Possibilities of shelf-life prolongation of commercial fresh
squeezed carrot juice by HPP pasteurisation

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Summary. This paper describes studies of the microbiological and sensory quality of high
pressure processed freshly squeezed non-pasteurised carrot juice and possibility of
inactivation of pathogenic micro-organisms in carrot juice samples by high pressure
treatment. Juice in original commercial containers was taken from the market following
direct delivery from the manufacturer.

Pressure treatment of fresh carrot juice was performed at pressure of 500 MPa at
temperature 2°C for 5 minutes. Microbiological and sensory investigations were carried out
directly after pressurisation and subsequently every day until six days of refrigerator storage,
and compared with unpressured samples.

Significant reduction of total bacterial count a 3-log to 5-log reduction, good quality of
juice and preserved sensory quality of the fresh product were observed in the high pressure
treated samples up to 5 days of storage in refrigerator conditions. Some off-flavour of carrot
juice was detected after 5 days of storage.

Experimental samples of carrot juice were artificially contaminated with pure
cultures of Escherichia coli, Salmonella Enteritidis, Listeria monocytogenes, Staphylococcus
aureus, Yersinia enterocolitica, moulds and yeasts, and treated with pressure from 200 to
500 MPa. After 500 MPa at2°C for 5 minutes treatment 5-log reduction of these pathogenic
micro-organisms was observed.

Introduction

In the recent years, freshly squeezed non-pasteurised carrot juice with declared one
day shelf-life when stored in the refrigerator at the temperature lower than 6°C, has appeared
on the Polish market, produced by several small industrial companies.

In spite of such a short shelf-life period, in case of incidental contamination with
pathogenic bacteria that are able to multiply also in refrigerator conditions, the risk of
foodborne diseases becomes probable. Another cause of food poisoning might be improper, long-term storage of juice by consumers.

There are reports in the literature documenting the increasing number and severity of food poisoning following consumption of fresh non-pasteurised apple and citrus fruit juices in different countries (OMAF Food Inspection branch, 2002; Parish, 1997, p.109; Parish, 1998, p. 280). Most of the reported outbreaks have been associated with pathogens such as *Salmonella* spp., toxigenic *Escherichia coli* and *Listeria monocytogenes* (Hammermann, Dyrberg, 2002; Boase, Lipsky et al., 1999, p.582; Cody, Glynn et al., 1999, p.202; Cook, Dobbs et al., 1998, p.1504). Therefore, freshly squeezed juices carry health risk for consumers, as well as waste risk for the producer, related to the necessity to return products which were unsold in one day. The Office of Advocacy of the U.S. Small Business Administration recommended that “all juices regardless of the relative risk they impose on health and safety will have to be pasteurised” (U.S. SBA, Office of Advocacy, 2000). The recommended Microbiological Standards (Al.-Jedah, Robinson, 2002, p.79) for any fruit and vegetable juice sold in the Gulf Region are: total colony count – 5x10³ cfu/ml, coliform – 10 cfu/ml, yeasts and moulds – 100 cfu/ml.

Fresh carrot juices sold in Poland don’t fully meet these requirements even directly after delivery from manufacturer, total count of micro-organisms is usually 10⁴ - 10⁵ cfu/ml and sometimes reaches 10⁷ cfu/ml of at the end of 24 hours storage.

FDA is proposing a 5-log (99.999 %) reduction of pathogens in fresh apple juice as acceptable after pasteurisation (cited after U.S. SBA Office of Advocacy). Heat pasteurisation is the only currently approved method to achieve the 5-log reduction. HPP pasteurisation could be an alternative method which not only improves microbial quality but also preserves very good sensory values of the HPP-treated product.

Our previous investigations and recent literature indicate that HPP technology is a first-line option for pasteurisation of fresh fruit and vegetable juices (Fonberg-Broczek, Arabas et al., 1999, p.281; Fonberg-Broczek, Prestamo et al., 2002). Transfer of this method to small and medium enterprises (SMEs) could be a milestone in widespread the implementation of this novel preservation method, assuring safety of minimally-processed food products.

Institute of High Pressure Physics of the Polish Academy of Sciences, basing on its own experience in the field of HPP of food, collaborates with small producers of freshly squeezed vegetable and fruit juices, giving them the opportunity to use HPP technology.

The aim of the work presented in this paper was to establish HPP parameters enabling 5-log reduction of pathogens, optimisation of the process and prolongation of shelf-life of commercially produced freshly squeezed carrot juice.

**Materials and methods**
Carrot juice in original commercial package (250 mL bottles of DPE) was taken from the market following directly delivery from the manufacturer. These samples were high pressure processed in 500 MPa, initial temperature of 2ºC for 5 minutes. During HPP processing temperature was controlled by means of temperature detector.

Simultaneously prepared samples of carrot juice were inoculated with suspensions of micro-organisms in phosphate buffered saline. Initial counts were $10^6$ cells/ml for each strain of bacteria and processed from 200 to 500 MPa (2ºC, 5 minutes).

The studies included the following micro-organisms:
Gram(+) bacteria: *Staphylococcus aureus* spp, *Listeria monocytogenes* ATCC 19115 and sporogenic bacteria *Bacillus cereus*.
yeasts: *Candida albicans* and *Saccharomyces cerevisiae*,
moulds: *Aspergillus flavus*.

High pressure treatment was conducted in a high pressure food processor piston-cylinder type vessel with inner diameter of 110 mm, working volume of 1.5 L and maximum pressure of 700 MPa, equipped with heating/cooling jacket and temperature measuring system. A mixture of potable water with propylene glycol (50:50) was used as a pressure transmitting medium.

The investigations included:
1. microbiological quality
2. evaluation of appearance, colour, smell and taste during the storage time.

Quality studies and microbiological investigation of products were performed before pressure processing, directly after pressure processing and after 1, 2, 3, 4, 5 and six days of storage in a refrigerator.

Microbiological investigations were performed according to the method EN ISO 4833:2004 and the number of surviving micro-organisms was determined.

**Results**

Total bacterial count estimated at baseline in freshly squeezed carrot juice, taken from the market, averaged $10^5$ – $10^6$ cfu/ml, but some samples taken from the market after 24 hours of storage had higher counts of bacteria, some more than $10^7$ cfu/ml. Significant reduction of total bacterial count (3-log to 5-log), good quality of juice and preserved sensory quality of the fresh product were observed in the high pressure treated samples up to 5 days of storage at refrigerator conditions. Some off-flavour of carrot juice detected after 5 days of storage resulted from enzymatic activity. After six days of storage in a refrigerator, high pressure processed samples of carrot juice still presented good microbiological quality ($10^2$-
10^4 cfu/ml). In juice samples not subjected to HPP, increase of total colony count (10^7 cfu/ml) and count of moulds (10^3 cfu/ml) were observed after 48 hours of storage in refrigerator (Table 1).

Table 1   Microbiological quality of unpasteurised and HPP - pasteurised carrot juice*

<table>
<thead>
<tr>
<th>Carrot juice</th>
<th>TCC **</th>
<th>Yeast and moulds</th>
<th>TCC 24 h</th>
<th>TCC 48 h</th>
<th>TCC 72 h</th>
<th>Yeast and moulds 72 h</th>
<th>TCC 96 h</th>
<th>TCC 144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpasteurised</td>
<td>1.5x10^4 - 1.8x10^7</td>
<td>7.1x10^2 - 4.0x10^3</td>
<td>1.7x10^7</td>
<td>2.7x10^7</td>
<td>3.5x10^7</td>
<td>7.8x10^3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pasteurised</td>
<td>1.5x10^1 - 5.4x10^2</td>
<td>ND ***</td>
<td>1.0x10^2 - 8.0x10^2</td>
<td>9.0x10^2</td>
<td>2.4x10^2 - 6.0x10^2</td>
<td>ND ***</td>
<td>9.5x10^2</td>
<td>3.7x10^2 - 6.0x10^4</td>
</tr>
</tbody>
</table>

* 500 MPa, temp. 2 °C, time 5 minutes
** TCC = Total Colony Count, *** ND = None Detected

In the samples of carrot juice, prepared simultaneously, contaminated with moulds, yeasts, E. coli, S. Enteridis, L. monocytogenes, S. aureus, Y. enterocolitica pasteurisation effects (reduction of 5-log) for these micro-organisms were observed at 500 MPa This level of pressure caused 1-log reduction of sporogenic B. cereus(Table 2).

Table 2    High pressure inactivation of microorganisms in carrot juice (count of microorganisms  cfu/ml)

<table>
<thead>
<tr>
<th>Pressure MPa</th>
<th>TCC</th>
<th>Yeast and moulds</th>
<th>E. coli</th>
<th>S.Ente-ritidis</th>
<th>L. monocyo-genes</th>
<th>S. aureus</th>
<th>Y. enterocolitica</th>
<th>B. cereus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>5,4 x 10^5</td>
<td>2,1 x10^5</td>
<td>3,6 x10^6</td>
<td>2,2 x10^6</td>
<td>5,6 x 10^5</td>
<td>4,1 x 10^4</td>
<td>2,6 x10^6</td>
<td>4,6 x10^3</td>
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<tr>
<td>200 MPa</td>
<td>2,4 x 10^6</td>
<td>3,2 x10^4</td>
<td>8,2 x10^4</td>
<td>8,5 x10^5</td>
<td>1,0 x 10^5</td>
<td>2,1 x 10^4</td>
<td>1,5 x10^6</td>
<td>3,1 x10^3</td>
</tr>
<tr>
<td>300 MPa</td>
<td>8,3 x 10^3</td>
<td>4,4 x10^3</td>
<td>2,3 x10^3</td>
<td>2,2 x10^4</td>
<td>2,0 x 10^3</td>
<td>6,5 x 10^3</td>
<td>8,6 x10^5</td>
<td>5,5 x10^2</td>
</tr>
<tr>
<td>400 MPa</td>
<td>1,1 x 10^3</td>
<td>ND</td>
<td>4,0 x 10</td>
<td>ND</td>
<td>1,0 x 10</td>
<td>6,5 x 10^1</td>
<td>4,0 x10^2</td>
<td>6,5 x10^2</td>
</tr>
<tr>
<td>500 MPa</td>
<td>2,4x10^2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3,8 x10^2</td>
</tr>
</tbody>
</table>

* temp. 5 °C, time 5 minutes
If initial counts of pathogens were lower than $10^3 - 10^4$ cfu/ml it was sufficient to pressure samples of carrot juice in 400 MPa for total inactivation of vegetative micro-organisms and 1-log reduction of *B. cereus*.

**Conclusions**

High pressure treatment prolongs shelf-life of commercially freshly squeezed carrot juice to five days, assures good microbiological parameters: total bacterial count up to $10^2$ cfu/ml, absence of pathogenic micro-organisms, good sensory quality, natural colour, flavour, taste of juice and adequate safety for consumers.

**Acknowledgements:**

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**References**

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