

The combined effect of high pressure and ultrasounds on the germination and inactivation of *Alicyclobacillus acidoterrestris* spores in apple juice

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Alicyclobacillus acidoterrestris is a thermoacidophilic, nonpathogenic and sporeforming bacterium which has been found in commercial pasteurized fruit juices and beverages. High heat resistance combined with unique among sporeformers ability to grow under acidic conditions (optimal pH 3,5-5,0) make them one of the most serious problems for the fruit and vegetable processing industry.

Nonthermal treatments can be attractive alternatives to traditional heat treatments for producing high quality, convenient and safe food products and can be used as promising methods for microbial inactivation.

Knowledge about the spore germination process has increased considerably during the past two decades. This has allowed the development of novel strategies to inactivate bacterial spores in a two step process, i.e., germination followed by inactivation [4]. Moderate hydrostatic pressure can induce the germination of *A. acidoterrestris* spores [2, 5]

Dipicolinic acid (DPA) is an important factor in spore resistance to many environmental stresses, and in spore stability. DPA is a universal component of bacterial spores and its release can serve as an indicator of spore germination.

The purpose of this study was to investigate the combined effect of high hydrostatic pressure (HHP) and high power ultrasound (HPU) on the germination and inactivation of two strains *Alicyclobacillus acidoterrestris* spores in apple juice.

The spores of two spoilage *A. acidoterrestris* strains were inoculated into clarified apple juice from concentrate – FCAJ (11.30°Bx) and cloudy apple juice not from concentrate – NFCAJ (11.26°Bx), up to 7 log cfu/mL. Then samples were exposed to the following treatments with HHP: 300 MPa / 50°C / 15 min, HPU: 25-45 kHz / 30-50°C / 10 min. and combination of both treatments. The pressure 300 MPa at 50°C was chosen based on previous experiments as optimal for germination of spores [2,3].

The number of surviving population was determined by the plate method on BAT-agar (incubation for 5 days at 45°C). Pressure-induced inactivation was the difference between the plate count before, after HHP and HPU treatment.

Quantification of the DPA concentration in the samples was performed using the HPLC method [3].

In the case of HHP treatment in FCAJ, 3.3 - 4.37 log (cfu/mL) of *A. acidoterrestris* spores were germinated of which 2.3 - 3.7 log (cfu/mL) were inactivated. In a NFCAJ the number of *A. acidoterrestris* germinated spores were 2.3-3.8 log (cfu/mL) and 1.7-3.0 log (cfu/mL) were inactivated.

When HPU was applied at 30°C, no significant effect on *A. acidoterrestris* spores was observed in both kind of juices. Results showed that the effectiveness of HPU treatment increased when temperature increased. Conducting the HPU process at 50°C resulted in spores inactivation in the range 1.25 - 3.0 log depending on the strain and kind of juice. The better reduction (about 1.5 log) was obtained for FCAJ than for NFCAJ.

The combined treatment HHP and mild heat (300 MPa / 50°C / 15 min) followed by HPU (25 kHz / 50°C / 10 min) resulted in 5-log in case *A. acidoterrestris* TO-169/06. Spores of *A. acidoterrestris* TO-117/02 were more resistant to both methods and 3.9 log were inactivated. Our results are very promising, especially in comparison to study [1] in which thermosonication of orange juice pretreated with 600 MPa HPP for 15 min at temp below 39°C, allowing a 3 log reduction after 42 min treatment at 78°C. Probably HHP induced too lower germination at applied conditions.

The total amount of DPA in *A. acidoterrestris* spores were 42.7 - 50.3 μM depending on the strain. The amount of DPA released in FCAJ treated with 300 MPa at 50°C ranged from 24.6 to 29.3 μM. The amount of DPA released correlated to the count of the germinated *A. acidoterrestris* spores ($R^2=0.8992$) Fig.1 [3].

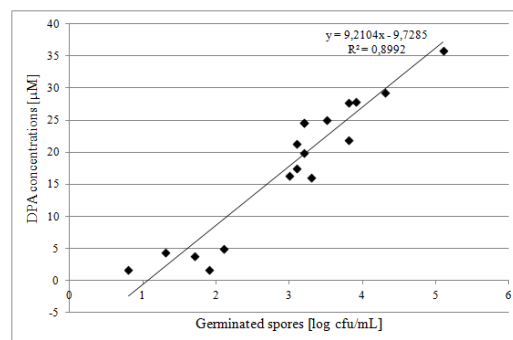


Figure 1. DPA released from of the spore suspensions vs number of germinated spores of *A. acidoterrestris* after HHP treatment

To enhance process effectiveness it is recommended to induce germination and transform spores into more susceptible vegetative form. Results showed that the effects of HPU and HHP were quite variable and strongly strain and temperature dependent. The protective effect of cloudy apple juice not from concentrate on *A. acidoterrestris* spores, during studied processes was observed. Overall it can be concluded, the potential of HHP and HPU treatments is promising in juice processing as an alternative to traditional thermal treatment.

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