Furthermore we could also detect a time-delayed spore germination followed by an inactivation step for both high pressure temperature conditions in acid buffer solution (pH 5,5). However only at 600 MPa/60°C for 2 min the acid pH-conditions yield in a significant increased spore inactivation up to 1.55 logs. The addition of Nisin or Lysozym to the spore suspension caused just at 600 MPa/60°C an increased spore inactivation at reduced treatment times (< 3 min). Our results reveal the potential of FCM as a useful technique for rapidly studying endospore germination and structural alterations following high pressure temperature treatment with chemical agents.

Keywords: high pressure, flow cytometer, spore inactivation mechanisms, chemical agents

## [P130] Inactivation mechanisms of bacterial spores during ultra high pressure treatments K Reineke\*, A Mathys, D Knorr Berlin University of Technology, Germany

The inactivation mechanisms of bacterial spores during high pressure thermal sterilization have to be completely known and understood to establish this technology successfully in the food industry. At low pressures (100-200 MPa) spore germination is triggered by an activation of the germinant receptors, but a part of the whole spore population is still in the dormant state, which could cause foodborne diseases. Spores that lack nutrient receptors germinate rapidly if treated at pressures above 500 MPa caused by an opening of the Ca<sup>2+</sup>–DPA channels, which triggers the cortex lytic enzymes (CLE). It is assumed that these activated CLEs are subsequently inactivated by pressure and temperature.

To proof this assumption and to gain a deeper insight in the inactivation mechanisms during ultra high pressure treatments, inactivation experiments with genetic modified *Bacillus subtilis* spores were performed. Two different strains of spores lacking the germinant receptors and one of the CLEs (FB114 [ $\Delta$ ger3  $\Delta$ sleB] and FB115 [ $\Delta$ ger3  $\Delta$ cwlJ]) and its wild type strain (PS832) were treated in ACES buffer solution (0.05 M, pH 7). All three samples were treated in time in a SITEC-high pressure unit (Type 0101-7000s) in the pressure- temperature range of 550-700 MPa and 37-80 °C.

The FB114 and FB115 spores germinated under atmospheric conditions very poorly  $(3*10^5 \text{ cells ml}^-1)$  due to the lack of germinant receptors. After a pressure treatment at 550 MPa, 37 °C and 60 min more than  $1.7*10^8$  cells ml<sup>-1</sup> could be germinated by high pressure and no inactivation was observed. Higher treatment intensities resulted in a strongly increased inactivation of all spore strains. All inactivation kinetics of the three strains runs similar under the equal pressure-temperature-conditions, which indicates that CLE are not the target enzyme system for spore inactivation under the investigated pressure-temperature conditions.

To validate these findings, further research under various pressure-temperature conditions is needed.

Keywords: high pressure, spore, mechanisms, cortex lytic enzymes

## [P131]

**Formulation and Quality Evaluation of a Clarified Mixed Fruit Juice** T.V. Candéa<sup>1</sup>, L.A. Nakano<sup>1</sup>, R.A. Mattietto<sup>2</sup>, L.M.C. Cabral<sup>3</sup>, V.M. Matta<sup>\*3</sup> <sup>1</sup>Federal Rural University of Rio de Janeiro, Brazil, <sup>2</sup>Embrapa Eastern Amazon, Brazil, <sup>3</sup>Embrapa Food Technology, Brazil

Fruit juice marketing has been continuously increasing all over the world due to the consumers search by natural and healthy products added to their potentiality as functional. The objective of this work was to study the formulation of a clarified mixed fruit juice based on their bioactive compounds. The raw materials were *açaí* pulp, pineapple and grape juice, chosen due to their composition on sugars and organic acids aiming at sensory acceptability and bioactive composition. *Açaí* and pineapple were clarified by microfiltration using ceramic membranes with 0.022m<sup>2</sup> of filtration surface and their permeate fractions were collected in sanitized packages

and afterwards frozen storage. The commercial pure grape juice is already clarified and did not require this process step. A two factor simplex-centroid design was used for the formulation, fixing the *açai* juice concentration and varying only the two other juices contents performing five treatments. The formulated juices were analyzed for determination of anthocyanins and total phenolics. The results showed an expected behavior of a positive correlation between the concentration is fixed, grape is the component richer in both phenolics and anthocyanins, which explain the results. The formulation containing 20% *açai*, 80% pineapple and no grape presented the lowest values, 4.34 mg/100g anthocyanins and 175.21 mg/100g phenolics were verified in the formulation that contained 20% *açai*, no pineapple and 80% grape. The Tukey test showed significant difference between the five formulations with 95% of probability.

Keywords: fruit juices, fruit drinks, clarification, tropical fruits

## [P132]

Effect of Pre-Treatment on Açaí Pulp Microfiltration Process F.S. Monteiro<sup>1</sup>, F.S. Gomes<sup>1</sup>, R.A. Mattietto<sup>2</sup>, L.M.C. Cabral<sup>3</sup>, V.M. Matta<sup>\*3</sup> <sup>1</sup>Federal Rural University of Rio de Janeiro, Brazil, <sup>2</sup>Embrapa Eastern Amazon, Brazil, <sup>3</sup>Embrapa Food Technology, Brazil

Açaí (Euterpe oleracea Mart.) is a palm tree from north region of Brazil. Its small fruit is rich in bioactive compounds making it an attractive product. The aim of this work was to evaluate the effect of pre-treatments on microfiltration of acaí pulp. Raw material was acaí pulp from Belém, Brazil. Pre-treatments were centrifugation, conducted in a basket centrifuge; refining, in a depulper with a 0.5 µm mesh; and enzymatic hydrolysis with DSM Rapidase and Picantase for 30 min at 35°C. Microfiltration was conducted in tubular membranes with 0.1µm pore size and 0.022m<sup>2</sup> of surface. Processes were carried out at 35°C/3bar in batch mode. Permeate flux was determined during the process. Total and soluble solids, pH and acidity were determined on initial and treated pulp, permeate and retentate. Initial pulp contained 13.89 (w/w), 2.85°Brix, 0.19g/100g (malic acid) and 4.93 of pH. Total solids of treated pulp from centrifugation (5.71%) were lower than the others (13.83 and 13.84%) which consequently caused lower total solids in retentate and permeate fractions. Acidity and pH of treated pulp were different (p<0.05) for the different pre-treatments. Similar behaviors were also verified to retentate and permeate fractions. Values varied from 0.10 to 0.29 g/100g malic acid and pH from 4.17 to 4.98, with no correlation to total solids content. Soluble solids were different for each fraction although there was no significant difference in the same fraction in function of used pre-treatment. The values were 2.87, 1.63 and 4.77°Brix for treated pulp, permeate and retentate, respectively. Average flux was affected by pre-treatment being the lowest values, around 70 L/hm<sup>2</sup>, obtained after centrifugation and the highest, 110 L/hm<sup>2</sup>, with the enzymatic action. As physical-chemical parameters did not present a behavior that contributes for the pre-treatment selection, permeate flux results suggest the use of enzyme as the recommended option.

Keywords: tropical fruits, membrane processes, fruit juice, enzyme

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Beer is a drink known for over six thousand years. Throughout its long history, it has always been one of the most favorite and widely spread drinks, present at all meridians in every season of a year, equally favourite with all society classes. The favourable effect of beer has been known for centuries, so, besides being used as a refreshment it has been used as a medicine recommended for kidneys treatments, appetite improvement, as a tranquilizer and for good sleep.