mature, seeded red watermelons (*Citrullus lanatus*) were purchased at a local supermarket and kept at 4 °C until washing, peeling, crushing, and filtration with a sterilized cotton filter. In sterile conditions, the filtered juice was separated in different aliquots (10 mL), and was aseptically placed in low permeability polyamide-polyethylene bags (PA/PE-90, Albipack – Packaging Solutions, Águeda, Portugal), previously sterilized by UV light irradiation for 15 min, using a laminar flow cabinet (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain) to avoid contaminations. The bags were manually heat sealed with care to avoid as much as possible to leave air inside. All samples were immediately stored at − 20 °C, being thawed and kept at 4 °C (for as much as 5 h) before each experiment.

2.3. Storage conditions

Storage experiments were performed in a 2 L high pressure equipment (FPG7100, Stanstead Fluid Power, Stansted, UK), with a pressure vessel of 100 mm inner diameter and 250 mm height, using a mixture of propylene glycol and water (40:60) as pressurization fluid. HS was performed at three pressure levels (50, 75 and 100 MPa) up to 10 days, at variable RT (18–23 °C). At the same time, two control samples were kept at AP, one at RT (18–23 °C) and the other under RF (4 °C), immersed in the same fluid (used in HS) and kept in the dark to avoid differences between samples.

2.4. Microbial inoculations

To evaluate the effect of HS/RT on the development of pathogenic surrogated microorganisms, juice samples were previously inoculated with two non-pathogenic surrogated strains: *L. innocua* ATCC 33090 and *E. coli* ATCC 25922. The inoculations were carried out by suspending plated pure cultures of these microorganisms in Ringer's solution, being adjusted each suspension to 0.5 McFarland (Jenway 6405 UV/Vis spectrophotometer, Stone, Staffordshire, UK). Decimal dilutions were prepared to obtain a final microbial load between 3.00 and 4.00 log CFU/mL for *E. coli* and *L. innocua*, respectively.

2.5. Microbial analyses

Microbial analyses were carried out by adding 1.0 mL of watermelon juice to 9.0 mL of Ringer's buffer solution. Then, decimal dilutions were made and plated in specific culture media, according to the microorganisms analysed: (1) TAM and TAP were enumerated in PCA culture media and the plates were stored at 30 ± 1 °C and 20 ± 1 °C during 72 h and 5 days, respectively (ISO 4833: 2003); (2) ENT were plated and counted in VRBDA after incubation at 37 ± 1 °C for 24 h (ISO 8523: 1991); (3) YM were counted in RBCA culture medium, after incubation at 25 ± 1 °C for 5 days (ISO 7984: 1987); (4) *Listeria innocua* ATCC 33090 was plated in PALCAM *Listeria* agar base with the selective supplement PALCAM (FD061), and incubated at 37 ± 1 °C for 48 h (ISO 11290: 1997); (5) *Escherichia coli* ATCC 25922 was plated and counted in CCA after incubation at 37 ± 1 °C for 24 h (ISO 9308-1).

The results were expressed as decimal logarithm of colony forming units per millilitre of watermelon juice (log CFU/mL). The maximum load considered in this study was 6.00 log CFU/mL, while the detection and quantification limits considered were 1.00 log CFU/mL and 2.00 log CFU/mL, respectively.

2.6. Physicochemical analyses

2.6.1. pH and titratable acidity

The pH-value was obtained at 25 °C by using a properly calibrated glass electrode (pH electrode 50 14, Crison Instruments, S.A., Spain). The titratable acidity of each sample was determined with an automatic titrator (Titromatic 1S, Crison Instruments, S.A., Spain) by titrating 10 mL of diluted watermelon juice (3 mL of watermelon juice and 7 mL of distilled water) with a standardized solution of 0.02 M sodium hydroxide until reaching a final pH of 8.1. The results were expressed as milligrams of citric acid per liter of watermelon juice (mg citric acid/L) (Liu, Hu, Zhao, & Song, 2012).