# Thermal inactivation of *Aspergillus lacinosus* ascospores as a function of temperature and soluble solids content in fruit jam

**VÁCLAV POHŮNEK – MARKÉTA ADAMCOVÁ – IRENA KULIŠANOVÁ – IVETA ŠÍSTKOVÁ – BO-ANNE ROHLÍK – RUDOLF ŠEVČÍK**

**Summary**

Moulds are the most frequently occurring spoilage microflora in jams, in particular the heat-resistant species of the *Byssochlamys*, *Neosartorya* and *Aspergillus* genera. An alarming number of uncommon heat-resistant isolates, such as *Aspergillus lacinosus*, with unknown thermal inactivation kinetic parameters, decimal reduction times (*D* values), were detected in recent studies. *Aspergillus lacinosus,* an ascospore-forming mould was isolated from commercially produced jams with reduced sugar content, during a thermal death study. The aim of our study was to determine *D* values for *A. lacinosus* in three types of matrices: physiological solution (PS, pH 6.6, refraction *Rf* = 1.2 °Brix), low-sugar jam (LSJ, pH 3.5, *Rf* = 57 °Brix) and extra-sugar jam (ESJ, pH 3.5, *Rf* = 65 °Brix) at 70–95 °C. ESJ was premium quality jam with minimal content of fruits 450 g per kilogram of product. Data obtained from thermal death curves showed various *D* values of *A. lacinosus* in these matrices, i.e. (23.25 ± 7.21) min for PS, (8.18 ± 3.56) min for LSJ and (4.89 ± 1.89) min at 85 °C for ESJ. It was observed that ESJ had the lowest *D* value from all matrices for each temperature. Calculation of the temperature sensitivity of microorganisms (*z* values) showed the same phenomenon, the values ranging from 17.2 °C to 38.7 °C for strawberry jams and from 20.3 °C to 21.8 °C for forest fruit jams.

**Keywords**

heat resistant mould; thermal treatment; inactivation; decimal reduction time; thermal sensitivity; thermal death curves

Moulds pose a detrimental risk for microbial stability of jams, since they can grow well under acid conditions and low water activity [1–3]. Apart from the fact that the mould spoilage causes significant economic losses worldwide, it is also a serious food safety issue due to the possible production of allergens or mycotoxins, which may have further carcinogenic or toxic effects on humans [4–6]. The main source of mould contamination is raw material used to produce jams. These materials are often contaminated by soil-borne heat-resistant, xerophilic or preservative-resistant moulds [6].

Heat-resistant moulds, mainly from the genera *Aspergillus* (*Neosartorya* morph), *Paecilomyces* (*Byssochlamys* morph) and *Penicillium* (*Eupenicillium* morph), are considered as some of the most resilient microorganisms due to their ability to survive not only pasteurization treatment, but low pH environment, limited headspace oxygen levels and high-pressure processing as well [4, 7]. The heat resistance of their vegetative cells is relatively low. However, these moulds form specific survival structures, so-called ascospores, that have extremely high heat resistance [4, 8]. These structures are able to resist the heat treatment at temperatures exceeding 75–80 °C for 30 min [5, 9]. It means that standard pasteurization conditions will not be sufficient to prevent the product from spoilage. In fact, the pasteurization treatment induces the opposite; i.e. it activates the ascospores, so the mould can germinate, grow and cause spoilage [5, 7, 10].

In order to prevent the growth of heat-resistant moulds, a number of approaches were studied and implemented in the food process chain. Be-

**Václav Pohůnek, Markéta Adamcová, Irena Kulišanová, Iveta Šístková, Bo-Anne Rohlík, Rudolf Ševčík**, Department of Food Preservation, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Technická 5, 16628 Prague 6 – Dejvice, Czech Republic.

*Correspondence author*:
Václav Pohůnek, e-mail: vaclav.pohunek@vscht.cz

 

sides keeping to the good hygiene practices (GHP) and good manufacturing practices (GMP), the potentially present ascospores can be inactivated by specific thermal and non-thermal processes, or by their combination [7, 8, 11]. Non-thermal processes, such as high-pressure processing [12], power-ultrasound [12] or even low-energy electron beam treatment of the fruit surface [11], are promising alternatives to thermal treatment, due to their minimal effects on the sensory and nutritional characteristics of the fruit product. Despite the positive effects of these technologies and the progress that has been made, the aforementioned treatments have one or more disadvantages. They are often unable to inactivate ascospores of some genera of heat-resistant moulds. Therefore, there is a need to combine these methods with thermal treatment [12, 13]. However, these methods, generally, require the use of expensive instrumentation and skilled personnel.

In order to inactivate the ascospores of heat-resistant moulds, the regime of pasteurization as a common thermal treatment in the food industry has to be optimized. It is essential to understand the thermal destruction kinetics of these specific microorganisms. The knowledge of the inactivation kinetics parameters, decimal reduction times ($D$ values) and thermal sensitivity of microorganisms ($z$ values), is a key to safely inactivate the microbes and to obtain a product of high quality at the same time [14].

Although the moulds with *Neosartorya*-type ascospores from the *Aspergillus* genus belong to the most commonly occurring and economically relevant heat-resistant moulds, only some of them were studied for their heat resistance [9, 10, 15, 16]. To the best of our knowledge, there is not a study that investigated the thermal resistance of *Aspergillus lacinosus* (*Neosartorya lacinosa*). In this study, we determined the inactivation kinetics parameters of *A. lacinosus* during heating in three different media, namely, physiological solution (PS), low-sugar strawberry jam (LSJ) and extra-sugar strawberry jam (ESJ) to evaluate the impact of the soluble solid content and temperature on $D$ and $z$ values.

## MATERIALS AND METHODS

### Samples

Low-sugar strawberry jam (LSJ; 57 °Brix, pH 3.5) and extra-sugar strawberry jam (ESJ; 65 °Brix, pH 3.5) were obtained from local stores in Prague, Czech Republic. ESJ was premium quality jam with a higher content of the fruit component, minimally 450 g per kilogram of product according to Czech legislation (Annex 2 to Decree No. 157/2003 Coll.) [17].

As an additional medium for assessment of thermal inactivation, a low-sugar premium quality jam from forest fruits was used, containing raspberries, blueberries, blackberries and blackcurrants (50 °Brix, pH 3.2; obtained from a local store in Prague, Czech Republic).

The pH value of the latter jam was adjusted from 3.2 to 4.2 and the refraction from 50 °Brix to 60 °Brix by adding solution of 0.1 $mol \cdot l^{-1}$ NaOH for pH adjustment and saccharose for refraction adjustment.

All obtained jams used in this work meet the parameters listed in Annex 2 to Decree No. 157/2003 Coll. [17], where premium jams are labeled as “Extra”. In this study, the term extra is taken to reflect a higher refraction than low-sugar jams.

### Isolation and identification of the mould

The study was carried out on the strain of *Aspergillus lacinosus* (syn. *Neosartorya laciniosa*) CBS 117721 (NRRL 35589, KACC 41657) isolated from spoiled premium quality low-sugar strawberry jam. *A. lacinosus* was described by Hong et al. [18] as a mould that is morphologically and genetically similar to *Neosartorya spinosa* and is characterized as a common soil-borne heat-resistant mould, which is able to contaminate food materials, produce secondary metabolites (aszonalenin, tryptoquivaline and tryptoquivalone) and eventually cause human mycotic diseases [19–22].

For *A. lacinosus*, the phenotype description was carried out according to Samson et al. [19] and the microscopic structures of the mould were observed by a differential interference contrast (DIC) microscope (BX51 Laboratory Microscope, Olympus, Tokyo, Japan). According to morphological characteristics, the mould was identified as a strain related to *Neosartorya laciniosa*, *N. spinosa*, *N. coreana* and *N. galapagensis* [19]. As stated by literature, these moulds currently belong to *Aspergillus* section *Fumigati* [23].

Additionally, molecular analysis was performed. DNA was extracted from the isolated mould using ArchivePure DNA yeast and Gram2+ kit (5PRIME, Gaithersburg, Maryland, USA). Afterwards, a part of the internal transcribed spacer (ITS) rDNA (including ITS1-5,8S-ITS2) was amplified by polymerase chain reaction using two mould-specific primers ITS5 and ITS4S. The purified products were sequenced using both end primers. The obtained sequences

were manually edited in BioEdit (version 7.0.0; T. Hall, Raleigh, North Carolina, USA) where the sequence errors and the unreadable areas were excluded from the analysis. Thereafter, the highest resemblance with the sequences of ex-type strains was examined using GenBank database and BLAST web client software (National Centre for Biotechnology Information, Bethesda, Maryland, USA). The isolation of mould (*A. lacinosus*) and identification was performed by the Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic.

#### Preparation of spores suspension

The isolated strain of *Aspergillus lacinosus* (syn. *Neosartorya laciniosa*) CBS 117721 was transported to the Department of Food Preservation, Faculty of Food and Biochemical Technology (University of Chemistry and Technology Prague, Prague, Czech Republic) on a malt extract agar (Merck, Darmstadt, Germany) at 8 °C. Immediately after transport, *A. lacinosus* was inoculated on yeast extract glucose chloramphenicol agar FIL-IDF (YGC agar, Merck) and incubated for 15 days at 30 °C and, thereafter, for 15 days at 2 °C. These conditions are known to enhance the production of ascospores due to the temperature shock and increase the heat resistance of the mould at the same time [10]. After incubation, a suspension of *A. lacinosus* ascospores was obtained as described by Evelyn and Silva [12] by filtration using a glass filter P16 (10–16 $\mu$m) and permeate was then centrifuged at 4000 ×*g*, 15 min and then stored in sterile distilled water at 3 °C until used.

To determine the concentration of ascospores, a part of the stored suspension was heated in a thermostatic bath (PolyScience WB05A12E, Polyscience, Niles, Illinois, USA) at 80 °C for 10 min to achieve activation the spores [9]. Then, appropriate dilutions of the suspensions in a range of $10^{-1}$–$10^{-5}$ were prepared and plated on YGC agar. The concentration of the ascospores was standardized at the level of $10^7$ spores per millilitre [9, 24].

#### Preliminary tests

Tests were carried out in physiological solution (PS, 8.5 g of NaCl and 1 g of peptone in 1 l of distilled water, 1.2 °Brix; pH 6.6), LSJ and ESJ. LSJ and ESJ samples were transferred into a sterile blender bag (VWR, Radnor, Pennsylvania, USA), then crushed in a laboratory blender Stomacher 3500/4500 (Seward, Worthing, United Kingdom) and used as a medium for the suspension of *A. lacinosus* ascospores ($10^7$ spores per millilitre). The inoculation preceded the thermal treatment to simulate natural contamination of fruits. The initial concentration of spores in the inoculated strawberries was approximately $10^6$ CFU·ml$^{-1}$. The mixture was aseptically filled into 100 ml glass flasks and subjected to thermal treatment as described in the next section.

#### Thermal resistance studies

To investigate the impact of the soluble solid content and the processing conditions (temperature and time) on the inactivation kinetics parameters of *A. lacinosus* ascospores *lacinosus*, a range of factors was applied. The ranges of the processing conditions were as follows: temperature 70, 75, 80, 85, 90, 95, 100 °C, time 5, 10, 20, 40, 60 min and soluble solid content 1.2; 50; 57; 60; 65 °Brix.

The determination of thermal inactivation kinetic parameters of *A. lacinosus* ascospores was performed in metallic 2 ml thermal death tubes a thermostatic bath. The tubes were aseptically filled with samples previously prepared in 100 ml glass flasks, concentration of ascospores being $10^6$ CFU·ml$^{-1}$. Immediately after cooling the tubes in an ice bath, microbiological analysis was performed as described in the next section.

Calculation of the decimal reduction times (*D* values) is defined in Eq. 1 and the temperature sensitivity of microorganisms (*z* value) is defined in Eq. 2 [25]:

$$D = \frac{\tau_2 - \tau_1}{log\ N_1 - log\ N_2} \quad (1)$$

where $N_1$ and $N_2$ represent surviving microorganisms at times $\tau_1$ and $\tau_2$, respectively.

$$z = \frac{T_2 - T_1}{log\ D_1 - log\ D_2} \quad (2)$$

where $(T_2 - T_1)$ represents the temperature change resulting in a tenfold ($\log D_1 - \log D_2$) change in *D*.

#### Enumeration of *A. lacinosus* ascospores in physiological solution and strawberry jams

The concentration of *A. lacinosus* ascospores in the physiological solution and in strawberry jams was determined before and after thermal processing. The samples were analysed according to European Standards ISO 6887-1:2017 [26], ISO 6887-4:2017 [27], and ISO 21527-2:2008 [28]. During each analysis, approximately 10 g of the examined sample was transferred into a sterile blender bag (VWR), blended with 90 ml of sterile physiological solution. This mixture was homogenized in a laboratory blender MixWell (Alliance Bio Expertise, La Nouëraie, France). One hundred microlitres of the appropriate dilution in a range of $10^{-1}$–$10^{-5}$ was equally spread over the surface of

the Petri dish with a diameter of 90 mm with YGC agar and incubated at 30 °C for 7 days to evaluate the efficiency of the thermal treatment [16]. Three parallel repetitions were performed for each measurement.

## RESULTS AND DISCUSSION

The identified *A. lacinosus* mould was isolated from a commercially produced jam with a reduced sugar content, where the mould grew on the surface of the product. After 14 days, the manufacturer inspects the batches of products when this mould was discovered under the lid and thus a technological defect was discovered. Despite the fact that the reduced-sugar jam had undergone the same thermal treatment as those used in extra jam production, it is most likely that the mould growth was due to a reduced sugar content in the final product and it was not caused by insufficient heat treatment.

A number of studies reported that the higher content of sugar protects the moulds against injury caused by the heat treatment [9, 29, 30]. This is due to the stress adaptation response in microbial cells [31–33]. However, foods are complex matrices and the relationship between particular microorganisms, the content of protective compounds and heat transfer may differ [24].

The study of Beuchat and Kuhn [29] reported that thermal inactivation characteristics differ not only for various microbial genera or species, but also for various serotypes. As was published in the study of Torregiani and Toledo [34], the type of the used carbohydrate in the heating and recovery media can have significant effect on heat inactivation, injury and repair of fungi. Jams with reduced content of sugar have to be enriched by polysaccharides to ensure the gel structure of the product [35]. These polysaccharides, in particular pectin, gellan, xanthan, carrageenan and other compounds, could have potentially a protective effect on the number of survivors after the heat treatment. It is well known that microorganisms, including moulds, can produce extracellular biopolymers, which protect the cells against harsh external environmental conditions. Extracellular polysaccharides form a protective layer of the cell and serve to store carbon and energy [36]. Polysaccharides added into a low-sugar jam can have a similar impact on the survival of mould as would have the microbially produced exopolysaccharides. Cebrián et al. [31] stated that an increase in complexity of the medium leads to increased heat resistance of present microorganisms. However, available studies do not give a satisfactory answer which components are the most relevant in this respect.

Mould thermostability tests confirmed that the studied isolate belongs to thermotolerant fungi, which are primarily found in soil but can contaminate raw materials, such as strawberries, and consequently jams produced from them. This mould type forms thermosetting ascospores that are able to survive the commonly used pasteurization regimes.

As to best of our knowledge, the literature does not provide any data on lethal curves for this specific mould. Our determined values are shown in Tab. 1 and Tab. 2. From the results of the microbiological analysis, decimal reduction times (*D* values) were calculated. Due to linear characteristics of the obtained inactivation curves, the temperature sensitivity (*z* value) of our *A. lacinosus* strain was determined. Our results of the deci-

**Tab. 1.** *D* and *z* values of *A. lacinosus* thermally inactivated in physiological solution and strawberry jam.

| | Physiological solution | Low-sugar jam | Extra-sugar jam |
|---|---|---|---|
| pH | 6.6 | 3.5 | 3.5 |
| Refraction [°Brix] | 1.2 | 57 | 65 |
| Temperature | ***D* value** [min] | | |
| 70 °C | 55.54 ± 20.41 | 45.57 ± 19.61 | 29.51 ± 12.59 |
| 75 °C | 37.96 ± 14.23 | 24.95 ± 12.24 | 14.26 ± 7.89 |
| 80 °C | 31.64 ± 11.88 | 14.37 ± 5.44 | 7.58 ± 3.21 |
| 85 °C | 23.25 ± 7.21 | 8.18 ± 3.56 | 4.89 ± 1.89 |
| 90 °C | 18.56 ± 6.59 | 5.01 ± 1.16 | 2.23 ± 0.64 |
| 95 °C | 12.56 ± 5.78 | 3.12 ± 0.86 | 1.04 ± 0.14 |
| | ***z* value** [°C] | | |
| | 38.7 | 21.5 | 17.2 |

**Tab. 2.** *D* and *z* values of *A. lacinosus* thermally inactivated in forest fruit jam.

| | Standard jam | Jam with adjusted pH | Jam with adjusted pH and refraction |
|---|---|---|---|
| pH | 3.2 | 4.2 | 4.2 |
| Refraction [°Brix] | 50 | 50 | 60 |
| Temperature | ***D* value** [min] | | |
| 70 °C | 46.26 ± 18.87 | 62.41 ± 22.14 | 51.32 ± 20.65 |
| 75 °C | 22.72 ± 10.61 | 38.24 ± 13.39 | 31.81 ± 11.49 |
| 80 °C | 9.23 ± 2.45 | 17.14 ± 6.87 | 16.36 ± 5.16 |
| 85 °C | 4.48 ± 1.16 | 10.31 ± 2.67 | 9.17 ± 2.32 |
| 90 °C | 2.63 ± 0.82 | 6.19 ± 1.97 | 5.16 ± 1.38 |
| 95 °C | 1.74 ± 0.61 | 4.01 ± 1.02 | 3.16 ± 0.95 |
| 100 °C | 1.14 ± 0.23 | 2.33 ± 0.79 | 1.76 ± 0.67 |
| | ***z* value** [°C] | | |
| | 20.3 | 21.8 | 20.7 |

mal reduction times were similar to the *D* values of *Aspergilli* with *Neosartorya*-type ascospores, which range between 1.5–6 min at 90 °C, as was published in previous studies of Souza et al. [9] and Berni et al. [16].

With known *D* and *z* values of *A. lacinosus,* thermal inactivation of microorganisms in final products within food processing can be described. The *D* value of *Neosartorya fischeri* ascospores for the temperature of 85 °C was determined to be 35.25 min in the neutral environment [37]. The *D* value at 88 °C in acidic juices ranges from 4.2 min to 16.2 min [29]. *N. fischeri* ascospores are also able to survive a four-hour heating cycle at 85 °C in citrus juice [38] and at 100 °C for 4–12 min [29]. Results of these studies correlate with the *D* values of *A. lacinosus* in physiological solution, extra sugar jam and low sugar jam which were obtained in our study.

Although the standard deviations of *D* values at the lowest studied temperatures (70–75 °C) in Tab. 1 and Tab. 2 are significant, these values are similar to the deviations reported in the studies of Souza et al. [9], Scaramuzza and Berni [10], Rajashekhara et al. [38] and Beuchat [29]. Temperatures 70–75 °C are not sufficient for inactivation of ascospore-forming moulds and, therefore, these temperatures are not usually used for pasteurization of fruit jams. In some cases, such temperatures may cause germination of spores instead of their inactivation [7, 30] and, therefore, the increased number of colony-forming units during the microbiological analysis. There is also an additional but important factor of the age of microorganisms present in the food matrix. It was well described that the microorganisms in the stationary phase of growth are more resistant to heat treatment than the microorganisms in the logarithmic phase. Thus, it can be expected that fully grown mycelium is of a different heat resistance than conidia or ascospores [32, 33]. Hence, the high standard deviations were probably caused by the combination of factors: insufficient inactivation (and possible activation) of ascospores [7, 30], different heat resistance of individual microbial forms [32, 33] and possible inaccuracy of the plate count technique used for analysis [39]. Unfortunately, to best of our knowledge, there is no study that would clarify these uncertainties in detail. At higher temperatures (80–95 °C), the standard deviations correlated with the results obtained in studies of Berni et al. [16], Sant'Ana et al. [24] and Rajashekhara et al. [38]. This range of temperatures is important in particular from the technological point of view especially since these are standard pasteurization temperatures.

Wyatt et al. [40] found that any heat treatment of *N. fischeri*, has a low effect on its germination rate. However, heat treatment of several seconds at 85 °C is sufficient to activate the germination. Maximal activation of germination of this mould was observed at heat treatment at 85 °C for 2 min. This is an important finding but Wyatt et al. [40] also observed that no *N. fischeri* ascospores survived the heat treatment at 85 °C for 30 min. We found in this study that when the pH value was increased, the heat resistance and temperature sensitivity increased from 20.3 °C to 21.8 °C (Tab. 2) in a case of forest fruit jams with adjusted pH values and refraction. The higher pasteurization temperature (100 °C) had a higher inactivation effect, but this temperature is generally

not used for thermal treatment of fruit jams due to the loss of nutritionally and sensory substances. For this reason, further investigation of this phenomenon is necessary.

## CONCLUSION

It was found previously that reducing the sugar content in jams can affect the durability and safety of the product. An *A. lacinosus* mould culture was isolated from a commercial jam sample. This mould is capable of forming ascospores that are resistant to standard pasteurization temperatures. To best of our knowledge, our study was one of the first to determine the lethal curves and the thermal inactivation kinetic parameters (*D* and *z* values) of *A. lacinosus* in several types of matrices. The literature does not mention this particular mould and, therefore, the determined data were compared with a related mould species, namely, *N. fischeri*. Therefore, it is necessary to carry out further measurements, for example, at different pH values of the matrices and the matrix composition as such, and to focus on measuring lethal death curves for different pasteurization temperatures. Based on our findings, jams with a low sugar content should undergo heat treatment at 80 °C for at least 20 min under contemporary pasteurization conditions.

**Acknowledgements**

This research was financially supported from an internal university research grant MSMT No 21-SVV/2019 (Ministry of Education, Youth and Sports, Prague, Czech Republic). We thank to Department of Botany, Faculty of Science (Charles University in Prague, Prague, Czech Republic) for identification of the mould.

## REFERENCES

1. Baker, R. A. – Berry, N. – Hui, Y. H. – Barrett, D. M.: Fruit preserves and jams. In: Barret, D. M. – Somogyi, L. – Ramaswamy, H. S. (Eds.): Processing fruits: Science and technology. 2nd ed. Boca Ralton : CRC Press, 2005, pp. 113–127. ISBN: 9780849314780.
2. Worobo, R. W. – Splittstoesser, D. F.: Microbiology of food products. In: Barret, D. M. – Somogyi, L. – Ramaswamy, H. S. (Eds.): Processing fruits: Science and technology. 2nd ed. Boca Ralton : CRC Press, 2005, pp. 261–284. ISBN: 9780849314780.
3. Adams, M. R. – Moss, M. O.: Plant products. In: Adams, M. R. – Moss, M. O. (Eds.): Food microbiology. 3rd ed. Cambridge : RSC Publishing, 2008, pp. 145–153. ISBN: 978-0-85404-284-5. DOI: 10.1039/9781847557940.
4. Dijksterhuis, J.: Heat-resistant ascospores. In: Dijksterhuis, J. – Samson, R. A. (Eds.): Food mycology: a multifaceted approach to fungi and food. Boca Ralton : CRC Press, 2007, pp. 101–120. ISBN: 9780849398186.
5. Frąc, M – Jezierska-Tys, S. – Yaguchi, T.: Occurrence, detection, and molecular and metabolic characterization of heat-resistant fungi in soils and plants and their risk to human health. Advances in Agronomy, *132*, 2015, pp. 161–204. DOI: 10.1016/bs.agron.2015.02.003.
6. Rico-Munoz, E. – Samson, R. A. – Houbraken, J.: Mould spoilage of foods and beverages: Using the right methodology. Food Microbiology, *81*, 2019, pp. 51–62. DOI: 10.1016/j.fm.2018.03.016.
7. dos Santos, J. L. P. – Samapundo, S. – Biyikli, A. – Van Impe, J. – Akkermans, S. – Höfte, M. – Abatih, E. N. – Sant'Ana, A. S. – Devlieghere, F.: Occurrence, distribution and contamination levels of heat-resistant moulds throughout the processing of pasteurized high-acid fruit products. International Journal of Food Microbiology, *281*, 2018, pp. 72–81. DOI: 10.1016/j.ijfoodmicro.2018.05.019.
8. Rico-Munoz, E.: Heat resistant molds in foods and beverages: recent advances on assessment and prevention. Current Opinion in Food Science, *17*, 2017, pp. 75–83. DOI: 10.1016/j.cofs.2017.10.011.
9. Souza, P. B. A. – Poltronieri, K. F. – Alvarenga, V. O. – Granato, D. – Rodriguez, A. D. D. – Sant'Ana, A. S. – Peña, W. E. L.: Modeling of *Byssochlamys nivea* and *Neosartorya fischeri* inactivation in papaya and pineapple juices as a function of temperature and soluble solids content. LWT – Food Science and Technology, *82*, 2017, pp. 90–95. DOI: 10.1016/j.lwt.2017.04.021.
10. Scaramuzza, N. – Berni, E.: Heat-resistance of *Hamigera avellanea* and *Thermoascus crustaceus* isolated from pasteurized acid products. International Journal of Food Microbiology, *168–169*, 2014, pp. 63–68. DOI: 10.1016/j.ijfoodmicro.2013.10.007.
11. Etter, D. – Rupp, A. – Prange, A. – Drissner, D.: Inactivation of mould spores in a model system and on raisins by low-energy electron beam. Food Control, *92*, 2018, pp. 357–361. DOI: 10.1016/j.foodcont.2018.05.006.
12. Evelyn, F. – Silva, F. V. M.: Inactivation of *Byssochlamys nivea* ascospores in strawberry puree by high pressure, power ultrasound and thermal processing. International Journal of Food Microbiology, *214*, 2015, pp. 129–136. DOI: 10.1016/j.ijfoodmicro.2015.07.031.
13. Evelyn, F. – Silva, F. V. M.: Resistance of *Byssochlamys nivea* and *Neosartorya fischeri* mould spores of different age to high pressure thermal processing and thermosonication. Journal of Food Engineering, *201*, 2017, pp. 9–16. DOI: 10.1016/j.jfoodeng.2017.01.007.
14. Ramaswamy, H. S.: Thermal processing of fruits. In: Barret, D. M. – Somogyi, L. – Ramaswamy, H. S. (Eds.): Processing fruits: Science and technology. 2nd ed. Boca Ralton : CRC Press, 2005, pp. 173–200. ISBN: 9780849314780.
15. Kikoku, Y. – Tagashira, N. – Nakano, H.: Heat resistance of fungi isolated from frozen blueberries.

Journal of Food Protection, *71*, 2008, pp. 2030–2035. DOI: 10.4315/0362-028X-71.10.2030.
16. Berni, E. – Tranquillini, R. – Scaramuzza, N. – Brutti, A. – Bernini, V.: *Aspergilli* with *Neosartorya*-type ascospores:heat resistence and effect of sugar concentration on growth and spoilage incidence in berry products. International Journal of Food Microbiology, *258*, 2017, pp. 81–88. DOI: 10.1016/j.ijfoodmicro.2017.07.008.
17. Sbírka zákonů č. 157/2003. Vyhláška ze dne 12. května 2003, kterou se stanoví požadavky pro čerstvé ovoce a čerstvou zeleninu, zpracované ovoce a zpracovanou zeleninu, suché skořápkové plody, houby, brambory a výrobky z nich, jakož i další způsoby jejich označování. (Decree laying down requirements for fresh fruit and fresh vegetables, processed fruit and processed vegetables, dry nuts, mushrooms, potatoes and products thereof, and other means of labeling.) Sbírka zákonů České republiky, *59*, 2003, pp. 3327–3358. ISSN: 1211-1244. In Czech.
18. Hong, S. B. – Shin, H. D. – Hong, J. – Frisvad, J. C. – Nielsen, P. V. – Varga, J. – Samson, R. A.: New taxa of *Neosartorya* and *Aspergillus* in *Aspergillus* section *Fumigati*. Antonie Van Leeuwenhoek, *93*, 2008, pp. 87–98. DOI: 10.1007/s10482-007-9183-1.
19. Samson, R. A. – Hong, S. – Peterson, S. W. – Frisvad, J. C. – Varga, J.: Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph *Neosartorya*. Studies in Mycology, *59*, 2007, pp. 147–203. DOI: 10.3114/sim.2007.59.14.
20. Malejczyk, K. – Sigler, L. – Gibas, C. F. – Smith, S. W.: Invasive sino-orbital mycosis in an aplastic anaemia patient caused by *Neosartorya laciniosa*. Journal of Clinical Microbiology, *51*, 2013, pp. 1316–1319. DOI: 10.1128/JCM.02919-12.
21. Raper, K. B. – Fennell, D. I.: The genus *Aspergillus*. Baltimore : Williams and Wilkins, 1965. DOI: 10.1126/science.150.3697.736-a.
22. Peterson, S. W.: Phylogenetic analyses of *Aspergillus* species using DNA sequences from four loci. Mycologia, *100*, 2008, pp. 205–226. DOI: 10.1080/15572536.2008.11832477.
23. Samson, R. A. – Visagie, C. M. – Houbraken, J. – Hong, S. B. – Hubka, V. – Klaassen, C. H. – Perrone, G. – Seifert, K. A. – Susca, A. – Tanney, J. B. – Varga, J. – Kocsubé, S. – Szigeti, G. – Yaguchi, T. – Frisvad, J. C.: Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology, *78*, 2014, pp. 141–173. DOI: 10.1016/j.simyco.2014.07.004.
24. Sant'Ana, A. S. – Rosenthal, A. – Massaguer, P. R.: Heat resistance and the effects of continuous pasteurization on the inactivation of *Byssochlamys fulva* ascospores in clarified apple juice. Journal of Applied Microbiology, *107*, 2009, pp. 197–209. DOI: 10.1111/j.1365-2672.2009.04195.x.
25. Adams, M. R. – Moss, M. O.: Heat processing. In: Adams, M. R. – Moss, M. O. (Eds.): Food microbiology. 3rd edition. Cambridge : RSC Publishing, 2008, pp. 63–80. ISBN: 978-0-85404-284-5. DOI: 10.1039/9781847557940.
26. ISO 6887-1:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Geneva : International Organization for Standardization, 2017.
27. ISO 6887-4:2017. Microbiology of the food chain – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 4: Specific rules for the preparation of miscellaneous products. Geneva : International Organization for Standardization, 2017.
28. ISO 21527-2:2008. Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 2: Colony count technique in products with water activity less than or equal to 0,95. Geneva : International Organization for Standardization, 2008.
29. Beuchat, L. R. – Kuhn, G. D.: Thermal sensitivity of *Neosartorya fisheri* ascospores in regular and reduced-sugar grape jelly. Journal of Food Protection, *60*, 1997, pp. 1577–1579. DOI: 10.4315/0362-028X-60.12.1577.
30. Beuchat, L. R.: Ectraordinary heat-resistance of *Talaromyces-Flavus* and *Neosartorya-fisheri* ascospores in fruit products. Journal of Food Science, *51*, 1986, pp. 1506–1510. DOI: 10.1111/j.1365-2621.1986.tb13846.x.
31. Cebrián, G. – Condón, S. – Maňas, P.: Physiology of the inactivation of vegetative bacteria by thermal treatment: Mode of action, influence of environmental factors and inactivation kinetics. Foods, *6*, 107. DOI: 10.3390/foods6120107.
32. Adams, M. R. – Moss, O. M.: Factors affecting the growth and survival of micro-organisms in foods. In: Adams, M. R. – Moss, O. M. (Eds.): Food microbiology. 3rd ed. Cambridge : RSC Publishing, 2008, pp. 20–62. ISBN: 978-0-85404-284-5. DOI: 10.1039/9781847557940-00020.
33. Jay, J. M. – Loessner, M. J. – Golden, D. A.: Food protection with high temperatures. In: Heldman, D. R. (Ed.): Modern food microbiology. 7th ed. New York : Springer Science+Business Media, 2005, pp. 415–442. ISBN: 0-387-23180-3.
34. Torreggiani, D. – Toledo, R. T.: Influence of sugars on heat inactivation, injury and repair of *Saccaromyces cerevisiae*. Journal of Food Science, *51*, 2006, pp. 211–215. DOI: 10.1111/j.1365-2621.1986.tb10872.x.
35. Ateş, O. – Oner, E. T.: Microbial xanthan, levan, gellan, and curdlan as food additives. In: Gupta, V. K. – Treichel, H. – Shapaval, V. – de Oliveira, L. A. – Tuohy, M. G. (Eds.): Microbial functional foods and nutraceuticals. Hoboken : John Wiley and Sons, 2018, pp. 149–174. ISBN: 9781119049012. DOI: 10.1002/9781119048961.ch7.
36. Sigha, T. K.: Microbial extracellular polymeric substances: production, isolation and application. IOSR Journal of Pharmacy, *2*, 2012, pp. 276–281. DOI: 10.9790/3013-0220276281.
37. Rajashekhara, E.: Influence of different rating media on thermal resistance of *Neosartorya fischeri*

isolated from papaya fruit. Journal of Applied Microbiology, *81*, 1996, pp. 337–340. DOI: 10.1111/j.1365-2672.1996.tb04336.x.

38. Rajashekhara, E. – Suresh, E. R. – Ethiraj, S.: Thermal death rate of ascospores of *Neosartorya fischeri* ATCC 200957 in the presence of organic acids and preservatives in fruit juices. Journal of Food Protection, *61*, 1998, pp. 1358–1362. DOI: 10.4315/0362-028X-61.10.1358.
39. Nemati, M. – Hamidi, A. – Dizaj, S. M. – Javaherzadeh, V. – Lotfipour, F.: An overview on novel microbial determination methods in pharmaceutical and food quality control. Advanced Pharmaceutical Bulletin, *6*, 2016, pp. 301–308. DOI: 10.15171/apb.2016.042.
40. Wyatt, T. T. – Leeuwen, R. – Golovina, E. A. – Hoekstra, F. A. – Kuenstner, E. J. – Palumbo, E. A. – Snyder, N. L. – Visagie, C. – Verkennis, A. – Hallsworth, J. E. – Wösten, H. A. B. – Dijksterhuis, J.: Functionality and prevalence of trehalose-based oligosaccharides as novel compatible solutes in ascospores of *Neosartorya fischeri* (*Aspergillus fischeri*) and other fungi. Environmental Microbiology, *17*, 2015, pp. 395–411. DOI: 10.1111/1462-2920.12558.

Received 27 March 2019; 1st revised 16 May 2019; 2nd revised 7 June 2019; accepted 11 June 2019; published online 10 September 2019.