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INACTIVATION OF THE NATIVE MICROFLORA IN BEETROOT JUICE BY HIGH PRESSURE CARBON DIOXIDE COMBINED WITH TEMPERATURE

Barbara Sokołowska, Izabela Porębska, Justyna Nasiłowska, Jolanta Niezgoda, Agnieszka Dekowska

Institute of Agricultural and Food Biotechnology
Department of Fruit and Vegetable Product Technology

K e y w o r d s: high pressure carbon dioxide, beetroot juice, spoilage microorganisms, lactic acid bacteria, yeasts and moulds.

A b s t r a c t

Commercially available unpasteurized, freshly-squeezed beetroot juice with a 24–72 hour shelf-life in cold storage, retains its natural flavour and nutritional value but can be a source of undesirable microflora. In this paper, the suitability of high pressure carbon dioxide (at 20 and 60 MPa, and a temperature of: 20, 35 and 60°C) to inactivate the native microflora in this juice has been studied.

The results show that high pressure carbon dioxide was effective in the inactivation of the studied groups of microorganisms only when combined with increased temperature. The reduction in the total count of spoilage microorganisms and lactic acid bacteria was 5–6 log when 20 or 60 MPa and 60°C for at least 30 min were used. Yeasts treated with carbon dioxide at 20 MPa and 60°C were totally (>6 log) inactivated. The reduction in moulds count above 3 log, was observed in this conditions.

INAKTYWACJA NATURALNEJ MIKROFLORY SOKU Z BURAKÓW ĆWIKŁOWYCH DITLENKIEM WĘGLA POD WYSOKIM CIŚNIENIEM W POŁĄCZENIU Z TEMPERATURĄ

Barbara Sokołowska, Izabela Porębska, Justyna Nasiłowska, Jolanta Niezgoda, Agnieszka Dekowska

Instytut Biotechnologii Przemysłu Rolno-Spożywczego im. prof. Wacława Dąbrowskiego
Zakład Technologii Przetworów Owocowych i Warzywnych

S ł o w a k l u c z o w e: ditlenek węgla pod wysokim ciśnieniem, sok z buraków, mikroorganizmy psujące, bakterie fermentacji mlekowej, drożdże i pleśnie

Address: Barbara Sokołowska, Institute of Agricultural and Food Biotechnology, Department of Fruit and Vegetable Product Technology, ul. Rakowiecka 36, 02-532 Warszawa, Poland, phone: +48 22 606 39 25, e-mail: sokolowska@ibprs.pl

Abstrakt

Dostępny w handlu niepasteryzowany, świeżo wyciskany sok z buraków ćwikłowych posiada 24–72 godzinny okres przydatności do spożycia. Zachowuje swój naturalny smak i walory odżywcze, ale może być źródłem niepożądanej mikroflory. W artykule przedstawiono ocenę przydatności ditlenku węgla pod wysokim ciśnieniem (20 i 60 MPa w temperaturze: 20, 35 i 60°C) do inaktywacji naturalnej mikroflory soku z buraków ćwikłowych.

Wyniki badań wskazują, że działanie ditlenku węgla jedynie w połączeniu z podwyższoną temperaturą skutecznie eliminuje poszczególne grupy badanych drobnoustrojów. Przy zastosowaniu ditlenku węgla pod ciśnieniem 20 lub 60 MPa i temperatury 60°C przez co najmniej 30 min, osiągnięto redukcję ogólnej liczby drobnoustrojów psujących i bakterii fermentacji mlekowej o 5–6 log. Drożdże ulegały całkowitej inaktywacji (>6 log) po zastosowaniu ditlenku węgla pod ciśnieniem 20 MPa i temperatury 60°C. W tych samych warunkach redukcja liczby pleśni wynosiła powyżej 3 log.

Introduction

High pressure carbon dioxide (HPCD) processing has been developing rapidly over the past decades as an innovative, non-thermal pasteurization method for the preservation of liquid food. It has the ability to inactivate different microorganisms without exposing foods to the adverse effects of heat and therefore they can retain their fresh-like physical, nutritional, and sensory qualities (DAMAR and BALABAN 2006, KINCAL et al. 2006, GARCIA-GONZALES et al. 2007, CHEN et al. 2009, FERRENTINO et al. 2009b). HPCD processing has been also proven effective to inactivate certain enzymes, including polyphenol oxidase and peroxidase which cause fruit, vegetable and juice browning (LIU et al. 2008 a, LIU et al. 2010, XU et al. 2011).

The efficacy of HPCD on gram-positive bacteria, gram-negative bacteria, bacterial spores, fungi (SPILIMBERGO et al. 2002, ZHANG et al. 2006, LIAO et al. 2007, BAE et al. 2009, LIAO et al. 2010a, YUK et al. 2010, YUK and GEVEKE 2011), and the native microflora in juices (LIM et al. 2006, FERRENTINO et al. 2009a, FERRENTINO et al. 2009b, LIAO et al. 2010b, XU et al. 2011) has been studied over the past few years. It is known that, microbial inactivation is accelerated with increasing CD pressure. As a consequence, at higher pressure, a shorter exposure time is needed to inactivate the same level of microbial cells. The microbial inactivation is also sensitive to the applied temperature. In general, the inactivation rate increases with increasing temperature.

Beetroot juice is a popular beverage in Poland and is commercially available as an unpasteurized, freshly-squeezed juice with a 24–72 hour shelf-life (depending on the season) in cold storage, or as a product preserved by pasteurization. The attractive colour of beetroot juice is associated with betalain pigments, which belong to the group of cation antioxidants. Red betalain pigments, betacyanins, show anticancer activity and play an important role in preventing degenerative diseases (KANNER et al. 2001). Due to the

lack of heat treatment, freshly-squeezed beetroot juice retains most betacyanins, as well as its fresh taste and odour, but it can be a source of undesirable microflora.

The number of spoilage microorganisms in Polish commercial beetroot juice, supplemented with 5% apple juice, ranged from 2.1 x 10^6 to 2.4 x 10^7 cfu/mL, most of which were lactic acid bacteria and the rest were yeasts and moulds (SOKOŁOWSKA et al. 2011). The 25 g juice samples analyzed were negative for *Salmonella* spp. *Listeria monocytogenes* was found in 66.7% of the tested samples, but did not exceed 100 cfu/mL, which is the legal limit for such bacteria in food. The contamination of beetroot juices with *E. coli* was 1–800 cfu/mL. All the tested-juice samples met the microbiological safety criteria for raw juices as required by Commission Regulation (EC) No 2073/2005.

The elimination of pathogenic microorganisms and the reduction in the risk of microbial spoilage of industrially processed juices are usually accomplished by means of thermal pasteurization. Thermal preservation has some disadvantages such as biochemical and nutritional changes in processed products; in the case of beetroot juice the main problem is a decrease in betalains and betacyjanins content resulting in the degradation of the colour of the juice (CZAPSKI 1990, KIDOŃ and CZAPSKI 2007, CHANDRAN et al. 2012).

For these reasons new alternatives to the thermal treatment technologies of beetroot juice have been studied. The use of high hydrostatic pressure of 400 MPa at 20°C for 10 min, extended the shelf-life of freshly-squeezed beetroot juice from 1 day to 10 days, in refrigerated storage (SOKOŁOWSKA et al. 2014 a). The reduction in the *E. coli* cell number was 6.2 log under the same conditions and *L. innocua* cells were completely inactivated after 1 min at 400 MPa, 20°C (SOKOŁOWSKA et al. 2014b). The process conditions provided satisfactory product quality and safety.

The HPCD preservation method has several advantages. The CO_2 used in this method is inert, non-toxic, accessible, and inexpensive. In ambient conditions, CO_2 is a gas and does not leave any residues in the treated product, and furthermore, it is considered to be a GRAS solvent, which means it can be used in food products.

The aim of this work was to evaluate the suitability of the HPCD technique for the inactivation of native microflora in beetroot juice.

Material and Methods

Commercial unpasteurized beetroot juice, supplemented with 5% apple juice, pH 4.35, produced by Marwitt Sp. z o.o., was used. The juice samples (7–8 mL) were treated with HPCD at 20 and 60 MPa at a temperature of 20, 35 and

60°C with holding time of up to 40 min, using modified Applied Separations *Spe-ed* SFE device. The HPCD treatment times reported do not include the come-up and come-down times. The temperature was measured in the chamber. To evaluation impact of temperature 60°C, juice samples were held under the same treatment time, but without the flow of carbon dioxide.

Samples of the HPCD-treated juice were analyzed immediately after treatment. Raw juice was also used as a control. Samples were serially diluted in maximum recovery diluents (Merck), and spread on DRBC agar (Oxoid) in duplicate for the enumeration of yeasts and moulds (incubation: 5 days at 25°C, in accordance with PN-ISO 21527-1:2009). Mesophilic lactic acid bacteria was determined using the pour plate technique in duplicate on MRS agar (Merck) according to PN-ISO 15214:2002 (incubation: 3 days at 30°C). To enumerate the total count of spoilage microorganisms the pour plate technique was used in duplicate on Orange Serum Agar (Merck). In accordance with IFU Method no. 2:1996, 3 days incubation at 30°C was carried out.

An analysis of the variance and the Tukey multiple-range test, using StatSoft&Statistica 7.1, was used to test the significance of the differences ($p < 0.05$) between the mean log values of the survival microflora count.

Results and discussion

The inactivation of native microflora in beetroot juice samples exposed to HPCD treatment are presented in Figures 1–4. The reduction in the number of living microorganisms depended on their type, the pressure, time and temperature of the HPCD treatment.

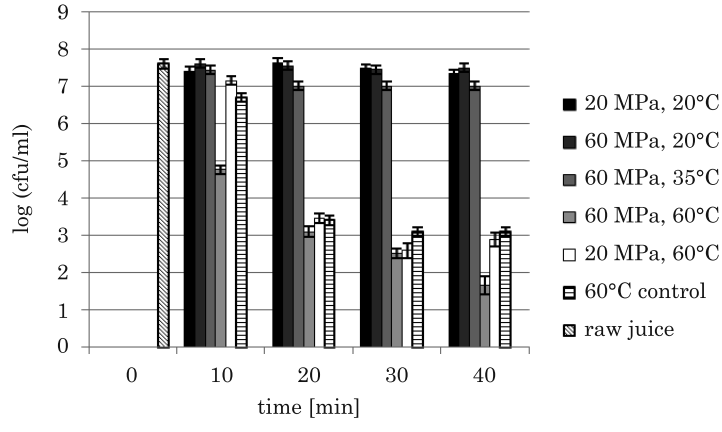

Fig. 1. Survival of spoilage microflora in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

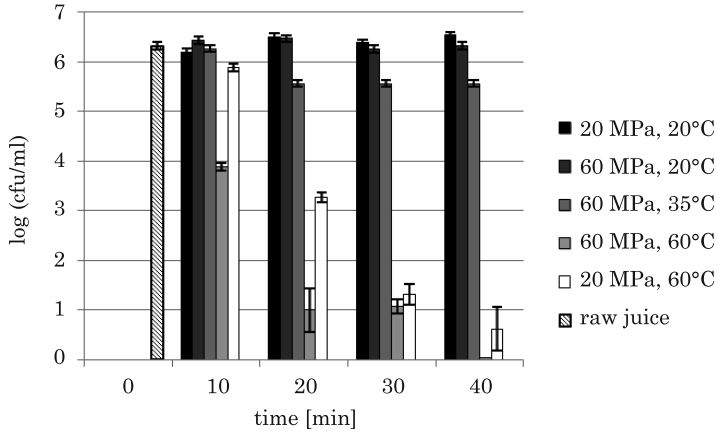

Fig. 2. Survival of lactic acid bacteria in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

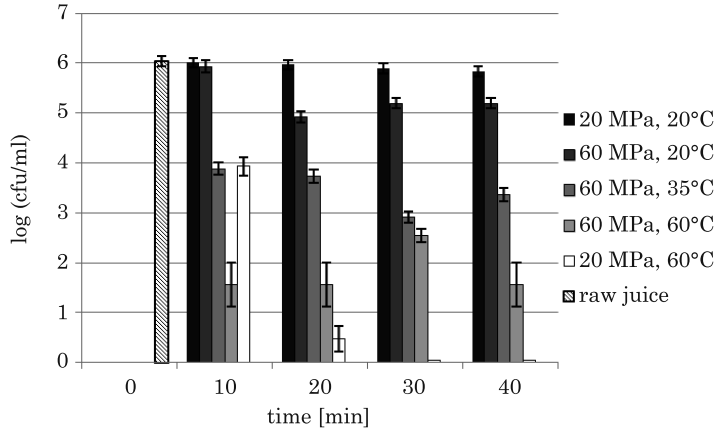

Fig. 3. Survival of yeasts in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

Only slight changes in the population of spoilage microorganisms were observed when HPCD under 20 MPa at 20°C and 60 MPa at 20 or 35°C was used (Fig. 1). When the HPCD treatment was combined with a temperature of 60°C, a significant reduction in spoilage microorganisms was achieved. The reduction in the total count of spoilage microorganisms was 2.8, 4.5, 5.1 and 5.9 log after 10, 20, 30 and 40 min treatment with HPCD at 60 MPa. Unfortunately, the reduction was mainly caused by temperature treatment. They achieved 0.9, 4.2, 4.5 and 4.5 log after 10, 20, 30 and 40 min treatment at 60°C, respectively. Perhaps it could be related to small volume of sample in our device. Decreasing the HPCD pressure to 20 MPa resulted in a similar

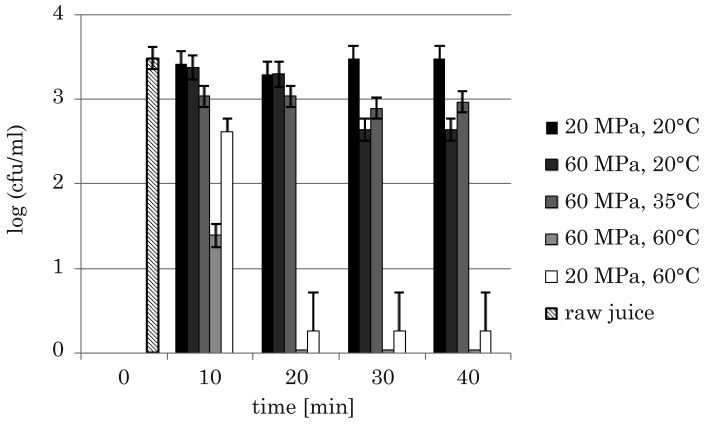

Fig. 4 Survival of moulds in beetroot juice supplemented with 5% apple juice treated with HPCD. The bars on the figures indicate the mean standard deviation for data points

reduction in the total count of spoilage microorganisms. Under these conditions the reductions were significantly lower ($p< 0.05$) than when using 60 MPa, except for the result after 30 min, which did not differ significantly ($p>0.05$). These results show that temperature plays a prominent role in the inactivation of spoilage microorganisms when combined with HPCD whereas the impact of the pressure value was less relevant.

Similar observation were reported in earlier studies carried out on apple juice. The microbial inactivation of 5 log of natural flora was achieved using the HPCD process at 16 MPa, 60°C and 40 min (FERRENTINO et al. 2009 a). LIAO et al. 2010 a obtained a 3.9 log reduction in the natural microorganisms in apple juice with HPCD at 20 MPa and a temperature of ≥52°C. The total aerobic flora in apple juice was reduced by 3.72 log after 10 min treatment at 22 MPa and 60°C (XU et al. 2011). Similar results were also observed in other juices. In litchi juice, a reduction of 4.19 log of aerobic bacteria was achieved with HPCD at 10 MPa and 52°C for 15 min (LI et al. 2012). A 5 log reduction for total aerobic microorganisms occurred with 34.5 MPa at 40°C and 7 min treatment in red grapefruit juice (FERRENTINO et al. 2009b).

The use of the temperature of 60°C could have serious disadvantages to beetroot juice. The stability of beetroot pigments such as betanin and isobetanin under HPCD was affected by both pressure and temperature. The study LIU et al. 2008 b shown, that treatments with a pressure above 30 MPa and temperature more than 55°C, led to a more rapid loss of betanin and isobetanin, and color change from violet to orange-red, when the aqueous solution of pigments was used.

As shown in Fig. 2, a significant reduction in lactic acid bacteria in beetroot juice was achieved, similarly to spoilage organisms, when HPCD was combined

with a temperature of 60°C. Inactivation reached 5.0 and 5.7 log after 30 and 40 min treatment with 20 MPa and respectively 5.2 and 6.3 log when 60 MPa was used. After 30 min treatment the results did not differ significantly ($p>0.05$).

Only a few articles concerning the inactivation of lactic acid bacteria with HPCD were found. The inactivation of *Lactobacillus plantarum* by 8 log was observed within 120 min under HPCD of 6.9 MPa at 30°C (HONG and PYUN 1999). The inactivation of *Lactobacillus plantarum* in apple cider reached 5 log after 20 min treatment at 7.6 MPa and 42°C, using a continuous system (YUK and GEVEKE 2011). The authors concluded that both CO_2 concentration and temperature contributed to microbial inactivation. Another lactic acid bacteria – *Leuconostoc dextranicum* was inactivated by at least 8 log at 35°C in 15–20 min under CO_2 pressure 6.9 or 20.7 MPa (LIN et al. 1993).

The results of our study confirmed the thesis that it is more difficult to inactivate natural microorganisms in real foods than inoculated microorganisms in real foods or buffers, which could be due to the complexity of natural microflora in real foods and the fact that they differ from inoculated pure strains in their susceptibility to HPCD.

Yeasts in beetroot juice were inactivated, when 60 MPa HPCD was conducted for 20 min even at 20°C, but at 35°C the reduction was higher and reached 2.7 log after 40 min (Fig. 3). The greatest reduction in yeasts, reaching 6.0 log, was achieved after HPCD treatment for 30 and 40 min at 20 MPa and 60°C. Contrary to previously described results, increasing the pressure to 60 MPa did not result in the higher inactivation of yeasts.

A significant reduction in moulds, above 3 log, was observed when HPCD both at 20 and 60 MPa was applied at a temperature of 60°C for at least 20 min (Fig. 4). In this case also, temperature played a predominant role in the inactivation of moulds; the pressure value was less significant.

In recent years there have been several studies on the inactivation of fungi using HPCD. The yeasts and moulds in apple juice treated with HPCD at ≥42°C were totally inactivated after 30 min, with a 3.9 log reduction (LIAO et al. 2010 b). XU et al. 2011 showed that yeast and moulds were completely inactivated (>4 log) in apple juice after 3 min treatment at 22 MPa and 60°C. The inactivation of yeast and moulds in litchi juice was complete (2.60 log reduction) at 10 MPa and 32°C for 30 min, or at 42°C for 15 min and at 52°C for 5 min (LI et al. 2012). Five log reduction for yeasts and moulds occurred at 34.5 MPa at 40°C and 7 min treatment in red grapefruit juice (FERRENTINO et al. 2009 b).

Studies on the effect of HPCD on yeasts were also conducted using *Saccharomyces cerevisiae* as the target microorganism. VALVERDE et al. 2010 reported that a 5 log inactivation of *S. cerevisiae* with HPCD in the Conference pear took place at 55°C. The required pressure and exposure times were

relatively low ≥6 MPa and 10 min. SPILIMBERGO et al. 2007 achieved an above 4 log reduction of *S. cerevisiae* in apple juice treated with HPCD 20 MPa at a temperature in the range of 25–50°C.

Conclusion

Studies of the effectiveness of preserving techniques, in which natural microorganisms in real foods are considered the targets, are very important for process design. A significant reduction in the native microflora in beetroot juices with HPCD using laboratory apparatus has been achieved. The study showed that the inactivation of native microflora in beetroot juice using HPCD was greatly affected by the treatment temperatures. The results indicate that HPCD combine with temperature may be a useful technique for preserving beetroot juices, but further studies on a larger half technical scale and shelf-life study are needed.

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