Patterns of activation of *Neosartorya fischeri* and *Talaromyces flavus* ascospores in phosphate buffers (pH6 and 7) and in distilled water at temperatures 70^0, 75^0 and 80^0C.

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Abstract: Activation of ascospores of the two strains of *N. fischeri* designated E7 and 3C and two strains of *T. flavus* designated 2E and 5A were studied at three different temperatures (70^o ,75^o and 80^oC) in 0.1 phosphate and compared to enumeration in the haemocytometer. The optimum temperature/time for activation of *N. fischeri* (E7 and 3C) were75^o/50min or 80^o/30min in PB(pH7) and 75^o/30min or 80^o/10min in PB(pH7) respectively. For *T. flavus* (5A and 2E) the optimum temperature/time for activation were 80^o/150min in PB (pH7) and 80^o/130min in PB (pH6) respectively. There are some very practical implications which can be drawn from these activation studies. The heat processing schedule used to manufacture some types of fruit products may result in activation of dormant mold ascospores. Low recoveries may indicate that the many spores were truly non-viable or that they are merely highly dormant and could not be activated by our activation and cultural conditions and that the means for breaking this dormancy remains to be discovered.

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Introduction

As with many other microbial spores, ascospores of heat resistant fungi (HRF) possess a dormancy that can be broken by heat treatment. Heat activation of ascospores to interrupt dormancy and increase percent germination has become a common practice. Germination is the process by which a spore is transformed from a dormant state of metabolic activity to one of high activity. Gillespy (1938) obtained the highest percentage germination of *Byssochlamys fulva* ascospores by heating at 75^oC for 10min. Hull (1939) recognised that unheated ascospores either do not germinate or do so sporadically. Ascospores of *Talaromyes flavus* require heat activation for maximum outgrowth and the time for activation was reduced as the heating temperature increased (King and Halbrook, 1987). *Neosartorya fischeri* has also been reported to show enhanced ascospore germination when heat shocked prior to introduction into culture media (Beuchat, 1986).

Several temperature/time combinations have been used by scientists for activation. To determine whether better recoveries could be achieved by using different periods and temperatures for activation, 10 strains of *Neosartorya* were heated at 70^o and 75^oC for 30, 60, 90 and 120 min and at 85^oC for 15, 30, 45 and 60min (Splittstoesser *et al*., 1993). The results showed that 70^oC for 30 and 60 min generally gave similar recoveries and that raising the temperature to 75^o and 80^oC did not activate more spores. Eight of ascospore suspension gave lower counts when heated longer than 15min at 80^oC. Apparently 80^oC was a temperature where lethal activity occurred. Splittstoesser *et al*. (1969, 1970) reported similar results using cultures of *Byssochlamys*. At 80^oC, the counts were usually were lower than at 70^oC indicating that this temperature was lethal for the ascospores of many strains. It was concluded from these that 70^oC was the optimal activation temperature. Contrarily, Gillespy (1938) obtained the highest percentage of germination when a suspension of ascospores of *B. fulva* was heated for 10min at 75^oC in potato-sucrose medium and Hull (1939) found that activation was most extensive when ascospores were heated for 10 min at 75 to 80^oC.

Rajashekhara *et al*. (1996) reported 80°C for 15-30min as optimum heat activation temperature/time combination for ascospores of a strain of *N. fischeri* isolated from spoiled papaya fruit.

Studies on the breaking of dormancy of *B. fulva* have shown an interaction between temperature of heat shock and the nature of suspending medium (Splittstoesser *et al*., 1970). The influence of the suspending medium was less pronounced when the spores were heated at higher temperatures (70° to 80°C). When spores in Concord grape juice were heated at 60°C, activation was completed in 60min or less, whereas heating in water for as long as 3hrs produced no detectable increase in the viable count. Activation was enhanced even more in solution containing NaCl (Splittstoesser *et al*., 1972). Another study carried out by Splittstoesser *et al*. (1977) showed an interaction between temperature and heating medium and species. Maximal activation of *B. fulva* and *N. fischeri var glaber* ascospores were obtained by heating at 70°C for 30min in grape juice; in distilled water, 120 min were required for *B. fulva* while only 1% of the *N. fischeri* ascospores were activated. The effect of pH on activation was also studied by Splittstoesser *et al*. (1972) using HCl-NaCl (O.1N each) solution adjusted with 6N NaOH. Relatively small differences had a major effect; raising the pH only 0.4 units from 1.0 to 1.4 resulted in almost a 90% decrease in number of spores activated. Above pH 1.6, viable counts were similar to water controls indicating that activation no longer was benefited.

In an experiment carried out by Beuchat (1986) on activation it was shown that different species and also different strains within the same species might require different treatment times and temperatures to achieve maximal activation. The ascospores of *Byssochlamys spp* exhibited considerable difference compared to ascospores of *T. flavus* and *N. fischeri.* While activation of *B. nivea* and *B. fulva* ascospores was achieved within 30 min at 70°C and 75°C followed by complete inactivation at 90min, a large percentage of ascospores of *Talaromyces* and *Neosartorya* were not activated even though they were viable. This was true even when ascospores were heated for as long as 120 min. The optimum temperatures tested for two strains of *T. flavus* NFPA-2 and CBS317-63 were 85°C for 15-30 min or 80°C for ≥ 45-120 min for NFPA-2 and ≥ 90-120 min for CBS317-63. The optimum temperature for activating the third strain of FRR-1265 was 70°C for 45 min. For the three strains of *N. fischeri* FRR-1833, FRR 2334 and 110483, ascospores of strain FRR-2334 required the highest temperatures for activation. Maximum activation occurred only at 85°C for 15-105 min. Heating at 70° and 75°C failed to activate ascospores of this strain. Strain FRR-1833 behaved somewhat differently in that all test temperatures (70°, 75°, 80° and 85°C) activated ascospores in a similar fashion. Likewise, ascospores of strain 110483 were activated similarly at all temperatures. However, treatment at 85°C for ≥ 45 min caused a rapid decline in viable ascospores. Ascospores heated at 70°, 75° and 80°C for longer than 45 min lost viability, but at a much slower rate than at 85°C.

Repeated activation trials conducted on three ascospore crops showed marked differences between crops as well as between strains of *Neosartorya* to the same activation treatment (Splittstoesser *et al*., 1993). Crop 1 ascospores were harvested from cultures grown 3-4 weeks at 30°C on oatmeal agar and stored in water at 5°C after harvest and sonication that lasted for 2min. Crop 2 ascospores were grown on a medium containing Bacto malt extract (pH5.5) and harvested after 30 days incubation at 30°C. The ascospores were stored at –15°C after 20 min sonication. Crop 3 ascospores were treated in a manner identical to the crop 2 ascospores except that the cultures were grown on oat meal-wheat germ agar. Crop I of *N. fenelliae* yielded recoveries that were ten fold higher than crops 3.

Materials and Methods

Production and harvesting of ascospores

The fungi used in this study were isolated from soils of south eastern/middle belt in Nigeria were identified as *N. fischeri* and *T. flavus* according to the methods described by Amaeze *et al*. (2010). Two strains were selected for each and designated as E7 and 3C for *N. fischeri* and as 5A and 2E for *T. flavus.* The test fungi were grown on potato dextrose agar in 500ml capacity universal bottles. The cultures were incubated at room temperature (28°-30°C) for 30 days to ensure ample time for ascospore formation and maturation. To harvest ascospores at the end of the incubation, slants were flooded with sterile deionized water and asci and/or cleistothecia were scrapped off the culture surface with sterile wire loop. The cleistothecia / asci suspension prepared were treated to release free ascospores using a Virsonic model 16-850 cell disrupter (The Virtis Company Gardiner New York). The suspensions were filtered. The suspensions were separately sonicated in a sterile 50ml beaker while being cooled by ice at 0-2°C with a 5 min treatment of 60w to disrupt the asci and cleistothecia and release the free ascospores using a Virsonic model 16-850 cell disrupter (The Virtis Company Gardiner New York). The suspensions

were filtered separately through sterile glass wool to remove hyphal fragments. After this, the ascospore suspensions were stored in sterile 0.2cm (diameter) glass beads in 50mls capacity universal bottles. The ascospore suspensions were stored at 4°C in the refrigerator.

Enumeration of ascospore

Direct microscopic counts were determined with a haemocytometer. Ascospores were readily recognizable and countable at 40-fold magnification. Direct microscopic counts were taken and total ascospore population/ml of sample was determined as described in the manual of haemocytometer.

Determination of optimum activation temperature and time

The temperature and time necessary for activation of ascospores were determined by a modification of the method described by Beuchat (1986). Phosphate buffers (PBs), pH 6 and 7 and distilled water adjusted to 70°, 75° or 80°C (9.9mls each) were inoculated with 0.1ml of the counted ascospores and completely dispersed by vigorous shaking. Samples were withdrawn from the continuously agitated suspension at 20min intervals over a period of 120min. Following activation, appropriate decimal dilutions were made in sterile water in test tubes maintained at room temperature. 0.1ml of the appropriate dilution was withdrawn and plated out by pour plate method using single strength potato dextrose agar (PDA). Incubation was carried out for 3-5 days and colonies were counted.

Results

Total count of ascospores (haemocytometer count)

Standardized ascospores population for each test organism is shown on Table 1.

Table 1 Haemocytometer counts of ascospores/ml

Isolate	No of ascospores /ml
N. fischeri (E7)	1.1×10^8 /ml
N. fischeri (3C)	8.2×10^7/m1
T. flavus (5A)	5.8×10^7/m1
T. flavus (2E)	1.3×10^7/m1

Activation Profile

Pattern of Activation of *N. fischeri* (E7) Ascospores in 0.1 M Phosphate Buffers (pH6 And 7) and Distilled Water at 70°C.

The pattern of activation of ascospores of E7 at 70°C is shown on fig 1 (and appendix 1 table i). Activation profiles were similar in all media and all the curves showed considerable fluctuations in viable population count. There was no indication of spore inactivation and heating for long periods (up to 150min) did not have any obvious effect on viable ascospore recovery except in distilled water. After heating for 70min in distilled water, the recovery of viable ascospore remained lower than the number obtained at 70min though irregular for the rest of the heating period. A comparison of direct microscopic ascospores count (table 1) with ascospore count as determined on PDA (appendix 1) revealed that the recovery of viable ascospores was low at this temperature. The highest number of viable ascospores (1.6×10^7) recovered following activation in PB (pH 6) after 130 min was only 15% of the population obtained in direct (microscopic) ascospores count. The heating medium appeared to have considerable impact on activation. Peak activation was attained earlier in distilled water than in the phosphate buffers where it was difficult to point at sharp peak activation. In PB (pH 6) the highest viable count was recorded after 130min of heating.

Pattern of Activation of *N.fischeri* (E7) Ascospores in 0.1M Phosphate Buffers (pH 6 and 7) and Distilled Water at 75°C.

The effects of heat at 75°C on ascospores of *N. fischeri* (E7) suspended in buffers, PB (pH 6 and 7) and distilled water for a period of 150min are shown in fig 2 (and on appendix 1 table ii). Peak activation was rapidly achieved after 50min in the phosphate buffers (fig 2). In water, the peak count of recoverable spores was recorded after 70min. A substantial increase in viable count was noticed at 75°C relative to 70°C. A higher peak recovery of 3.6 x 10^7 cfu/ml was obtained compared to 1.6 x 10^7cfu/ml obtained at 70^0C (an 18% increase). Peak population obtained at this temperature represents 33% of the total spores as counted in direct microscopy. The effect of the heating medium on the activation of ascospores was significant at this temperature as higher recoveries were obtained in the buffers than in distilled water. The profiles of activation in the phosphate buffers were similar. The population of viable counts declined after 50min probably due to spore inactivation. In water, lower counts were recorded after 70min. After 70min there were slight fluctuations in all the heating media

Pattern of Activation of *N. fischeri* (E7) Ascospores in 0.IM Phosphate Buffers (pH 6 and 7) and Distilled Water at 80°C.

The pattern of activation temperature to 80°C is shown in fig 3 (and on appendix 1 table iii). Raising the activation temperature did not significantly increase the percentage recoveries (appendix 1). Optimum activation leading to recovery of 3.6 x

10^7ascospores /ml was achieved in PB (pH7) after 30min which is comparable with the highest number obtained at 75°C in PB (pH7) after 50min of heating (table ii and fig 2). Thus the optimum temperature/time for activation of E7 is 75°C/ 50min or 80°C/ 30min in PB (pH 7). The effect /importance of heating medium on recovery of ascospores was apparent at 80°C as higher recoveries were obtained in the phosphate buffers that in distilled water. There was also a rapid reduction in viable counts in the phosphate buffers whereas in water reduction was gradual even though lower counts were recorded. The decline in counts of viable ascospores obtained after 30min in PBs and 50min in water was probably due to ascospore inactivation. The same was the case at 75°C and at 70°C. The highest recovery of ascospores made was low at 33% compared to the direct microscopic count.

When figs 1,2 and 3 were compared, it was clear that the fluctuations in the population of viable ascospores recovered reduced with increasing temperature

Pattern of Activation of *N. fischeri* (3C) Ascospores in Phosphate Buffers (pH 6 And 7) and Distilled Water at 70°C.

The pattern of activation of *N. fischeri* (3C) ascospores at 70°C is as shown in fig 4 (and on appendix 1 table iv). Recovery of viable ascospores fluctuated considerably for the duration of the activation. At 70°C, the composition of the heating medium affected the activation profile. Highest recoveries of viable ascospores were obtained in PB (pH6) followed by recoveries in PB (pH7) and the least in distilled water (Table iv). There was no indication of spore inactivation and heating for long periods of up to 130min did not seem to have any effect on viable ascospore recovery. Ascospore recovery was very low at this temperature. The highest viable count of spores (7.7×10^6/ml) represents only 9% of the direct microscopic count (8.2×10^7/ml)

Pattern of Activation of *N. fischeri* (3C) Ascospores in 0.IM Phosphate Buffers (pH6 and 7) and in Distilled Water at 75°C.

The pattern of activation of *N. fischeri* (3C) ascospores at 75°C is illustrated in fig 5 (and on appendix1 table v). Raising the activation temperature from 70°C to 75°C increased the recovery/activation of ascospores by 3%. The highest recovery of viable count was 9.9×10^6/ml in PB (pH7) after 30min. This represents only 12% of the direct microscopic count. In PB (pH6) and water, peak recoveries were obtained after 50min. Thus raising the activation temperature by 5°C did not only achieve higher yields of ascospores but also reduced the time at which the peak viable counts were recorded (at 70°C the highest count of 7.7×10^6/ml was recorded after 90min of heating).

The profiles were slightly irregular due to fluctuations in viable population count. For each curve, the count dropped after each peak count probably due to spore inactivation.

Pattern of Activation of *N. fischeri* (3C) Ascospores in Phosphate Buffers (pH6 and 7) and Distilled Water at 80°C.

The pattern of activation of *N. fischeri* (3C) ascospores at 80°C is shown in fig 6 (and on appendix 1 table vi). Peak activation was rapidly achieved in the three media after a shorter period of 10min. This was followed by a decline in viable ascospores count probably due to spore inactivation. The curves were however irregular due to fluctuation in the viable count. The highest viable ascospore count for *N. fischeri* (3C) was 1.0×10^7 at 80°C and was obtained after 10min (Table vi). This represents only 12% of the total haemocytometer count as was obtained at 75°C. Least recovery was obtained in distilled water followed by PB (pH6), while PB (pH7) had the highest recovery. The highest viable ascospores counts were less than the numbers obtained at 75°C in water and PB (pH6).

From the results, the optimum temperature/time for activation of *N. fischeri* ascospores (3C) was 80°C/10min in PB (pH7) or 75°C/30min in PB (pH7). The peak counts were similar at these temperature/time combinations.

Pattern of Activation of *T. flavus* (2E)Ascospores in Phosphate Buffers (pH7 and 6) and Distilled Water at 70°C.

The profile for activation of *T. flavus* (2E) ascospores at 70°C is shown in fig 7 (and on appendix 1 table vii). It is remarkable to note that after 10min of heating in the three media, there were no colony forming unit (cfu) probably due to the absence of activated spores. The number of viable spores (cfu) fluctuated considerably throughout the heating period. The highest ascospores recoveries achieved for *T. flavus* (2E) at 70°C was 1.4×10^4 in PB (pH6) and distilled water after 150min and 110min respectively. This number is just 0.01% of the direct microscopic count. The effect of pH was observed at this temperature as lowest recoveries were obtained in PB (pH7).

Pattern of Activation of *T. flavus* (2E) Ascospores in Phosphate Buffers (pH7 and 6) and Distilled Water at 75°C.

The pattern of activation of ascospores (2E) at 75°C is shown in fig 8 (and on appendix 1 table viii). Increasing the activation temperature from 70° to 75°C resulted in greater yields/recovery of viable ascospores especially after 90min when recovery sharply increased (fig 8). The cfu increased with activation period and continued for the duration of the activation study. The highest cfu was recorded after 190min in PB (pH6) as 6.0 x 10^4spores/ml. This however represents only 0.05% of the direct microscopic count. Better yields were obtained in PB (pH6) than distilled water and PB (pH7) indicating an influence of pH on activation. No viable spore was obtained after 10min of heating at 75°C as was the case following activation at 70°C showing that no spore activation had occurred.

Pattern of Activation of *T. flavus* (2E) Ascospores in Phosphate Buffers (pH6 and 7) and Distilled Water at 80°C.

The pattern of activation of ascospores (2E) at 80°C is shown on fig 9 (and on appendix 1 Table ix). When the activation temperature was increased to 80°C, the recovery of viable ascospores increased drastically compared to the results obtained at 70°C and 75°C. There was a ten thousand percent increase on the population obtained at 75°C and 70°C. This shows that temperature rather than time and heating medium composition is the principal component in activation of *T. flavus* ascospores. Activation was higher in the phosphate buffers (pH6 and 7) than in distilled water. Optimum temperature/time for activation of *T. flavus* ascospores (2E) was 80°C/150min in PB (pH7). Recoveries were however low compared with the haemocytometer count. The highest cfu obtained in the experiment (1.7 x 10^7 in PB (pH7) is only 14% of the direct microscopic count. The three curves in fig 9 followed a similar pattern showing physiologic uniformity. There was a gradual increase to peak counts followed by rapid decline in viable counts in three heating media. Beyond 170min in buffers and 150min in water inactivation of ascospores started.

Pattern of Activation of *T. flavus* (5A) Ascospores in Phosphate Buffers (pH7 and 6) and Distilled Water at 70°C.

The effects of heating ascospores of *T. flavus* (5A) in buffers, PBs (pH6 and 7) and distilled water for periods of time ranging to 150min at 70°C are shown in fig 10 (and on appendix 1 Table x). The curves on fig 10 were irregular due to fluctuations in viable population counts. The highest viable count obtained for *T. flavus* ascospores (5A) at 70°C was 2.4 x 10^4 in distilled water after 150min, which is only 0.04% of the direct miscroscopic count. The viable counts obtained in water and PB (pH6) was comparable. Lowest recoveries were obtained in PB (pH7). As was obtained with ascospores of *T. flavus* (2E) there was no cfu observed on PDA plates until after 30min of heating in the three media at 70°C.

Pattern of Activation of *T. flavus* (5A) Ascospores in Phosphate Buffers (pH7 and 6) and Distilled Water at 75°C.

The pattern of activation of ascospores of *T. flavus* (5A) is shown in fig 11 (and on table xi). Ascospores of *T. flavus* (5A) responded faster to heat than the ascospores of *T. flavus* (2E). Raising the activation temperature from 70°C to 75°C resulted in greater yields (10% increase) of viable ascospores of *T. flavus* (5A) (Table xi). The highest viable count achieved for *T. flavus* (5A) at 75°C was 8.0 x 10^4 in PB (pH6) which represents only 0.1% of the direct microscopic count, followed by 6.5 x 10^4 in PB (pH7) and the least in distilled water. At 75°C the buffers enhanced activation. Although the cfu fluctuated slightly counts increased with activation period.

Pattern of Activation of *T. flavus* (5A) Ascospores in Phosphate Buffers (pH6 And 7) and Distilled Water at 80°C.

The pattern of activation of *T. flavus* ascospores (5A) is illustrated in fig 12 (and on appendix 1 Table xii). Recovery of viable ascospores obtained at 80°C was very high compared to the results obtained at 70° and 75°C. There was over a ten thousand percent increase on the population obtained at 70°C and over a thousand percent increase on the population obtained at 75%. However recoveries were low when compared with the direct microscopic count. The highest cfu obtained was 1.46 x 10^7 in PB (pH6) after 130min that represents 25% of the direct microscopic count. Even though the curves were slightly irregular due to fluctuations in viable counts (fig 12) there was a gradual increase to peak counts followed by a decline in viable counts in three heating media. This shows physiologic uniformity of ascospore population in this specie. Higher recoveries (1.46 x 10^7 and 1.38 x 10^7) were obtained in the phosphate buffers pH6 and 7 respectively compared to the peak recovery of 1.29 x 10^7 in distilled water. Optimum temperature/time for activation of *T. flavus* (5A) was 180°C/130min in PB (pH6). Beyond 130min in buffers and 110min in water rapid inactivation of ascospores started.

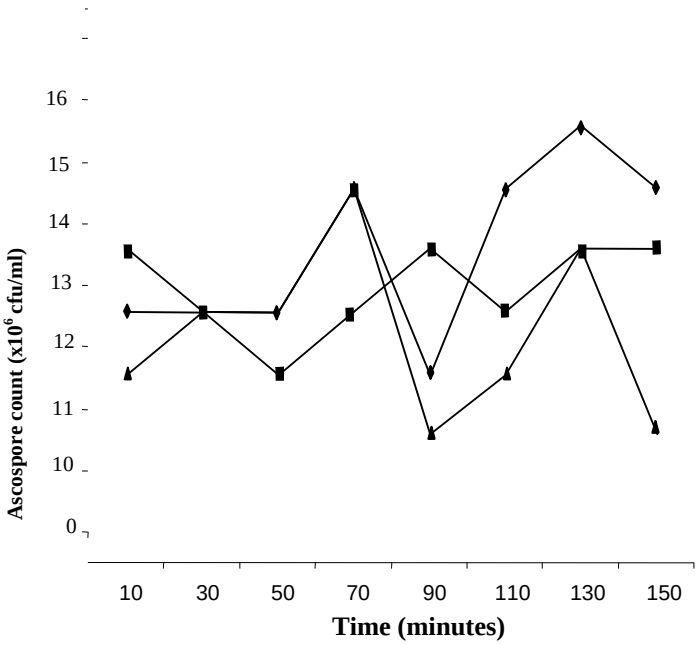

Fig 1: The pattern of activation of *N. fischeri* ascospores (E7) in PBs (pH 6 and 7) and distilled water at 70°C over a period of 150min

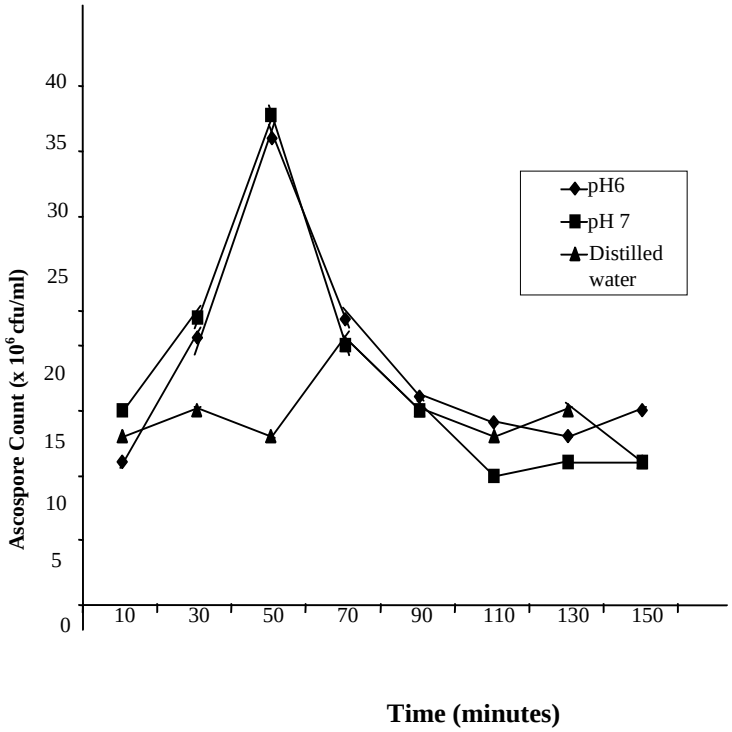

Fig.2: The pattern of activation of *N. fischeri* ascospores (E7) in PBs (pH 6 and 7) and distilled water at 75°C over a period of 150 min

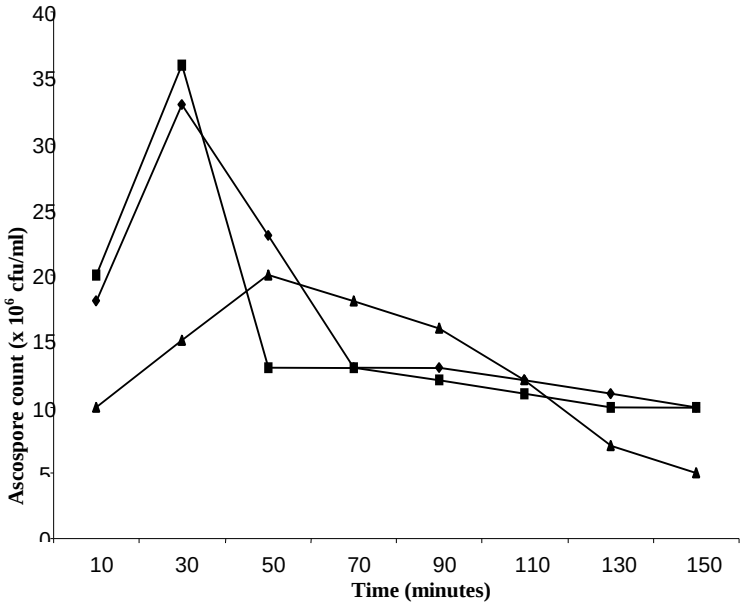

Fig. 3: The pattern of activation of *N. fischeri* ascospores (E7) in PBs (pH 6 and 7) and distilled water at 80° C over a period of 150 min

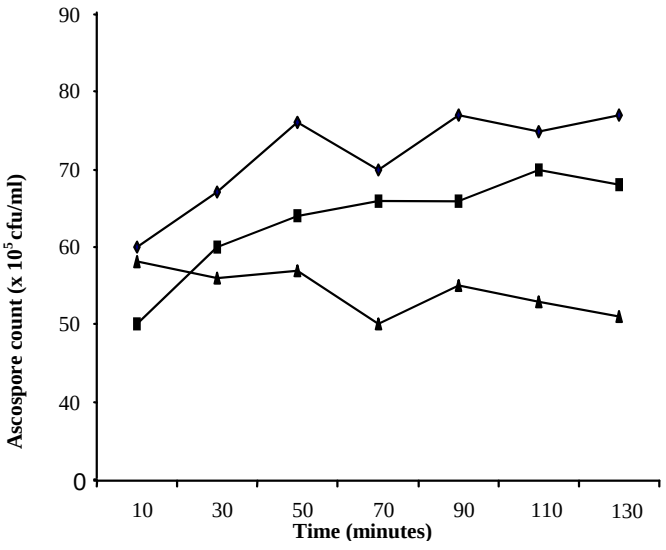

Fig. 4: The pattern of activation of *N. fischeri* ascospores (3C) in PBs (pH 6 and pH 7) and distilled water at 70°C over a period of 130 min

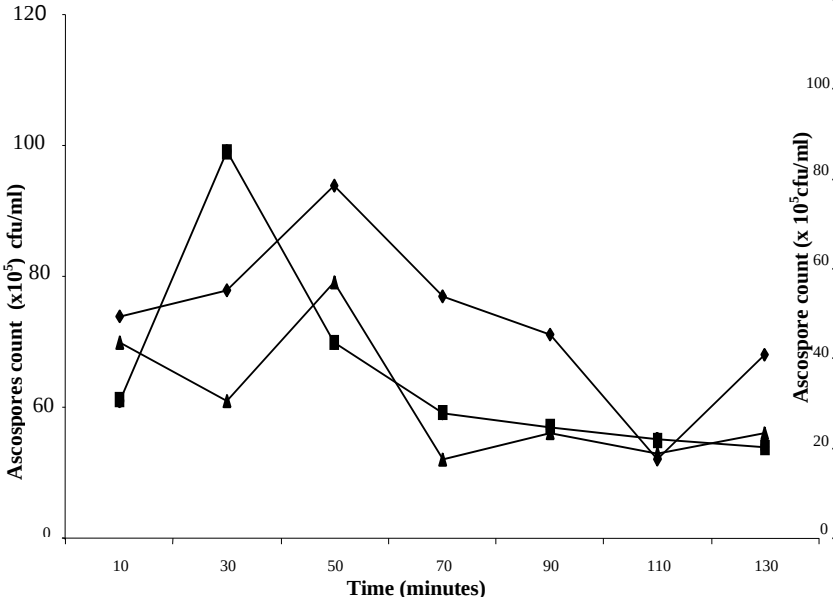

Fig. 5: The pattern of activation of *N. fischeri* ascospores (3C) in PBs (pH 6 and pH 7) and distilled water at 75^{o}C over a period of 130 min

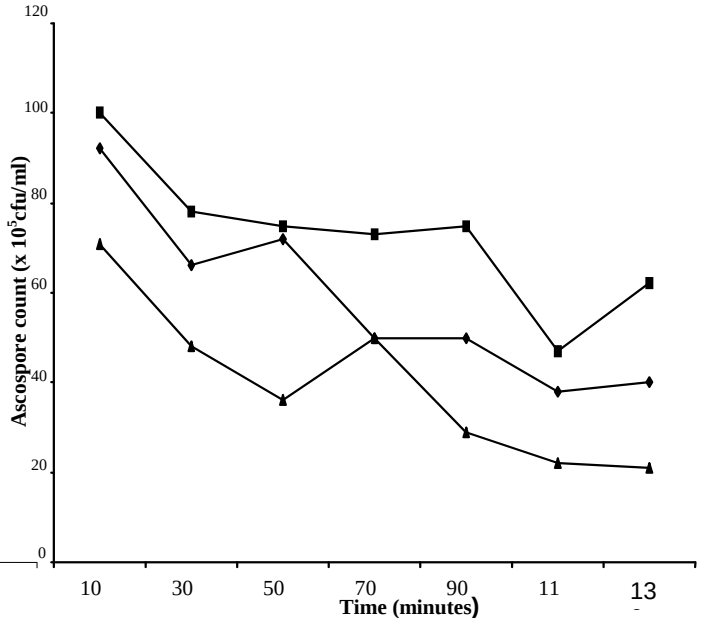

Fig.6: The pattern of activation of *N. fischeri* ascospores (3C) in PBs (pH 6 and pH 7) and distilled water at 80^{o}C over a period of 130 min

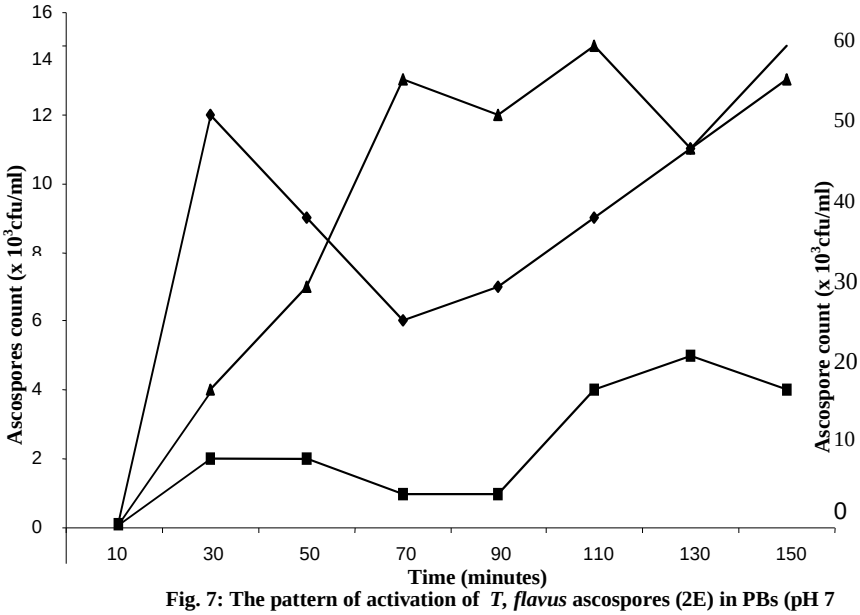

Fig. 7: The pattern of activation of *T, flavus* ascospores (2E) in PBs (pH 7 and pH 6) and distilled water at 70^{o}C over a period of 150 min

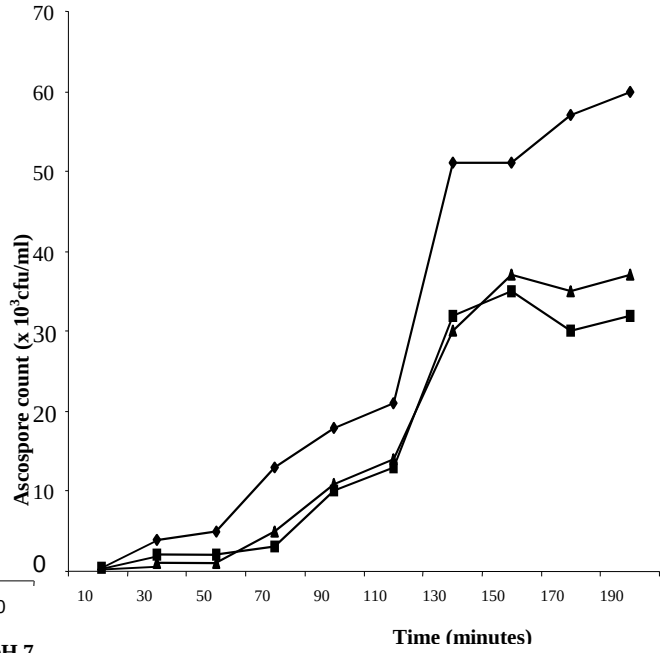

Fig. 8: The pattern of activation of *T. flavus* ascospores (2E) in PBs (pH 6 and pH 7) and distilled water at 75^{o}C over a period of 190 mins

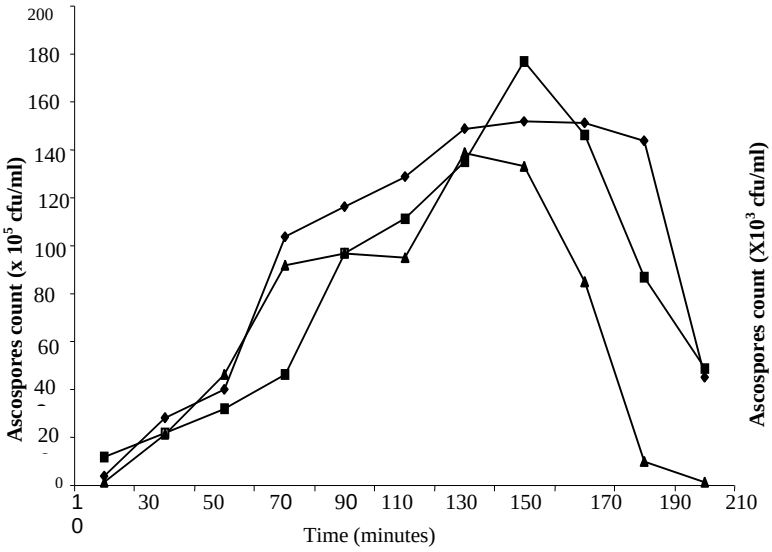

Fig. 9: The pattern of activation of *T. flavus* ascospores (2E) in PBs (pH 6 and pH 7) and distilled water at 80°C over a period of 210 min

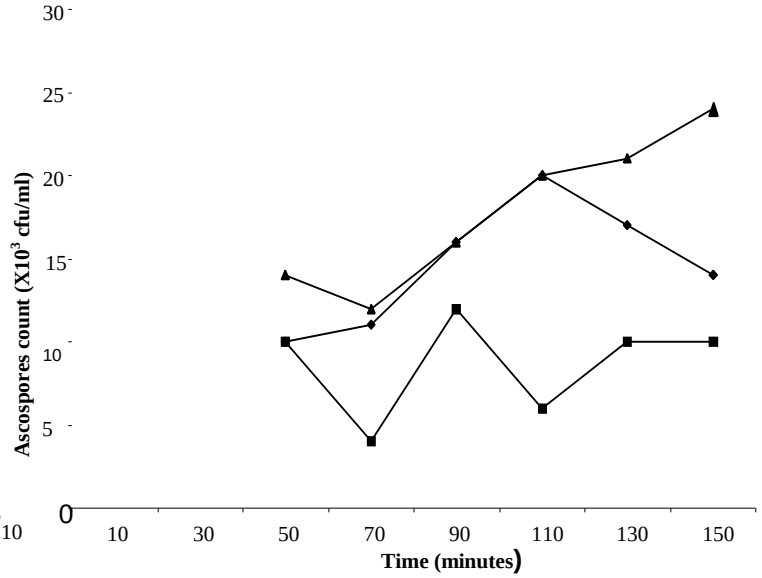

Fig. 10: The pattern of activation of *T. flavus* ascospores (5A) in PBs (pH 7 and pH 6) and distilled water at 70°C over a period of 150 min.

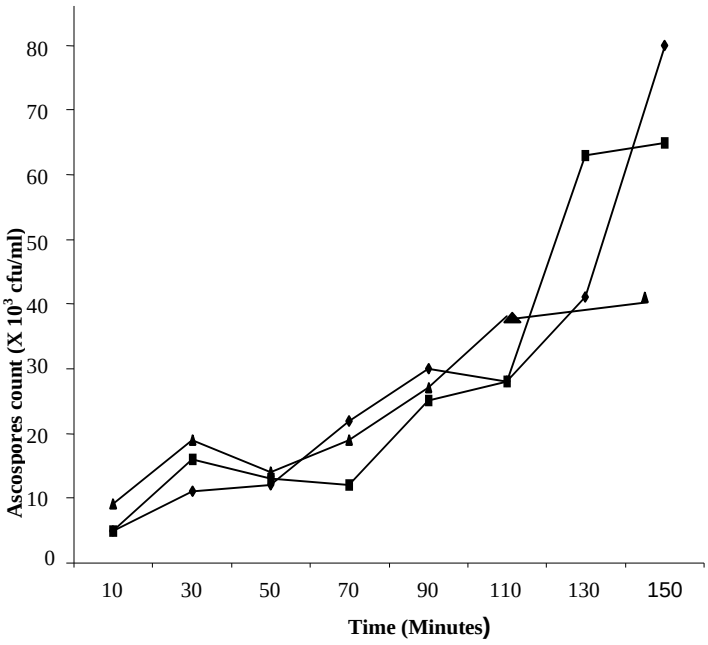

Fig. 11: The pattern of activation of *T. flavus* ascospores (5A) in PBs (pH 7 and pH 6) and distilled water at 75°C over a period of 150 mins

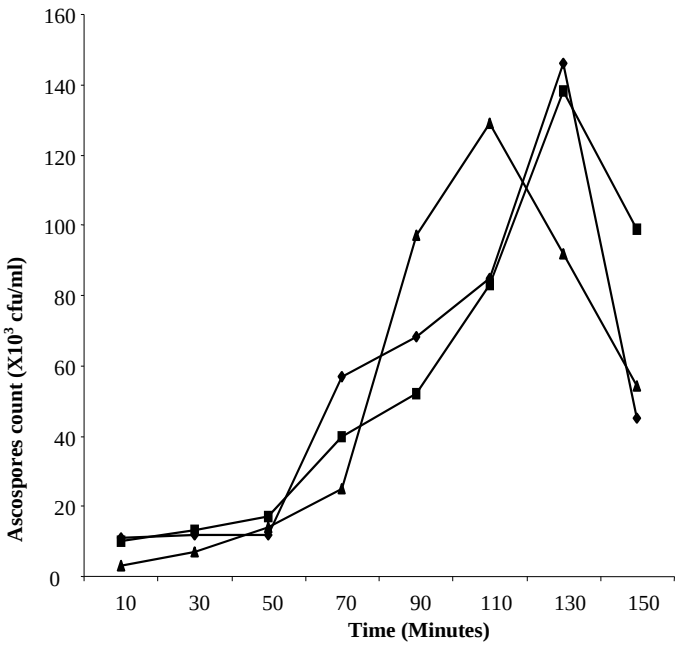

Fig. 12: The pattern of activation of *T. flavus ascospores* (5A) in PBs (pH 7 and pH 6) and distilled water at 80°C over a period of 150 min

Discussion

Repeated activation trials conducted on the ascospores of the four test isolates showed that dormacy of ascospores can be broken by heat and also marked differences in temperature/time combination for optimum recovery of ascospores. This is as a result of interactions between temperature, heating medium and specie/strain. This agrees with the findings of Beuchat, 1986; Splittstoesser *et al.*, 1993; Splittstoesser *et al.*, 1969, 1970 and 1977. The optimum temperature/time schedules for activation of *N. fischeri* (E7 and 3C) were 75°C/50min or 80°C/10min and 75°C/30min or 80°C/10 min respectively in PB (pH 7.0). Beuchat (1986) and Suresh *et al.* (1996) have reported that heating of ascospores of different strains of *N. fischeri* in phosphate buffers of pH 7.0 at 75°C for 15 to 60 min or at 80°C for 15 min was optimum for activation of ascospores. Rajashekhara *et al.* (1996) have also reported a similar schedule of 15-30min at 80°C as optimum heat activation temperature/time schedule for ascospores of a strain of *N. fischeri.* For *T. flavus* (5A and 2E) the optimum temperature/time schedules for activation were 80°C/150min in PB (pH 7.0) and 80°C/130min in PB (pH6.0). At 70° and 75°C, a very large percentage (> 99%) of the ascospores of *T. flavus* (2E) was not activated to the extent that they would not germinate and form colonies on PDA until after 10min. For *T. flavus* (5A) ascospores heating for 30min at 70^{o}C failed to activate ascospores. In Beuchat's work (1986) a similar behaviour and temperature/time schedule for activation of *T. flavus* strains NFPA-2 and CBS317-63 were noticed. This pattern of behaviour exhibited by ascospores of *T. flavus* marks its extraordinary resistance to heat compared to those of *N. fischeri.*

The general low yield of ascospores of *N. fischeri* and *T. flavus* compared to enumeration in the haemocytometer has also been reported by Splittstoesser *et al.* (1993). The non-uniform response of ascospores to heat shows heterogeneity in physiology in the ascospores. The low recoveries did not appear to be related to the strain or specie of the isolates. Low recoveries may indicate that the many spores were truly non-viable and thus do not have the potential to produce new vegetative growth. The other possibility is that they are merely highly dormant could not be activated by our activation and cultural conditions and that the means for breaking this dormancy remains to be discovered.

There are some very practical implications which can be drawn from these activation studies. The heat processing schedule used to manufacture some types of fruit products may result in activation of dormant mold ascospores. Data presented here from a limited number of strains nevertheless demonstrate a wide range of temperature/time combinations necessary for heat activation. This range encompasses processing schema necessary to achieve desired sensory characteristics while also inactivating microorganisms with less heat resistance. Thus, heat processing may also guarantee activation of ascospores of some strains of heat resistant molds.

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References

[1] Amaeze,N.J.,Ugwuanyi,J and Obeta, J.A.N. (2010).Studies of heat resistant in the soil:*Talaromyces flavus* isolated in Nigerian soils. *New York Sci. J.*, 3(12), 8-18.

[2] Beuchat,L.R.(1986).Extraordinary heat resistance of *Talaromyces flavus* and *Neosatorya fischeri* ascospores in fruit product. *J. of Food Sci.*, 51, 1506- 1510.

[3] Gillespy, T.G. (1938). Studies on the mould *Byssochlamysfulva.*Ann.Report.Univ.fruit Bristol.Campden, England. Pp 67-68.

[4] Hull, R. (1939). Study of *Byssochlamys fulva Appl. Bio*, 26, 800-822.

[5] King, A.D.Jr. and Halbrook, W.U. (1987). Ascospores heat resistance and control measures of *Talaromyces flavus* isolated from fruit juice concentrate. *J.Food Sci.*, 52, 1252-1254, 1266.

[6] Rajashekhara, E., Suresh, E.R. and Ethiraj, S. (1996]. Influence of different heating media on thermal resistance of *Neosartorya fischeri* from papaya fruit. *J. Appl. Bacteriol.*, 81, 337-340.

[7] Splittstoesser, D.F., Nielsen, P.V. and Churey, J.J. (1993). Detection of viable ascospores of *Neosartorya. J. of Food Prot.*, 56 ,599-603.

[8] Splittstoesser, D.F., Cadwell, M.C.and Martin, M .(1969). Ascospore production of *Byssochlamys fulva. J. Food Sci*, 34,248- 250.

[9] Splittstoesser, D.F, Wilkson, M. and Harison, W. (1972).Heat activation of *Byssochlamys fulva* ascospores. *J. Milk Food Tech*, 35,399- 401.

[10] Splittstoesser, D.F., Kuss, F.R. and Harrison, W. (1970).Enumeration of *Byssochlamys* and other heat resistant molds. *Applied Microbiol.*,20, 393-397.

[11] Splittstoesser,D.F. and Splittstoesser,C.M.(1977) .Ascospores of *Byssochlamys fulva* compared with those of a heat resistant *Aspergillus. J. Food Sci.* 4,685-688.

[12] Suresh, E.R., Ethiraj,S. and Jayaram,H.L.(1996). Heat resistance of *Neosartorya fischeri* isolated from grapes. *J. of Food Sci. and Tech.-mysore*, 33, 76- 78.

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Appendix 1

Table i: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (E7) at 70^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	13	14	12
30min	13	13	13
50min	13	12	13
70min	15	13	15
90min	12	14	11
110min	15	13	12
130min	16	14	14
150min	15	14	11

Table ii: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (E7) at 75^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	11	15	13
30min	20	22	15
50min	35	36	13
70min	22	20	20
90min	16	15	15
110min	14	10	13
130min	13	11	15
150min	15	11	11

Table iii: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (E7) at 80^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	18	20	10
30min	33	36	15
50min	23	13	20
70min	13	13	18
90min	13	12	16
110min	12	11	12
130min	11	10	7
150min	10	10	5

Table iv: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (3C) at 70^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	60	50	58
30min	67	60	56
50min	76	64	57
70min	70	66	50
90min	77	66	55
110min	75	70	53
130min	77	68	51

Table v: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (3C) at 75^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	74	61	70
30min	78	99	61
50min	94	70	79
70min	77	59	52
90min	71	57	56
110min	52	55	53
130min	68	54	56

Table vi: Population count (x 10^6cfu^{-ml}) in different treatment media for *N. fischeri* ascospores (3C) at 80^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	92	100	71
30min	66	78	48
50min	72	75	36
70min	50	73	50
90min	50	75	29
110min	38	47	22
130min	40	62	21

Table vii: Population count (x 10^6cfu^{-ml}) in different treatment media for *T. flavus* ascospores (2E) at 70^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	-	-	-
30min	12	2	4
50min	9	2	7
70min	6	1	13
90min	7	1	12
110min	9	4	14
130min	11	5	11
150min	14	4	13

Table viii: Population count (x 10^6cfu^{-ml}) in different treatment media for *T. flavus* ascospores (2E) at 75^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	-	-	-
30min	4	2	1
50min	5	2	1
70min	13	3	5
90min	18	10	1
110min	21	13	14
130min	51	32	30
150min	51	35	37
170min	57	30	35
190min	60	32	37

Table ix: Population count (x 10^6cfu^{-ml}) in different treatment media for *T. flavus* ascospores (2E) at 80^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	4	12	1
30min	28	22	29
50min	40	32	46
70min	104	46	92
90min	116	97	97
110min	129	11	95
130min	149	135	139
150min	152	177	133
170min	151	146	85
190min	144	87	10
210min	45	49	1

Table x: Population count (x 10^6cfu^{-ml}) in different treatment media for *T. flavus* ascospores (5A) at 70^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	-	-	-
30min	-	-	-
50min	10	10	14
70min	11	4	12
90min	16	12	16
110min	20	6	20
130min	17	10	21
150min	14	10	24

Table xi: Population count (x 10^6cfu^{-ml}) in different treatment mediafo *T. flavus* ascospores (5A) at 75^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	5	5	9
30min	11	16	19
50min	12	13	14
70min	22	12	19
90min	30	25	27
110min	28	28	38
130min	41	63	30
150min	80	65	32

Table xii: Population count (x 10^6cfu^{-ml}) in different treatment media for *T. flavus* ascospores (5A) at 80^0C.

Treatment time	PB(pH6)	PB(pH7)	Distilled water
10min	11	10	3
30min	12	13	7
50min	12	17	14
70min	57	40	25
90min	58	52	97
110min	85	83	29
130min	14	38	92
150min	45	99	59