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Comparing thermal inactivation to a combined process of moderate heat and high pressure: Effect on ascospores in strawberry puree

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ABSTRACT

High pressure processing is a mild preservation process that inactivates pathogenic and spoilage micro-organisms in food products, but preserves the fresh characteristics of a product. Compared to untreated product, an enhanced shelf life is obtained during refrigerated storage. Knowledge on the use of high pressure pasteurisation aimed for ambient storage is limited.

The aim of this research was to investigate if a combination of high pressure and moderate heat could be used to produce a shelf-stable high-acid fruit product. Ascospores of the heat resistant fungi *Talaromyces macrosporus* and *Aspergillus fischeri* were added to fresh strawberry puree that served as a model system. The effect of the processing steps and storage at ambient temperature for 2 weeks was studied on viability of the ascospores.

A preheating step at 69 °C/2 min resulted in full or partial activation of *A. fischeri* and *T. macrosporus* spores, respectively. The pressure build-up by the process without any holding time resulted in additional activation of spores. A combination of moderate heat (maximum 85–90 °C) and high pressure (500–700 MPa) for holding times up to 13 min inactivated these highly resistant spores much faster than a heat treatment alone. At T_{max} = 85 °C and 600 MPa the spores of *T. macrosporus* and *A. fischeri* were inactivated by 5.0 and 5.5 $\log_{10}$ after 13 and 7 min, respectively. At T_{max} = 85 °C the heat treatment alone did not reduce the viability of these spores up to 60 min of treatment. At T_{max} = 90 °C the holding time of the combined pressure-heat treatment could be reduced to obtain the same degree of inactivation of the heat resistant fungi.

In addition, treated and untreated ascospores in strawberry puree were stored for 14 days at room temperature to evaluate delayed outgrowth of spores. Untreated ascospores of *A. fischeri* were activated by storage in the puree. However, at conditions combining high pressure ≥ 600 MPa with T_{max} ≥ 85 °C for 13 min, heat resistant fungi were successfully inactivated.

This research showed that a combination of moderate heat and pressure can drastically improve the effectiveness to inactivate heat-resistant ascospores in a high-acid fruit product compared to a heat treatment, potentially resulting in a better product quality.

1. Introduction

Thermal treatment is widely used to extend the shelf life of fruit products. Heat-resistant fungal ascospores that contaminate these products can persist after pasteurisation (Dijksterhuis, 2007; Dijksterhuis, 2019; Santos et al., 2018a). These sexual spores are characterized by a profound (constitutively) dormant state where germination is only occurring after an extreme environmental trigger, such as heat (70–85 °C) or high pressure (at 600 MPa) (Dijksterhuis and Samson, 2006; Dijksterhuis and Teunissen, 2004; Reyns et al., 2003).

High hydrostatic pressure treatment is a non-thermal food processing technology that is used as an alternative to thermal treatment. It results in processing at lower temperatures and/or shorter holding times than a thermal treatment, aiming for a safe product with an improved quality. High pressure pasteurisation is a combination of pressure and time, typically at 400–600 MPa for 1–10 min holding time carried out at ambient temperature (Matser and Timmermans, 2016). This leads to inactivation of vegetative micro-organisms and is often followed by a chilled storage of the treated product to control the outgrowth of survived spores. Many applications of this technique have been commercially introduced, for instance the treatment of vegetable products, meat products, seafood and fish, juices and beverages (Huang et al., 2017; Tonello, 2011).

High pressure sterilisation is a more intense treatment and combines

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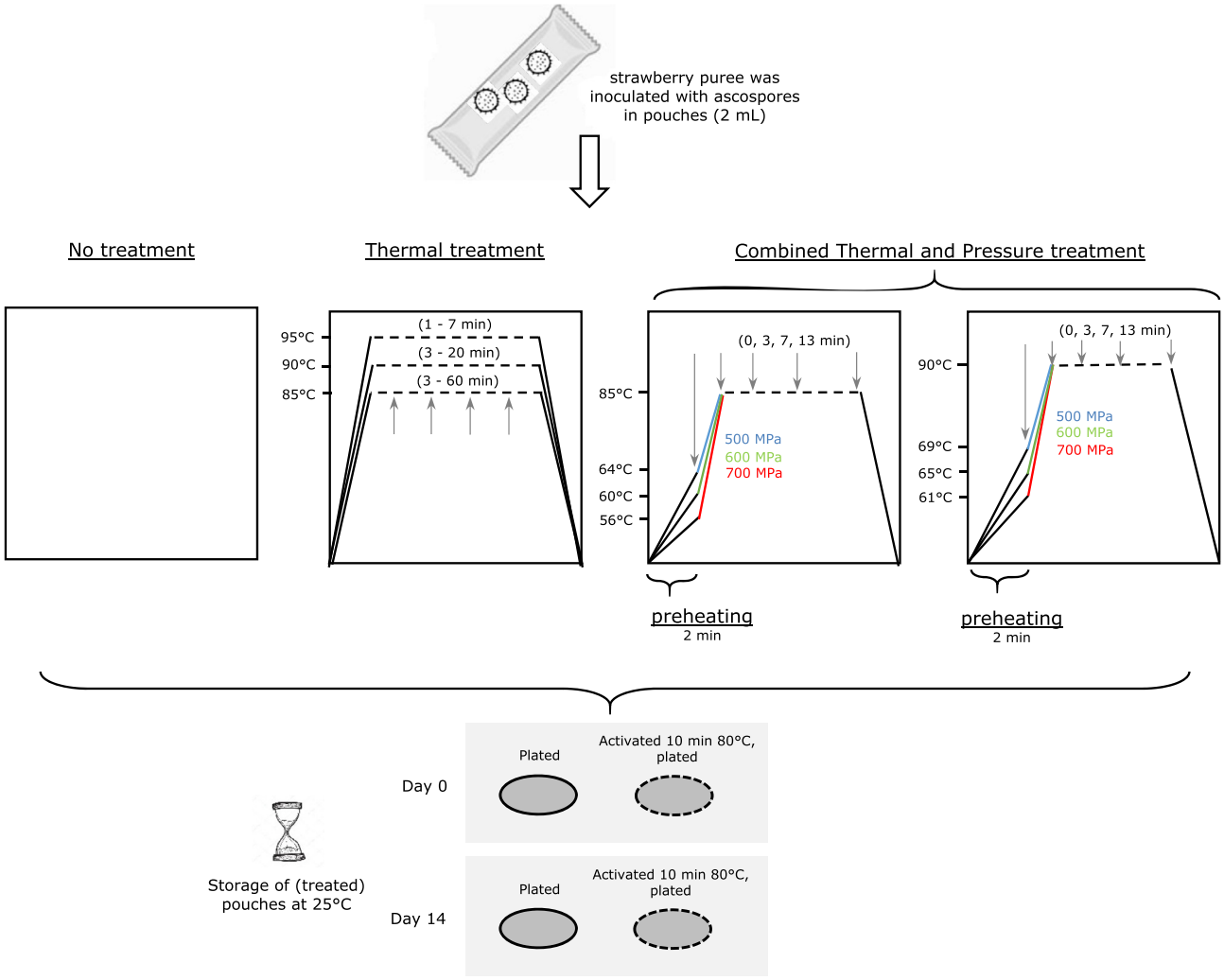

Fig. 1. Schematic overview of the experiment, where arrows indicate different sampling points during processing.

high pressure and preheating temperatures of ~90 °C. Higher temperatures are reached due to adiabatic heating of the product up to 120 °C (dependent on the pressure and preheating temperature) and can inactivate heat resistant fungal – and bacterial spores, implying that products can be stored at ambient temperature. Although many studies have been published showing the effects on inactivation of microbes and enzymes with retention of quality (Matser et al., 2004; Sevenich et al., 2014), this process is still under investigation and not yet applied by industry. Limited knowledge is available about the use of high pressure pasteurisation aimed for ambient storage. High-acid products could be an interesting model for this purpose, as some heat resistant fungal spores are able to germinate at these storage conditions and cause food spoilage (Berni et al., 2017; Beuchat, 1986; Silva and Gibbs, 2004). The combination of application of high pressure with moderate temperatures of 50–75 °C was evaluated to study the inactivation of heat-resistant ascospores (Evelyn and Silva, 2015; Evelyn et al., 2016). *Neosartorya fischeri* and *Byssochlamys nivea* (currently known as *Aspergillus fischeri* and *Paecilomyces niveus*, respectively) ascospores of 4 weeks old showed 3.3 $\log_{10}$ and 1.4 $\log_{10}$ reduction, respectively, when exposed at most intense conditions of 600 MPa - 75 °C for 10 min, while no inactivation was observed at thermal reference at 75 °C for 30 min (Evelyn & Silva, 2015, Evelyn et al., 2016).

The aim of this research was to investigate if the combination of high pressure and moderate heat could be used as alternative to thermal treatment to inactivate heat-resistant ascospores in a high-acid fruit product that is subsequently stored at ambient conditions.

2. Materials & methods

2.1. Preparation of fungal ascospores

The fungi *Talaromyces macrosporus* and *Aspergillus fischeri* (with a neosartorya-morph) were selected as model systems as they produce highly stress-resistant ascospores and are associated with the spoilage of high-acid fruit products after pasteurisation (Dijksterhuis, 2019). The fungi produce numerous ascospores in closed fruiting bodies (cleistothecia) and highly homogenous suspensions can be obtained from Petri-dish cultures. Furthermore, these species provide suspensions containing isolated single cells, in contrast to *Paecilomyces* species, such as *P. niveus*, in which the asci are hard to disrupt resulting in a mixture of eight-celled asci and single ascospores. The latter complicates interpretation of inactivation kinetics after treatments.

The ascospores of *T. macrosporus* (CBS 112372, originally isolated from pineapple concentrate in The Netherlands) and *A. fischeri* (CBS 317.89, neosartorya-morph, originally isolated from desert soil in Namibia) were cultivated on oatmeal medium at 25 °C for 88 days. The long incubation time of 88 days was selected as heat resistance of ascospores of both fungal species increase in time and maximal heat-resistance is needed for inactivation studies (Dijksterhuis and Teunissen, 2004; Wyatt et al., 2015a). Ascomata (fruiting bodies) were collected by means of a T-spatula in ice-cold ACES-buffer as described previously (Wyatt et al., 2015b). The mass of hyphae and fruiting bodies was vortexed in 50 mL Greiner tubes with a mixture of 1 mm and 100 µm

glass beads (Sartorius) and subsequently filtered over the sterile glass wool. Spores were washed twice in ACES buffer and the spore counts were obtained with a Coulter Counter (Beckman Multisizer 3, Beckman Coulter, Indianapolis, USA) and with a haemocytometer (Bürker Türk, VWR, Amsterdam, The Netherlands), both with similar results. The morphology of the ascospores was confirmed by light microscopy as described in (Samson et al., 2010) (See Supplementary Figs. 1 and 2 for spore images). The heat resistance of ascospores was tested and confirmed in advance by heating at 85 °C/30 min and a strong increase in germination was observed on agar plates (MEA, malt extract agar; Samson et al., 2010) and by light microscopy. After harvesting, the ascospores were stored on ice for 5–6 days (*T. macrosporus)* or for 7–8 days (*A. fischeri*) before they were added to the strawberry puree.

2.2. Sample preparation

Fresh strawberries (cv. Elsanta) were carefully sorted, crowns were removed and poor quality fruit was discarded before they were washed in tap-water (4 °C). Strawberries were pureed in a food blender (KitchenAid). The puree (pH = 3.3, 8.5°Brix) was packed in pouches (~200 g) and stored at −20 °C until use. One day before the experiment, the sample was thawed and heated (120 °C/20 min) to inactivate the background microflora. The puree was inoculated with 2.0×10^6/g (*T. macrosporus*) or 2.7×10^6/g (*A. fischeri*) ascospores. 2 mL aliquots of the inoculated puree were distributed into small pouches of 2.5 × 5 cm made of low-density polyethylene (LDPE) granule, 65 μm thickness (Stomacher®400, classic bag, Seward, UK). Inoculated pouches were stored in ice-water before and immediately after the treatment. The inoculated pouches were used within 2–5 h.

2.3. Experimental design

The inoculated strawberry puree was subjected to several treatments and process conditions, as schematically shown in Fig. 1, where arrows indicate different sampling points at thermal treatment or combined thermal and pressure treatment at variable preheating temperatures, pressure build-up, variable holding time or maximum temperature. The number of viable counts was determined directly after processing (t = 0 days) and after 14 days of storage at 25 °C.

2.4. Thermal treatment

Heat resistance of the ascospores was evaluated at three temperatures (85, 90 and 95 °C). The thermostatic water bath (Julabo MV-basis, Seelbach, Germany) was heated until the treatment temperature was reached. The inoculated pouches were submerged into the preheated water bath and heated up instantaneously due to the small volume, and kept at that temperature for various holding times between 1 and 60 min. Heating up and cooling down of the pouch occurred within seconds, as was verified with a pouch that contained very sensitive thermocouples (HYP-O T-type, Omega). After treatment the samples were kept on ice-water until plating at t = 0 d.

Samples for the storage test were kept at 25 °C for 14 days.

2.5. Combined moderate heat and pressure treatment

High pressure treatment was carried out in a pilot-scale equipment, custom made by Resato (The Netherlands). Samples were treated at three different pressures (500, 600 and 700 MPa) and at two maximum temperatures (85 and 90 °C). Two to four small sample pouches were packed in a larger pouch (and the larger pouches were vacuumized at 95% vacuum), and larger pouches were taped to the perforated cylindrical holder (polyoxymethylene acetale polymer (POM), 8.4 cm outer diameter, 29.4 cm outer length) that was placed in a sample holder (POM, 9.5 cm outer diameter, 36 cm length, with movable stopper) and preheated for 2 min in the high pressure vessel. Preheating was done with the help of an outer heating jacket and bottom heater, at variable temperatures between 56 and 69 °C. Variable preheating temperatures were used depending on the chosen pressure (increase of ~4 °C/100 MPa) to reach the same target maximum temperature. Temperature increase per 100 MPa was determined previously, and is described with the following equation: $T_{increase}$/100 MPa (°C) = 0.026 × $T_{initial}$ + 2.26. The pressure builds up in the pilot unit at a rate of ~30 MPa/s (Vervoort et al., 2012). After the treatment, the pressure was released and the temperature decreased.

Samples and control samples were taken after different stages of the treatment including preheating, pressure build-up (0 min holding time) and pressure treatment for 3, 7 or 13 min (Fig. 1). Pouches were transferred to ice-water for additional cooling, and kept on ice-water until plating at t = 0 d or kept at 25 °C for 14 days of storage.

2.6. Spore enumeration

Each sample was serially diluted in sterile peptone physiological salt diluent and 100 μL was plated in duplicate on malt extract agar (MEA). Part of the (stored) samples were heat activated (80 °C/10 min) prior to plating. Colonies on the agar plates were counted after 3 and 7 days of incubation at 25 °C.

2.7. Data acquisition

Presented results are the average number of colonies (surviving ascospores) determined on four plates coming from one pouch of puree (taken from two consecutive countable serial dilutions, plated in duplicate). Plates of the undiluted samples with 1 colony were used as a detection limit, meaning an ascospore concentration of 1 $\log_{10}$ cfu/g. When at least one colony was detected on one of the four plates, this was visualised as 1 $\log_{10}$ cfu/g, even if the average number of ascospores was < 1 $\log_{10}$ cfu/g, since for ambient storage even a single spore can grow out and spoil the product. If no colonies were detected on none of the four plates, this was indicated as < 1 $\log_{10}$ cfu/g.

The colony counts of the samples that have been stored for 14 days may result from a mixture of spores and hyphae, resulting in a lower number of counted colonies for those samples due the entanglement of fungal cells.

3. Results and discussion

3.1. Influence of heat activation and storage on untreated spores

Ascospore pouches that received no thermal or high pressure treatment were subjected to a 10 min heat activation treatment at 80 °C and showed a 2 $\log_{10}$ increase of colony counts for *T. macrosporus* and an increase > 3 $\log_{10}$ for *A. fischeri* (Fig. 2). These data indicate that < 0.1–1% of the ascospores may germinate without heat activation treatment (“spontaneously”), and 99.0–99.9% of the ascospores formed colony forming units after the heat treatment (“activated”). Thus, this heat activation treatment was sufficient to activate almost all the spores present in the strawberry puree. Germination without heat activation was markedly increased (especially in the case of *A. fischeri*) after 14 days of storage in strawberry puree at room temperature (Fig. 2B). This observation shows that dormancy is (partially) lost after storage. Low pH is a factor shown previously to lower the temperature required for heat activation (Dijksterhuis and Samson, 2006; Kikoku, 2003; Reyns et al., 2003), although this effect might also be influenced by the presence of organic acids (Beuchat, 1988; Kikoku et al., 2009). Dormancy of ascospores can also be broken in part of the ascospores after a very long storage period (Beuchat, 1992; Dijksterhuis, 2007; Nagtzaam and Bollen, 1994; Scholte et al., 2001). This indicates that ascospore germination in a stored fruit puree may happen without any triggering treatment. This is a species dependent treat as the majority of *T. macrosporus* spores still needed heat activation after 2 weeks of storage at

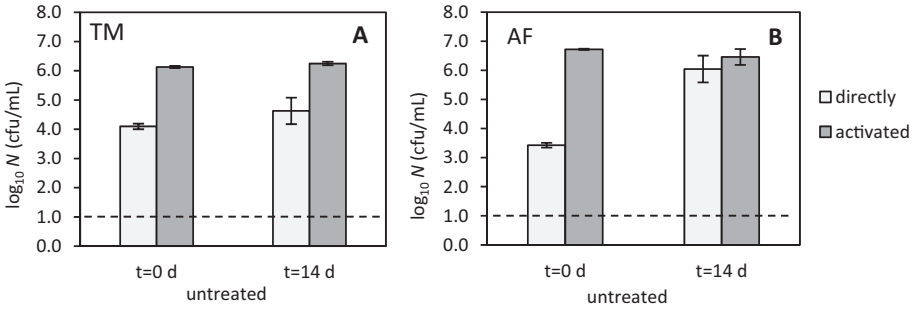

Fig. 2. Viable counts of *Talaromyces macrosporus* (TM)(A) and *Aspergillus fischeri* (AF)(B) suspended in strawberry puree that received no treatment, and were plated directly (light grey) or after an activation step of 10 min 80 °C (dark grey) on t = 0 days and after 14 days of storage at 25 °C. Dashed line indicates the detection limit.

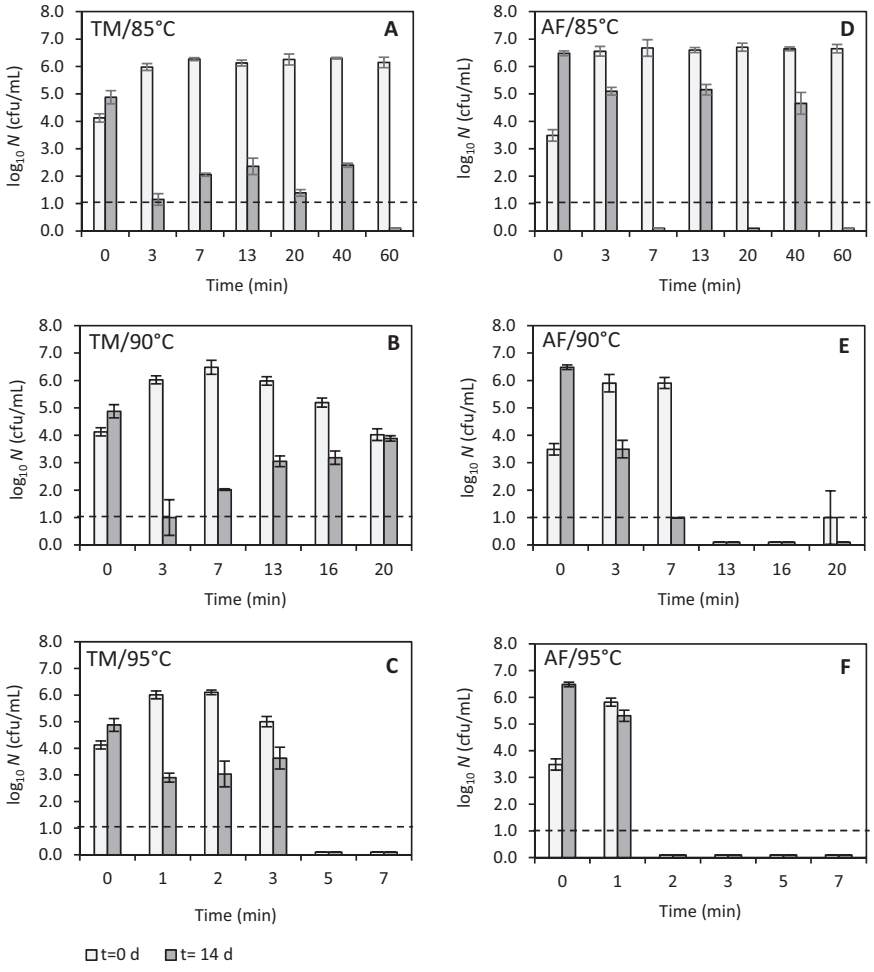

Fig. 3. Viable counts of *Talaromyces macrosporus* (TM)(A + B + C) and *Aspergillus fischeri* (AF)(D + E + F) suspended in strawberry puree that received a thermal treatment at 85 °C (A + D), 90 °C (B + E) or 95 °C (C + F) for indicated times. Results of directly plating at t = 0 days (light grey) and after 14 days (dark grey) storage at 25 °C are shown. Dashed line indicates the detection limit.

ambient conditions in order to germinate. The nature of activation of dormant ascospores is still an enigma; it is highly intriguing that different fungal species show different characteristics in this respect.

3.2. Inactivation of spores by thermal treatment

Ascospores in strawberry puree were heat-treated with heat at 85 °C, 90 °C and 95 °C (Fig. 3). At 85 °C no inactivation was observed (Fig. 3A, D, light grey columns). Even after 60 min holding time, no viable counts were reduced and full activation was reached after 3 min. of treatment at this temperature. These values are characteristic for *T. macrosporus* and relatively high for *A. fischeri*. The specific environment of the strawberry puree may affect heat resistance of these spores as a result of, for example, the presence of sugar (8.5°Brix). However, Beuchat (1992) does not confirm such effects for *A. fischeri*, but King and Whitehand (1990) do for *T. macrosporus* (in this paper designated as *Talaromyces flavus*).

When ascospores heat-treated at 85 °C were stored in puree for 14 days, subsequently obtained colony counts were significantly lower (Fig. 3 A, D, dark grey columns) compared to the directly plated samples (light grey columns). This indicates that the storage most likely resulted in the outgrowth of activated spores producing mixtures of different cellular states in the samples, such as ascospores and hypha. This makes the plating method not suitable for quantification of the number of survived ascospores, since the entangled fungal hypha and entrapped spores may grow as a single colony. However, this method

still gives an indication of the presence of viable fungal cells. Surprisingly in both cases, no viable counts were observed after a 60 min heat treatment and storage for 14 days.

T. macrosporus spores in puree were more heat resistant compared to *A. fischeri*, and were only inactivated (> 5 $\log_{10}$ cfu/g) after 5 min at 95 °C, whereas *A. fischeri* spores were inactivated (> 5.5 $\log_{10}$ cfu/g) after 2 min at 95 °C or 13 min at 90 °C (Fig. 3). In these samples with > 5 $\log_{10}$ reduction, there was no growth detected also after 14 days of storage.

In Fig. 3B the colony counts of *T. macrosporus* detected after 14 days of storage are increasing in correlation with the treatment time. This indicates that ascospores in puree were viable after up to 20 min exposure to 90 °C, but may have formed less hyphal networks in case of longer heating times compared to shorter heating times. Heat causes a delay in germination (Dagnas et al., 2017; Santos et al., 2018b) but outgrowth of colonies was not slowed down. Counterintuitively, resulting colony counts in such a case may be higher, as ascospores (with short germ tubes grown from spores that showed a delay in germination) still can be dispersed and are not captured between hyphae after dilution. This was not observed for *A. fischeri* treated at 90 °C and 95 °C (Fig. 3E, F).

3.3. The effect of preheating on spores

The effect of preheating the spores for 2 min at different temperatures was evaluated (Fig. 4). Preheating at 69 °C resulted in full or partial activation of *A. fischeri* and *T. macrosporus* spores respectively (Fig. 4A, C). These data indicate that dormancy of *T. macrosporus* spores was more profound compared to *A. fischeri* under these conditions.

Mixed populations of activated and dormant ascospores occur upon short heat treatments depending on the fungal species and the temperature during the 2 min pre-treatment. If these mixtures are subjected to a 14 days storage at room temperature, it has to be expected that part of the spores germinate and form fungal mycelium. *T. macrosporus* shows essentially the same response at 56, 60 and 64 °C preheating, with an approximately hundred-fold (2 $\log_{10}$) increase of germination after a further "activation step" at heat treatment at 80 °C (Fig. 4B). After a preheating at 69 °C a marked decrease of spontaneous and activated colonies occurs after the same treatment. This outcome could be the result of inactivation of germinated cells, with still colonies formed by a subpopulation of dormant spores. This might be complicated by capture of loose spores in a network of hyphae. It clearly shows that breaking of dormancy has occurred after 2 min at 69 °C, 2 weeks earlier. This shows that activation of *T. macrosporus* ascospores occurs at a lower temperature in strawberry puree (pH = 3.3) compared to a buffer at pH 7 (Dijksterhuis et al., 2002; Kikoku, 2003). Activation at lower pH is also observed in the case of pressure activation of ascospores of *T. macrosporus* (Reyns et al., 2003) and discussed in Dijksterhuis and Samson (2006).

For *A. fischeri*, the effect at 69 °C is even more profound after 14 days of storage in puree, with extensive inactivation (Fig. 4D) of the fungus after treatment at 80 °C. Nearly all ascospores survived thermal treatment at 85 °C for 60 min in fruit puree (Fig. 3A, D), while a 2 min treatment at 69 °C followed by the 2nd treatment of 10 min at 80 °C after 2 weeks of storage were able to reduce the viable count to 1 colony out of 5,4 × 10^6 ascospores (> 5.5 $\log_{10}$ cfu/g reduction). This is an example of Tyndallisation, where two subsequent heat treatments of lower intensity, due to activation of dormant spores, have a near complete eradication effect.

3.4. Inactivation of spores by combination of heat and high pressure

Ascospores in strawberry puree were subjected to combined high pressure/heat treatments with a maximum process temperature of 85 °C (Fig. 5). Preheating and pressure build-up to 500, 600 and 700 MPa (corresponding to t = 0 min, light grey bars) in all cases did not significantly decrease spore viability (Fig. 5A, C). Furthermore, an effective activation of both ascospores upon the combination of pre-treatment and pressure build-up in comparison to pre-treatment only (Fig. 4) was observed, e.g. at preheating temperature of 56 °C and any pressure about 1.5 $\log_{10}$ activation of *T. macrosporus*.

Concerning the inactivation at pressures of 500 MPa, a gradual decrease in viable count of *T. macrosporus* was observed when increasing the holding time, with a maximum of 2 $\log_{10}$ inactivation after 13 min and maximum temperature of 85 °C. *A. fischeri* ascospores were not inactivated at 500 MPa.

At 600 MPa (85 °C) the spores of *T. macrosporus* and *A. fischeri* were inactivated by 5.0 and 5.5 $\log_{10}$ after 13 and 7 min respectively (Fig. 5A, C). At this temperature the heat treatment alone did not reduce the viability of these spores after 60 min holding time (Fig. 3A, D).

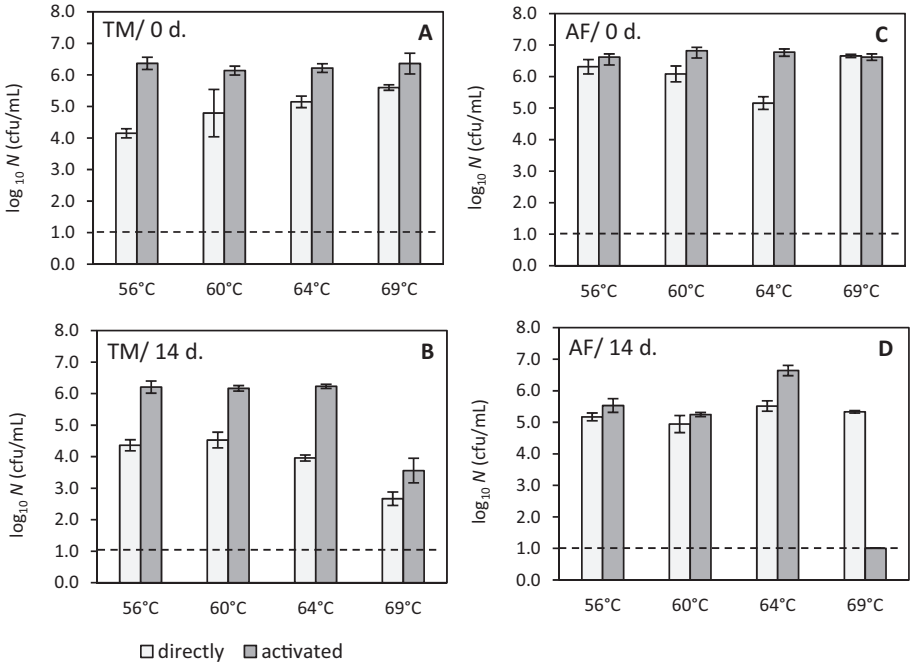

Fig. 4. Viable counts of *Talaromyces macrosporus* (TM)(A + B) and *Aspergillus fischeri* (AF)(C + D) suspended in strawberry puree that received a preheating step of 2 min at indicated temperatures. Results of counts obtained after directly plating (light grey) or after an activation step of 10 min at 80 °C (dark grey) are shown at t = 0 days (A + C) and after 14 days storage at 25 °C (B + D). Dashed line indicates the detection limit.

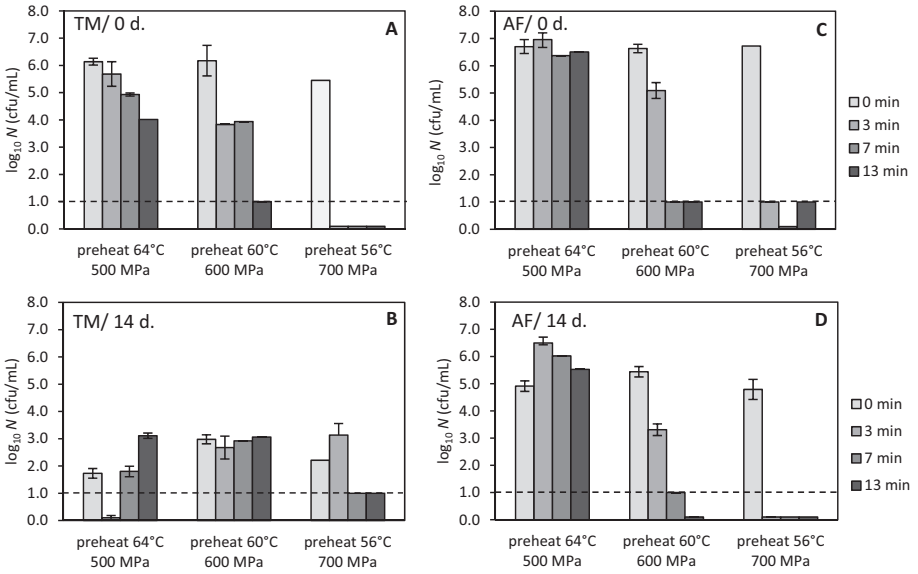

Fig. 5. Viable counts of *Talaromyces macrosporus* (TM) (A + B) and *Aspergillus fischeri* (AF) (C + D) suspended in strawberry puree that received a pre-heating step of 2 min at indicated temperature followed by high pressure treatment at indicated pressure and holding time at a maximum temperature of 85 °C. A + C) directly plated at t = 0 days, or B + D) plated after 14 days storage at 25 °C. Dashed line indicates the detection limit.

These data show that combination of heat with high pressure made the process much more effective in inactivation of these heat-resistant ascospores, providing an effective alternative to reduce processing time when compared to only moderate heat treatment.

In line with these observations, the value of combining high pressure with heat treatment for inactivation of heat resistant ascospores has been reported previously. *Byssochlamys nivea* (now *Paecilomyces niveus*) ascospores in strawberry puree were inactivated at 600 MPa/75 °C by 1.5 $\log_{10}$ in 10 min, whereas heating only at the same temperature only activated those spores (Evelyn and Silva, 2015). In another study *Neosartorya fischeri* (now *Aspergillus fischeri*) ascospores in apple juice were inactivated at 600 MPa/75 °C by 3.3 $\log_{10}$ in 10 min, whereas in case of heat only treatment at 85 °C the same $\log_{10}$ reduction was reached only after 60 min (Evelyn et al., 2016).

At 600 MPa 13 min as well as 700 MPa for 3 min very low or even absent counts were observed after direct plating *T. macrosporus*, but a 3 $\log_{10}$ cfu/g viability count was observed after 14 days of storage (Fig. 5B) indicating that a few survived spores (≤10 cfu/g) may grow out during storage or may form dispersed fungal mycelium. This is supported by the fact that the heating of the stored sample (80 °C/10 min) before plating eradicated the viability (not shown), which shows that the 3 $\log_{10}$ cfu counts detected in the stored sample were non-heat resistant structures (data not shown). These data illustrate that very low numbers of ascospores can lead to outgrowth in a sample. This is very relevant as ascospores in many fruit samples are present in only very low numbers/g product (Berni et al., 2017; Santos et al., 2018a).

At 700 MPa (≥3 min) no viable ascospores were detected for *T. macrospores* directly after treatment (Fig. 5A), though after 14 days of storage viability was detected in the sample treated for 3 min. This is of importance for products with ambient shelf life, since immediate measurements can predict "false" eradication. Indeed, fungal spores may restore their capacity of germination after a heat shock when kept under certain conditions, as observed with conidia of the fungus *Penicillium expansum* (Baldy et al., 1970). For *A. fischeri* this spore damage seemed to be irreversible, since also after 14 days of storage of those samples (700 MPa/3–13 min) no growth was detected (Fig. 5C, D).

A selected number of pressure-time combinations have been evaluated at the maximum temperature of 90 °C (Fig. 6). No ascospores were detected after 7 min at 600 or 700 MPa (90 °C), neither in directly plated, nor in stored samples for both species (Fig. 6). *A. fischeri* spores were inactivated already after 3 min at 600 MPa, while *T. macrosporus* had ≤1 $\log_{10}$ surviving spores after 13 min of treatment, which were no longer detectable after the storage. These observations indicate that 3–7 min treatment at 90 °C and 600 MPa resulted in > 5 $\log_{10}$ inactivation of two fungal species that form highly stress-resistant spores. At this temperature, the use of high pressure (600 MPa) reduces the processing time by > 3 fold for inactivation of the ascospores, which survived the heat-only treatments of up to 20 min (Fig. 3B).

4. Conclusion

In this study the combination of high pressure and moderate heat was applied as alternative process to thermal inactivation of heat-resistant ascospores in a high-acid fruit product. Results showed that combining the moderate heat (T_{max} = 85 or 90 °C) with the pressures of 500–700 MPa can drastically improve the effectiveness of the process compared to the treatment using heat only. This may lead to a significant reduction of the processing time and potentially to a better product quality, which is the subject of investigation in a forthcoming study. Furthermore, the storage of spores in strawberry puree at room temperature as well as the pre-heating step for 2 min at up to 69 °C and the pressure build-up process caused partial or full activation of ascospores, depending on the species. A Tyndallisation effect was observed for the spores of *A. fischeri* which were inactivated by > 5.5 $\log_{10}$ by a pre-heating step of 2 min/69 °C, followed by a 2-week storage and subsequent treatment of 10 min at 80 °C.

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Declaration of competing interest

Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108629.

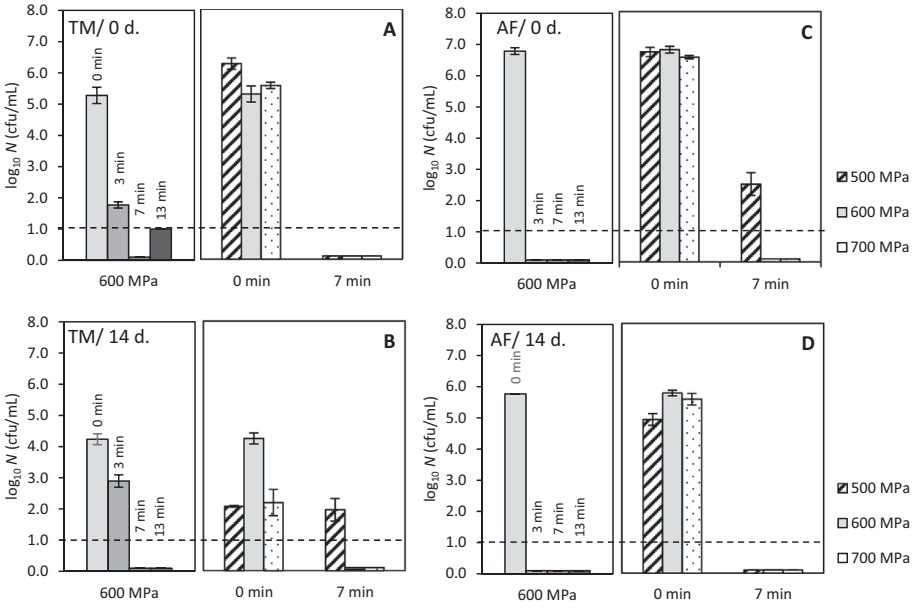

Fig. 6. Viable counts of *Talaromyces macrosporus* (TM) (A + B) and *Aspergillus fischeri* (AF)(C + D) suspended in strawberry puree that received a pre-heating step of 2 min followed by high pressure treatment at indicated pressure and holding time at a maximum temperature of 90 °C. A + C) directly plated at t = 0 days, or B + D) plated after 14 days storage at 25 °C. Dashed line indicates the detection limit.

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