

# **HEAT RESISTANT FUNGI**

**A literature study**

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# HEAT RESISTANT FUNGI

## A LITERATURE STUDY

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### INTRODUCTION

The most thermal resistant form of fungi is an ascospore. Vegetative cells of yeast, vegetative hyphae of moulds and asexual spores of moulds (sporangia and conidia) are not very heat resistant and are usually destroyed by pasteurizing processes at as low temperature as 60°C. Fungi, which produce ascospores, are classified within the class *Ascomycetes*. In this group of fungi are monocellular yeasts as well as hyphae-forming moulds. Ascospores are produced generally in groups of 8, very seldom in groups of 16 or 32. They are produced within a closed sac, the *ascus* (pl. *asci*), which is usually enclosed within larger bodies together with a large number of other asci. These bodies are diameter ranged between 40 and 80 µm and are called *fruit bodies* (*clestothecia*, *gymnothecia* etc.). Yeasts produce ascospores in free asci without fruit bodies. With exception of fungi within genus *Byssoschlamys*, where asci are borne singly, stalked to the aerial hyphae, all other moulds produce asci enclosed within fruit bodies.

Studies of heat resistance have concentrated on a little number of species, which cause large spoiling in the food industry. Investigation of heat resistance of *Byssoschlamys species* is very frequently reported in the literature. Information on this topic has been comprehensively reviewed by Beuchat and Rice (1979). Also fungi belonging to genus *Neosartorya*, which are often isolated from heat processed foods have high heat resistance (3, 5, 6 and 7). Other fungi, which less frequently cause spoilage in food, but produce ascospores with high heat resistance are *Talaromyces spp.* and *Eupenicillium spp.* (3, 10, 11 and 12). A special group of ascospore-forming fungi is yeast. Ascospores, which are produced by yeasts, have usually lower heat resistance than ascospores, which are produced by moulds (14, 15 and 16).

### ASCOSPORES PRODUCED BY YEAST

Heat resistance of ascospores, which are produced by yeast, proved to be 30-350 fold higher than the heat resistance of the corresponding vegetative cells (14 and 15), but much lower than the heat resistance of ascospores, which are produced by moulds (14, 15 and 16). Put and De Jong (1982) investigated heat resistance of ascospores from 21 strains of four different species of *Saccharomyces* and two different species of *Kluyveromyces* at 60°C and found the following D-values (Decimal reduction time value).

<i>Kluyvermocyces</i>	$D_{60^{\circ}} = 20 - 40 \text{ min}$
<i>Saccharomyces</i>	$D_{60^{\circ}} = 5.1 - 19.2 \text{ min}$
vegetative cells	$D_{60^{\circ}} = 0.1 - 0.3 \text{ min}$

In another work (15) Put and De Jong studied the thermal resistance of ascospores produced by four different species of *Saccharomyces* (*S. bailii*, *S. cerevisiae*, *S. chevalieri* and *S. uvarum*). They found  $D_{60^{\circ}}$ -values in the range between 1.0 and 22.2 min and z-values in the range between 4.0 and 6.5°C. Most heat resistant of the investigated species were *S. bailii* and *S. cerevisiae*.

They also noticed that ascospores of *Saccharomyces spp.* cannot be stored in water suspension at 5°C for a longer period then 2-3 weeks without loss of heat resistance. Su *et al.* (1985) studied the development of ascospores in McClary's acetate broth in the presence of two different sporulation inhibitors, ethanol and erythromycin. The presence of ethanol in growth media caused the increasing of heat resistance of the cells. They found a relationship between the content of fatty acids and chitin in the composition of cell walls and the sporulation degree. High content of triglycerides was associated with the initiation of sporulation. Increasing of the ratio between saturated and unsaturated fatty acids in triglycerides resulted in higher heat resistance of ascospores.

Put and De Jong also discussed the possibility of separation of ascospores from asci and vegetative cells using enzymes to digest the ascus wall with subsequent gradient centrifugation.

## ASCOSPORES PRODUCED BY MOULDS

Ascospores, produced by moulds, is the most heat resistant fungal form. A number of different factors such as composition of growth medium, the phase of growth medium (solid or liquid), incubation temperature, incubation time, pH of growth medium and ascospore age have an influence on heat resistance of ascospores. Another important factor, which has an effect on heat resistance is the composition of heating medium (Conner and Beuchat, 1987; King and Whitehand, 1990).

### Procedure for harvesting of ascospores

In the beginning of the preparation the fungal mass is a mixture of all different fungal forms as fragments of hyphae, asexual conidia, fruit bodies, free asci and free ascospores. The most of the hyphae can be removed from the mixture by filtration through sterile cheesecloth (Bayne and Michener, 1979) or sterile glass wool (Banner *et al.*, 1979). The rest of the hyphae fragments and vegetative conidia can be thermally destroyed at relatively low temperature such as 60°C. The heat transport to ascospores within fruit bodies is completely different from the heat transport to ascospores within free asci and both heat transports are different from the heat transport to free ascospores. Ratio between concentration of fruit bodies, free asci and free ascospores vary strongly from batch to batch. It is therefore very important to liberate as many ascospores as possible to separate ascospores from the rest of fruit bodies and free asci and to obtain nearly 100% free ascospores.

Conner and Beuchat (5) used sonication treatment of spore suspension to release ascospores of *Neosartorya fischeri* from asci. Another method for the liberation of ascospores of *Byssosclamyces fulva* from asci was used by Michener and King (1974). The mixture of asci and free ascospores was run through a French pressure cell with high presser (about 8 000 lb/inch<sup>2</sup>). The same harvesting treatment was later used for the destruction of walls and fruit bodies and asci and for the liberation of ascospores of *Talaromyces flavus* by King *et al.* (11, 12).

### **Genus *Byssochlamys***

Genus *Byssochlamys* has 25 different species. The anamorphic stages constitute genus *Paecilomyces*. The most heat resistant species is *Byssochlamys fulva*. Ascospores of that species appear to be among the most heat resistant fungal spores. Heat resistance can vary widely from isolate to isolate (Bayne and Michener, 1979; Hatcher *et al.*, 1979). Relationship between the chemical composition of ascospores and their heat resistance was studied by Banner *et al.* (1979). Cleaned spore suspension was analyzed for proteins, amino acids, fatty acids, lipids and minerals. The most significant difference was a higher quantity of fatty acids with the chain containing more than C<sub>20</sub> in the more heat resistant spores.

Thermal destruction of ascospores of two different strains *B. fulva* in 5° Brix Concord grape juice at 85°C was studied by Banner *et al.* (1979). D-values for 25 strains of *B. fulva* and *B. nivea* in a standard defined medium at 90°C and two different pH-values (3.5 and 5.0) were determined by Bayne and Michener (1979). They produced ascospores by cultivation of *B. spp.* for 30 days at 30°C in a broth medium containing sucrose as the primary carbohydrate source. D<sub>90°</sub>-values of ascospores range between 4 and 36 minutes.

The effect of cultivation temperature and percent soluble solids in the growth media (°Brix) on the detection of heat resistant species of *Byssochlamys* was described by Hatcher *et al.* (1979). They also studied thermal death of ascospores, produced by three isolates of *Byssocyhlamys*. The obtained z-values varied widely (7°, 9.5° and 14°F).

### **Genus *Talaromyces***

The anamorphic stages constitute genus *Penicillium*. *Talaromyces* is known for the production of yellow or white fruit bodies with fine structure of small hyphae on the outside (gymnothecium). The most common species is *Talaromyces flavus*. It is significant that all known heat resistant isolates have larger ascospores than normal (3 and 10). The heat resistance of ascospores of *T. flavus* is lower than the heat resistance of ascospores of *Byssochlamys spp.* or *Neosartorya fischeri* (3, 10, 11

and 12). The influence of growth conditions (phase of growth medium, cultivation temperature and time) and the influence of heating conditions such as composition of heating medium (brix, organic acids and pH) on heat resistance of ascospores of *T. flavus* was studied by King and Whitehand (12). They found  $D_{80^\circ}$ -value 191 min and  $D_{90^\circ}$ -value 6 min. Heat resistance increased with increased brix in the heating medium. Growth and sporulation at 20°C and 35°C resulted in higher heat resistance of ascospores than growth and sporulation at 30°C. Growth on solid medium resulted in higher heat resistance than growth in liquid medium.

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