



# Effects of thermal and high-pressure treatments on the carotene content, microbiological safety and sensory properties of acidified and of non-acidified carrot juice



Pierre A. Picouet <sup>a</sup>, Carmen Sárraga <sup>b</sup>, Silvia Cofán <sup>a</sup>, Nicoletta Belletti <sup>c</sup>,  
M. Dolors Guàrdia <sup>a,\*</sup>

<sup>a</sup> IRTA, XaRTA Food Processing Department, Finca Camps i Armet, 17121 Monells, Spain

<sup>b</sup> IRTA, XaRTA Functionality and Nutrition Department, Finca Camps i Armet, 17121 Monells, Spain

<sup>c</sup> IRTA, XaRTA Food Safety Department, Finca Camps i Armet, 17121 Monells, Spain

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## ABSTRACT

This study aims to assess the changes that are induced by thermal and non-thermal treatments on acidified and non-acidified carrot juice. Glucono-delta lactone (GDL) was used to reduce the pH of juice to 5.5. Carrot juice was treated using high pressure (HP) and mild heating (MH). The microbiological safety, physicochemical parameters,  $\alpha$  and  $\beta$ -carotene content and sensory characterisation were evaluated for 29 days of storage at  $5 \pm 2$  °C. Both treatments resulted in an equivalent reduction of microbial growth, and compared with the unprocessed samples, both HPP and MH processes induced modifications in the carotene content, colour, acidity and °Brix. These changes remained stable during storage. HPP processing provides carrot juice with better sensory properties than MH. Acidification by adding GDL did not significantly extend the shelf-life but had a detrimental effect on the sensory attributes.

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## 1. Introduction

There is a general consensus that fruit and vegetable consumption is a key factor for a balanced diet. In Europe, the fruit and vegetable intake varies among countries (Lobstein, 2004), but according to the compiled results of the EFSA (2008), the average consumption is 386 g per day. This value is below the 400 g per day of fruits and vegetables, excluding potatoes and other starchy tubers, that is recommended by the World Health Organisation (WHO, 2008).

Carrot juice is highly marketable because of its nutritional value (Kim, Park, Cho, & Park, 2001). Carrot juice has a pH of 6.0–6.5, which is prone to microbial spoilage (Patterson, McKay, Connolly, & Linton, 2012), and leads to a short shelf-life of 1–2 days (Alklint, Wadsö, & Sjöholm, 2004). To overcome this difficulty, the food industry prepares carrot juice in a mixture with other fruits, such as orange juice, to stabilise the product by decreasing the pH or a thermal process is employed. Although such processes or combination of processes guarantee food safety, in most cases, they have

a detrimental effect on the nutritional value (Torregrosa, Esteve, Frigola & Cortés, 2006) and the sensory attributes, such as colour, taste, flavour and texture (Chen, Peng, & Chen, 1995).

As suggested by several authors (Kim et al., 2001; Knockaert et al., 2011; Landl, Abadias, Sárraga, Viñas, & Picouet, 2010; Trejo-Araya et al., 2009; Vervoot et al., 2012), the High-Pressure Process (HPP) is an alternative technology to heat treatment to process fruit and vegetable juice and is considered to be acceptable for most consumers (Butz et al., 2003).

In general, the HPP at low or moderate temperature destroys microbial vegetative cells and inactivates enzyme without significantly changing the sensory and nutritional properties of the food. However, it may sometimes be necessary to use a hurdle type approach by combining the HPP with one or more other factors, such as acidity or mild heating (MH), which act synergistically (Alpas, Kalchayanand, Bozoglu, & Ray, 2000). The HPP affects the viability of microbial cells (Patterson et al., 2012) and the structure of proteins/enzymes (Basak & Ramaswamy, 1998; Palou, Lopez-Malo, Barbosa-Canovas, Welti-Chanes, & Swanson, 1999; Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007) but does not significantly change the food compounds with low molecular weights, such as vitamins, pigments, flavouring agents, other

\* Corresponding author. Tel.: +34 972 630052x1413; fax: +34 972 630373.  
E-mail address: [dolors.guardia@irta.cat](mailto:dolors.guardia@irta.cat) (M. Dolors Guàrdia).

sensory-related compounds and nutritional and health-related properties of the product (Butz et al., 2003; Fernández-García, Butz, Bognàr, & Tauscher, 2001; Nienaber & Shellhammer, 2001a, 2001b; Oey, Lille, Van Loey, & Hendrickx, 2008). However, the effect of the HPP on the nutritional and sensory properties depends on the specific characteristics of the fruit and vegetable products. In natural juices, such as smoothies, Keenan et al. (2010) found that mild thermal treatments affect the antioxidant activities less than the HPP at 450 MPa during a storage period of 30 days.

According to nutritional tables that are published by ANSES (2012), raw carrot juice contains  $8710 \pm 590$   $\mu\text{g}/100$  g of  $\beta$ -carotene.  $\beta$ -Carotene from plants is transformed into vitamin A in the small intestine.  $\alpha$ -Carotene can also be found in orange-coloured plants but has less vitamin A precursor activity than  $\beta$ -carotene. Technological treatments and processes can alter the bioavailability and antioxidant properties of the carotene content in carrot juice (Kim & Gerber, 1988) and decrease the nutritional value of the product.

The present work aimed to evaluate the effect of both MH and HP processes on the carotene content, microbial safety and sensory properties of acidified and non-acidified carrot juices in storage at  $5 \pm 2$  °C to obtain a “fresh-like” product.

## 2. Material and methods

### 2.1. Reagents

Ultrapure water was obtained using a Milli-Q Advantage system from Millipore Iberica (Madrid, Spain). Tetrahydrofuran (THF) and acetonitrile (ACN) were HPLC-grade from J.T. Baker (Deventer, The Netherlands). Glucono delta-lactone (GDL) and the standards of  $\alpha$ -carotene and  $\beta$ -carotene were purchased from Sigma–Aldrich (Madrid, Spain). Other reagents were of analytical grade.

### 2.2. Sample preparation

Fresh carrots (*Daucus carota* v. *Nantaise*, L.) were purchased from a local stoking house, peeled, washed, blended and squeezed to obtain juice. The fresh carrot juice, which had a pH of 6.48, was split into two batches. The first batch was added to 0.15 g/100 mL of GDL to obtain acidified juice (AJ) with a final pH of 5.5. In a preliminary trial, which was conducted at IRTA, the addition of 0.15 g/100 mL of GDL decreased the pH while maintaining the characteristic fresh carrot flavour, but higher GDL addition resulted in an unacceptably sour carrot juice (results not shown). The second batch was non-acidified juice (NAJ). The AJ and NAJ batches were processed using HP and MH, respectively. The carrot juice subjected to the HPP was packaged in 250 mL polyethylene terephthalate (PET) bottles (Sunbox, Madrid, Spain), whereas a specific HT300 pouch (Seal Air Cryovac, Milano, Italy) was used for the MH samples. Both types of packaging were selected for heat and high-pressure processing to avoid the effect of packaging materials on the juice quality of the juice. Two replicates of the experiment were performed.

### 2.3. Thermal and high-pressure processing

For MH processing, the samples were introduced into an ILRA-PLUS autoclave (Ilpra Systems, Mataró, Spain) and heated to 80 °C for 7 min, including the initial ramp of 5.7 °C/min; the total heating lasted 27 min. The HP processing consisted of pressurisation at 600 MPa for 5 min at an initial temperature of 10 °C in an HPP system (Wave 6500/120 of 120 l (Hyperbaric, Burgos, Spain)). The pressure ramp was 210 MPa/min, and the total processing time was 8.2 min. After processing, the samples were cooled and stored at  $5 \pm 2$  °C in darkness.

### 2.4. Sampling

Six independent treatments were considered: non-acidified (NAJ-Fresh) and acidified (AJ-Fresh) fresh juices; non-acidified (NAJ-HPP) and acidified (AJ-HPP) high- pressure-treated juices; and non-acidified (NAJ-MH) and acidified (AJ-MH) mild-heat-treated juices.

Different parameters were measured with unprocessed products (Day 0), after applying the MH and HP processes (Day 0<sub>AT</sub>) and throughout the refrigerated storage at  $5 \pm 2$  °C (days 7, 21 and 29). Microbiological and physicochemical analyses and instrumental colour measurements were performed on three independent samples (3 different 250-mL bottles) per sampling date.

### 2.5. Microbiological analyses

For each sample, an aliquot of 10 mL was diluted (1/10, v/v) with sterile saline peptone water, which contained 1 g/l Bacto Peptone (Difco Laboratories, Detroit, MI, USA) and 8.5 g/L NaCl (Merck, Darmstadt, Germany). Further decimal dilutions were made using the same diluent. Undiluted carrot juice was also sampled. Total mesophilic micro-organisms (TVC), anaerobes (TAB) and aerobic spore formers (Ae-Spores) were enumerated on Plate Count Agar (PCA, Merck, Darmstadt, Germany) after 72 h of incubation at  $30 \pm 1$  °C. The TAB plates were incubated under anaerobic conditions (Anaerogen, Oxoid). The aerobic spore formers were determined using samples that were previously treated for 1 min at 80 °C in a water bath. Chloramphenicol agar (YGC Agar, Merck, Darmstadt, Germany) that was incubated for 5 days at  $25 \pm 1$  °C was used to enumerate yeasts and moulds (Y&M). The pour plate method was used for all microbial determinations. The plate count results are expressed in  $\log_{10}$  [cfu/mL]. Determinations were performed in triplicate. The detection limit was 1.0 cfu/mL ( $0 \log_{10}$  cfu/mL).

### 2.6. Physicochemical analyses

The pH was measured using a Crison pH 25 coupled to a Crison 5053 puncture electrode, (Crison Instruments S.A., Barcelona, Spain). The total soluble solid content (TSS) was measured using a portable refractometer (Quick-Brix™ 90; Mettler Toledo GmbH, Giessen, Germany). One drop was placed on the refractometer glass prism, and the TSS was obtained as °Brix.

The titratable acidity (TA) was determined by titrating 10 g of a homogenised sample in 80 mL of water, which contained 0.1 mol/L NaOH pH 8.1, using a potentiometric titrator system (785 DMP Titrimo, Metrohm AG, Herisau, Switzerland) (AOAC, 1990, Part 942.15). The results were expressed as g of malic acid equivalents (MAE) per 100 g of juice.

### 2.7. Instrumental colour measurement

Instrumental colour parameters were measured using a Konica Minolta chroma meter (Model CR-410 HS, Minolta, Tokyo, Japan). The equipment was set to illuminate D<sub>65</sub> (2° observer angle) and calibrated using a standard white reflector plate. The readings were obtained by applying the standard CIE  $L^*$ ,  $a^*$  and  $b^*$  (1976) colour system. The browning index (B.I.) was calculated according to Maskan (2001).

$$B.I. = \left( \frac{100}{0.172} \times \left( \frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012 \times b^*} - 0.31 \right) \right) \quad (1)$$

where  $L^*$ ,  $a^*$  and  $b^*$  are individual readings at time  $t$ .

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