

High hydrostatic pressure

The use of high pressure processing (HPP) in the food industry was developed as an alternative to common thermal processes, such as pasteurization and sterilization, to produce a microbiologically safe food while avoiding and reducing undesirable changes in sensory, physiochemical and nutritional properties of foods. The use of high pressure at high temperatures as a tool for sterilization (high pressure thermal sterilization - HPTS) may lead to benefits in terms of food safety and food quality when compared to conventional retorting. This promising technology is not yet available at industrial scale level, however recent research may trigger the use of this application for certain foods. HPTS combines the synergistic effects of elevated temperatures (90 - 121°C) and pressures above or equal to 600 MPa to realize a quick and sufficient inactivation of endospores.

An additional benefit is the compression heating, which is caused by the compression work against intermolecular forces if pressure is applied. Depending on the food system this temperature increase can range from 3 – 9 °C per 100 MPa and helps to additionally heat up the product to the required temperatures; whereas the thermal load applied to the product can be reduced. Within the Prometheus project this promising emerging technology was used as a tool to mitigate and reduce the carcinogenic food PCs, which are the result of a high thermal load applied to the product during the processing. The aim was to increase the food quality without influencing the food safety of the product.

1 Results of the HPTS for the selected food systems:

Formation of furan and MCPD-ester in the fish systems:

Retorted cans were treated at 115°C for 28 min (total process time 55 min), which equals an $F_0 = 7$ min. The analyses of furan in fish samples showed that significant amounts could only be found in canned sardines in olive oil. In all pressure treated and retorted samples of tuna in brine (0.23 – 1.5 $\mu\text{g kg}^{-1}$) and tuna in sunflower oil (0.37 – 1.1 $\mu\text{g kg}^{-1}$) furan levels were nominal and close to the detection limit of the analytical method. The amounts of furan formed during retorting were 58 $\mu\text{g kg}^{-1}$ (28 min, 115°C). The data for the formation of furan clearly show that one of the main sources of furan, is the olive oil, in which PUFAs are probably the precursor. The formation of furan is also dependent on the treated food system and the treatment conditions. The reasons for the lower amounts of furan in the high pressure treated samples could be the shorter overall processing times which result in a reduction of the thermal load, and Le Chatelier's principle, which states that under high pressure conditions only reactions are favoured who have a negative reaction volume. The comparison of the retorted and the high pressure treated sardines in olive oil showed that depending on the treatment conditions, a reduction of furan in the high pressure treated samples is possible between 71 to 97 %. Even at sterilization conditions of 121°C, 600 MPa a reduction of 78 % is possible.

Formation of MCPD / MCPD-esters:

In all samples tested only very low amounts of free MCPD were found. The results of the preliminary trials indicate that the main focus concerning the formation of MCPD-esters should be put on tuna in sunflower oil. Since here the highest amounts of MCPD-esters are found. The quantities found in tuna in brine and in sardines in olive oil were nominal in all HP treated samples, the untreated sample and the retorted sample as well. In addition, the untreated sample of tuna in sunflower oil already contained quite high amounts of MCPD-esters (167 $\mu\text{g kg}^{-1}$) which are derived from the refined oil used (672 $\mu\text{g kg}^{-1}$). The quantities found in tuna in sunflower oil for the different temperature time combinations at 600 MPa showed no clear trend for any combination. Otherwise the results indicate that the use of non-refined oils or no oil in the tested food systems result in no or a low formation of MCPD-esters. Here the main aim should be to reduce the amounts of MCPD-esters in the food by changing the recipe towards non-refined oils.

Formation of furan in the baby food puree:

The quantities of furan formed in retorted baby food puree were $30.11 \pm 1.6 \mu\text{g kg}^{-1}$ at an $F_0 = 7 \text{ min}$ (115°C , 28 min). These values confirm the amounts of furan found in commercial available vegetable baby food purees with a mean concentration of $37 \mu\text{g kg}^{-1}$. The levels analyzed by both solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) and static headspace GC-MS (HS-GC-MS), were much lower than the levels common found in retorted vegetable baby food puree. The results of both methods are in most cases very comparable, and within the uncertainty of the methods (at such low levels $\leq 5 \mu\text{g kg}^{-1}$, 25-30 % is considered uncertainty), although the amounts analyzed by HS-GC-MS are a little bit higher than the ones analyzed by SPME-GC-MS. The main reasons for this can be twofold: the use of a different method to collect the volatiles and the samples that were sent to the two labs were not from the same batch and therefore variations/differences in the ingredients of the food system cannot be excluded. A reduction of furan within a range of 81 to 96 % is possible under HPTS conditions in comparison to the retorting. Even under sterilization conditions $F_0=7 \text{ min}$ (115°C , 28 min; 121°C , 7min) at 600 MPa a reduction of 81 to 86 % is possible.

Endospore inactivation:

To gain a better understanding of the T, t dependencies at 600 MPa a modeling of the spore inactivation, based on the inactivation kinetics obtained, for the selected food systems and the ACES buffer was conducted for an extrapolated $12 \log_{10}$ inactivation of *Bacillus amyloliquefaciens* (12 D-concept as for thermal retorting) for isothermal and isobaric conditions. The temperature-time combinations at 600 MPa, for the processing of the different food samples at pilot scale, were based on calculated and extrapolated isokinetic lines of the inactivation kinetics of *B. amyloliquefaciens* obtained at lab scale. Figure 1 shows the isokinetic lines in a temperature-time range of $90\text{-}115^\circ\text{C}$ and 0-40 min. The dotted and dashed line above the ACES-buffer (straight) (mainly stable under HP-conditions) represent as follows: sardine in olive oil (dotted) and tuna in sunflower oil (dashed). The progress shows that the oil within these systems has a protective effect on the spore inactivation. Whereas tuna in brine (dashed-dotted line) and the baby food puree (dot-dot-dashed line) are running beneath the ACES-Buffer and therefore represent food systems that have a rather low influence on the spore inactivation.

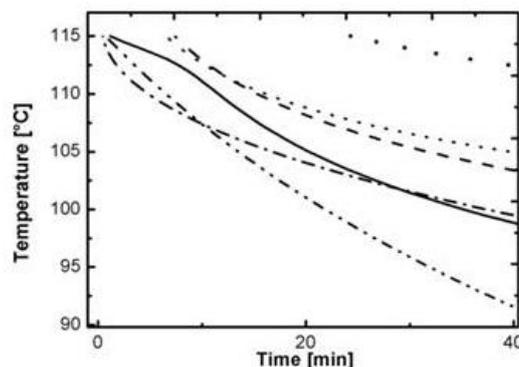


Figure 1. Isokinetic lines of selected food systems for an extrapolated $12 \log$ inactivation of *Bacillus amyloliquefaciens* at 600 MPa in a T,t-range of $90\text{-}115^\circ\text{C}$ and 0-40 min. Sardine in olive oil (small black dotted line); Tuna in sunflower oil (black dashed line); ACES-Buffer (solid black line); Baby food puree (black dash dotted line); Tuna in brine (dash dot dotted line); border of the thermal inactivation (big black dots).

This clearly indicates that the composition of foods plays an important role in the inactivation of spores. Due to this independent optimal treatment conditions could be obtained for the different foods without having to deal with an over-processing of the foods.

Upscaling of the HPTS-process from 4ml at lab scale to 55 L at pilot scale level:

Although pilot scale high-pressure systems as well as high-pressure-high-temperature stable packaging for HPTS are available on the market, up to now there is no HPTS treated product on the market. There has not yet been an approach to go from lab scale based modeled inactivation kinetic data of a high pressure high temperature resistant spore strain into an pilot scale system with economical T,t combination ($t \leq 10$ min) in connection with storage trials. Based on inactivation data derived in a 3.5 ml vessel under isothermal, isobaric conditions during pressure dwell-time a scaling up of the HPTS with tuna in brine, tuna in sunflower oil, sardine in olive oil and a baby food puree was conducted with the 55 L vessel HT from Hiperbaric at the AZTI-Tecnalia (Derio, Spain) to verify the findings in a large scale and non-uniform temperature. The foods were not inoculated with spores they were only treated at the calculated conditions for a $12 \log_{10}$ inactivation of *B. amyloliquefaciens* (T 100-115 °C; t 0.45-28min at 600 MPa) and later on stored after the French norm "Standard NF V 08 408" at room temperature and 37°C for 21 days to see if the calculated T,t-combinations at 600 MPa lead to a shelf stable product. The aim was 1) to use extrapolated temperature-time combinations obtained at lab scale; 2) Evaluate the formation of FPCs in comparison to lab scale; 3) Feasibility of HPTS as a pilot scale process.

Formation of PCs under Upscaling-conditions:

MCPD-esters in tuna in sunflower oil:

Due to the findings at lab scale only tuna in sunflower oil was investigated for the presents of MCPD-esters. The results at pilot scale level confirm the results at lab scale. The untreated samples already contained $84 \pm 19 \mu\text{g kg}^{-1}$ (lab scale $120 \pm 19 \mu\text{g kg}^{-1}$) and in comparison to the thermal sterilized samples with $64 \pm 15 \mu\text{g kg}^{-1}$ and $97 \pm 24 \mu\text{g kg}^{-1}$, the samples treated with the same T, t regime but at 600 MPa with $108 \pm 26 \mu\text{g kg}^{-1}$ were not significantly different. Nevertheless, the levels of MCPD-esters present in the samples were the same for a treatment at pilot and lab scale level. No significant formation could be detected for these two approaches and the retorting, since it is mainly due to the use of the refined oil.

Furan in sardine in olive oil and baby food puree:

Furan was found only at levels close to the detection limit ($\leq 0.1 \mu\text{g kg}^{-1}$) of the method in tuna in brine and tuna in sunflower oil for the retorted and high pressure sterilized samples and so will not be discussed further. The analysis of furan in sardines in olive oil showed that similar amounts of furan were obtained in the retorted samples at lab scale and pilot scale. The results also indicated the same trends as seen for the lab scale experiments that for the same F_0 -value (7) lower amounts of furan are measured under HPTS conditions as for normal retorting. At sterilization conditions under pressure (600 MPa) the amounts of furan were $17 \pm 2 \mu\text{g kg}^{-1}$ (lab scale 115°C, 28 min, 600 MPa) and $28 \pm 1 \mu\text{g kg}^{-1}$ (pilot scale 115°C, 28 min, 600 MPa). Overall the trials at pilot scale validated the trials conducted at lab scale. A reduction of furan in sardine in olive oil in comparison to the retorting was possible even for scaled up processes. For the pilot scale trials the reduction of furan was similar and between 42 % (115°C, 28 min, 600 MPa) to 77 % (113°C, 9.4 min, 600 MPa) in comparison to the retorting. The amounts of furan found in the baby food puree were similar to those for the formation of furan in sardine in olive oil. Although in general lower amounts of furan, between 30.1 to $0.4 \mu\text{g kg}^{-1}$, were detected in comparison to sardine in olive oil. The reduction of furan in baby food puree was 94-98 % in comparison to retorting, similar to the reductions obtained at lab scale, where the reduction for a broader temperature time range (T: 90-121 °C, t: 0-30 min) was between 81 and 96 %. The results of the formation of PCs under pilot scale conditions overall agree with the results obtained at lab scale.

Storage trials:

The results of the storage trials revealed that altogether three temperature-time combinations (107.5°C, 9.9 min; 115°C, 0.45 min for baby food puree and 107.5, 9.9 min for tuna in brine) were not suitable to produce a stable product. All of the undertreated (100°C, 10 min) and untreated samples were not stable and these are not discussed further. All other treatment conditions for the other tested foods proved to be suitable for the production of a high pressure sterilized stable product. To be certain that no spore recovery had occurred, all pouches were checked for revivable microorganisms. This shows that a storage trial performed after the treatment is a powerful tool to validate the safety of the applied extrapolated T, t – combinations at 600 MPa. The process window for the temperature-time domain at 600 MPa for the different food systems is shown in Figure 2. Here the assumption was made that if a sample at given T, t – combination would be stable it would also be stable if the temperature would be held constant and the time would be extended to a time ≤ 10 min. The connected symbols (for selected T, t-combinations) represent the treated and stable temperature-time combination at 600 MPa for the different food systems. In all cases the temperature-time combinations under HPTS-conditions that would lead to a safe product in terms of microbiological safety are lower in terms of time and temperature in comparison to the thermal retorting. Furthermore treatment conditions result in a lower formation of carcinogenic food PCs.

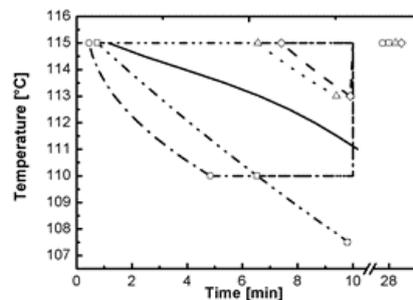


Figure 2. Process windows for the selected food systems for a 12 log inactivation of *B. amyloliquefaciens* that lead to stable product at 600 MPa in the temperature time range of 107.5-115°C and 0.45-28 min. Sardine in olive oil (Δ and small black dotted line); Tuna in sunflower oil (\diamond and black dashed line); ACES-Buffer (solid black line); Baby food puree (\circ and black dash dotted line); Tuna in brine (\square and dash dot dotted line).

2 Conclusion:

The industry can use the gathered data and new insights into the HPTS to produce food of a better overall quality without affecting the safety of the product. The results obtained at pilot scale verified the results at lab scale. It was possible to go from lab scale based modeled inactivation kinetic data of a high pressure high temperature resistant spore strain into an pilot scale system with economical T,t-combination ($t \leq 10$ min) in connection with storage trials for the selected food systems. The experiments showed that the storage trials were successful and that a suitable and that a feasible temperature-time combination at 600 MPa could provide a safe product. Furthermore over-processing can be avoided if HPTS is used as sterilization technique, resulting in a double benefit in terms of food quality and food safety. In the future more research needs to be conducted with more food systems and target microorganisms for the HPTS-process. Also since pilot scale and small industrial systems are available these need to be optimized to guarantee an economical process for the food industry. This signifies that the process line needs to be fine-tuned in terms of output, the heat up time of the vessel needs to be shorten and tools need to be developed to guarantee safe and constant temperature-pressure contribution in the packed food. Hence, the HPTS-process could lead to a new principle of application for high pressure processing, where the benefits of this emerging technology merge to create safer, healthier and high quality foods.

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