Heat Resistance And Inactivation of *Neosartorya fischeri* and *Talaromyces flavus* ascospores in water, phosphate buffers, fruit juices and fruit juices fortified with sugars and preservatives.

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Abstract: The work was designed to the study some heat resistance characteristics of the isolates obtained in relation to fruit processing parameters. The effect of heat (85^0, 90^0, 92.5^0 and 95^0C) in combination with common fruit products preservatives (sodium benzoate and citric acid) and sugars (fructose and sucrose) on inactivation of ascospores of *N. fischeri* and *T. flavus* were studied in water, phosphate buffers, grapefruit, orange and pineapple juices. The rate of thermal inactivation was highest in medium containing sodium benzoate. The presence of sugars (sucrose and fructose) in juices protected the ascospores against heat inactivation when compared with plain juices. Ascospores of *T. flavus* had $D_{85}{}^0{}_C$ values which ranged from 10.6 to 78.9min; $D_{90}{}^0{}_C$ values of <1 to 13.6min and $D_{92.5}{}^0{}_C$ values of <1 to 8.1min. Ascospores of *N. fischeri* had $D_{85}{}^0{}_C$ values which ranged from 61.9 to 217.5min; $D_{90}{}^0{}_C$ values of 5.0 to 62.6min; $D_{92.5}{}^0{}_C$ values of 1.1 to 19.7min and $D_{95}{}^0{}_C$ values of <1 to 13.6min. These results mark the high heat resistance of ascospores of heat resistant fungi isolated from the tropics. Heat resistant fungi not only cause spoilage of fruit products but also produce highly toxic and sometimes carcinogenic compounds; these findings are of utmost importance to fruit processing industries which are presently increasing in number in Nigeria and other developing countries.

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Introduction

Ascospores of HRF vary in their capacity to withstand thermal treatment. Heat resistance is influenced by many factors that include species, strain, nature of heating menstrum, the temperature of treatment, age, nature of suspending/storage medium and the presence of anti-mycotic agents among others. Ascospores from different cultures differ in heat resistance even though they represent same specie and are grown under similar conditions (King and Whitehand, 1990).

This work was designed to study some heat resistance characteristics of the heat resistant fungi isolated from the soils of vegetable and fruit producing zones of South Eastern/Middle belt in Nigeria in relation to fruit processing parameters.

Materials and methods

Isolation and Preparation of ascospores

Heat resistance fungi were isolated from soil samples were collected from gardens ,orchards and farmlands in South Eastern/ Middle belt zones in Nigeria according to the method of Okagbue (1989) (Amaeze *et al*, 2010). Large amounts of fully developed and mature ascospores were obtained by inoculating selected isolates on PDA slants and incubating for 30 days at room temperature (Splittstoesser *et al*., 1970, 1971; Splittstoesser and Splittstoesser, 1977; Amaeze *et al*, 2010). Ascospores in asci or cleistothecia were then dislodged by flooding each slant with 2-3ml of sterile 0.1M potassium phosphate buffer (PB), pH 6.0, followed by scrapping the culture surface with a sterile wire loop. Heat resistance was confirmed by heating ascospores for 1hour at 70^oC with the water level in the bath at least 3cm above the level of suspension in the tubes. The heating achieved inactivation of mycelia and conidia and activation of ascospores if any heat resistant ones were produced. Fungal growths, which occurred after the incubation, were considered to be heat resistant fungi. The colonies were further purified on PDA plates and transferred to slide cultures for preliminary identification (Amaeze *et al*., 2010). Cultures were also put on slants of PDA for preservation and deposited with Prof. J.O. Ugwuanyi of the Department of Microbiology, University of Nigeria, Nsukka.

Pure PDA cultures of representative confirmed heat heat resistant isolates (3C, E7, 2E and 5A), were inoculated onto slants of PDA in 50ml capacity universal bottles. The cultures were incubated at room temperature (28^o-30^oC) 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 scraping was poured off into another 50ml universal bottle. The procedure was repeated several times to achieve 40ml of asci/cleistothecia suspension and also ensure complete harvest of spores. The cleistothecia / asci suspension

prepared were treated to release free ascospores. The suspensions were separately sonicated in a sterile 50ml beaker while being cooled by ice at 0-2^{0}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 to minimize clumping in 50mls capacity universal bottles. Ascospore suspensions were stored at 4^{o}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.

2.4. 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 buffer, pH 6 and 7 and distilled water adjusted to 70^o, 75^o or 80^{o}C (9.9mls each) were inoculated with 0.1ml of the counted ascospores and completely dispersed by vigorous shaking in sterile 10mm wide and 20ml capacity screw capped test tube . 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 PDA. Incubation was carried out for 3-5 days and colonies were counted.

Preparation of fruit juices

Ripe healthy pineapple, grapefruit and orange fruits were purchased from Nsukka main market, Nsukka Local Government Area, Enugu State, Nigeria. The fruits were kept at room temperature in the laboratory to soften over 5 days in a clean insect proof chamber. The fruits then washed under running tap water with the aid of a soft brush. The fruits were then rinsed in sterile tap water. For the preparation of each fruit juice, the fruits were cut into 4 quarters longitudinally and vertically. The pieces were then squeezed into sterile 100ml beakers. The juice was then allowed to stand for 5-10mins to enable coarse particles to settle at the bottom of the beaker leaving a clear juice. The clear juice was filtered through sterile filter cloth and collected in a 250ml screw capped conical flask and stored at –5^{o}C in a freezer. 10mls of the juice was removed for determination of pH and total soluble carbohydrate before storage. The stored juice was used as such without any further treatment.

pH of the various fruit juices

The pH values of the three locally prepared fruit juices were determined using a pH meter within the hour of the juices preparation. The pH meter used was model 290mk 2 (PYE UNICAM).

Total soluble carbohydrate content in prepared fruit juices

Total soluble carbohydrate was determined in the fruit juices using the anthrone reagents method (Deriaz, 1961)

Preparation of anthrone's of reagent

Anthrone reagent was prepared by stirring in 760ml of sulphuric acid (approx 98% w/w H_2S0_4) into 330ml of water. The mixture was cooled and 1g each of thiourea and anthrone were added and stirred until dissolved. The mixture was stored in a refrigerator until needed. Immediately before use, 0.4g of anhydrous D (+)-glucose was dissolved in tap water and diluted to 500ml. 0, 5, 10, 15, 20 and 25ml of glucose stock solution were measured into 100ml graduated flasks and diluted to 100ml.

To 2ml of appropriately diluted juice and glucose solutions in test tubes, 10ml of anthrone reagent was rapidly added and mixed by inversion. The test tube was loosely covered with a glass bulb stopper and immediately placed in a boiling water bath for 20min. The mixture was cooled and the absorbance measured in a 10mm optical cell at 620nm in a digital photocolorimeter (Milton Roy). A standard curve of absorbance (nm) against concentration of glucose (mg/ml) was plotted from which total soluble carbohydrate of the juices were deduced.

Thermal resistance of ascospores

Thermal resistance/inactivation of ascospores in locally prepared fruit juices water and buffers

Thermal inactivation characteristics (D-values) of each test mold were investigated in fruit juices, water and acetate buffers (AB) prepared to correspond to the pH of the various fruit juices. 0. 1ml of the counted ascospores was added to 9.9mls each juice or buffer in sterile 10mm wide and 20ml capacity screw capped test tube adjusted to the desired temperature (85^o, 90^o, 92.5^o or 95^{o}C) in water bath. A similar test tube with 10ml of distilled water with a thermometer as a control to ensure that the desired temperature was obtained before addition of ascospores and during the investigation. For *N. fischeri* (3C and E7) the selected sampling intervals/duration of heating was 20min for 480min at 85^{o}C; 5 or 10min for 140min at 90^{o}C; 3min for 24min at 92.5^{o}C respectively. For *T. flavus* (2E and 5A) the selected sampling intervals/duration for heating was

10min at 85°C and 3min for 30min at 90° and 92.5°C. At sampling intervals, heating mixtures were agitated with the sterile micropipette inside the waterbath and 0.1ml was withdrawn into sterile 4.9ml of water in test tubes maintained at room temperature. O.1ml of each sample was withdrawn and plated out by pour plate method using single strength PDA. Plates were incubated at room temperature for 3-5 days before mold colonies were counted.. The decimal reduction values were calculated using the equation

$$D_{\text{-value}} = \frac{t}{(\log a - \log b)}$$ (Hersom and Hulland, 1969)

Where a and b are the initial count and the remaining survivors of ascospores/ml and t the heating time in minutes, at a given temperature, T. The highest obtained after heat activation before inactivation started was used as the term a

Effect of fructose, sucrose, citric acid and sodium benzoate on thermal resistance of ascospores in locally prepared juices

0.1% of citric acid and sodium benzoate and two concentrations (10 and 40%) of sucrose and fructose each in orange, pineapple and grapefruit juices were prepared. (All reagents used were obtained from BDH Poole England).

The preservatives (0.1%) and the sugars (10% and 40%) were prepared in sterile 250ml Erlenmeyer flask. 9.9ml of each solution was withdrawn into sterile test tubes and adjusted to the desired temperature (85°, 90°, 92.5° and 95°) in a water bath. 0.1ml of ascospore suspension of known total and viable population (of each test isolate) was added. Samples were heated for same periods and withdrawn at intervals described above and plated out by pour plate method. Incubation was at room temperature for 3-5 days.

Results

The isolates obtained were either *Aspergillus* spp (*Neosartorya fischeri* and *Emericella rugulosa*) *or as Penicillium* spp (*Paecilomyces variotii*, *Talaromyces flavus*, *Eupenicillium* spp) (Amaeze *et al*, 2010).

Total count of ascospores (haemocytometer count)

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

Activation profile

During all experiments, the population counts of *N. fischeri* and *T. flavus* ascospores were characterised by an initial rise in viable count caused by heat activation followed by a decline due to inactivation. Activation profiles were similar in all media and exhibited considerable fluctuations in viable population count. Recoveries were however low compared with the haemocytometer count. 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) (Tables 2 to 13).

The pH and soluble carbohydrate content of locally prepared fruit juices

The pH and soluble carbohydrate content of locally prepared fruit juices are shown in Table 14. The pH ranged from 3.6 (grape and orange) to 4.2 (pineapple), and soluble carbohydrate from 22.50mg/ml (grape) to 111.25mg/ml (pineapple).

Thermal resistance of ascospores of *N. fischeri* and *T. flavus*

The decimal reduction data (D-values) for test isolates are shown in Tables 15, 16, 17 and 18.

As in the activation profile, the population counts of *N. fischeri* and *T. flavus* ascospores were characterised by an initial rise in viable count caused by heat activation followed by a decline due to inactivation and was were similar in all media. Population counts also showed considerable fluctuations in viable population count. Recoveries were also low compared with the haemocytometer count.

Ascospores of *N. fischeri* (3C) were more heat resistant than ascospores of *N. fischeri* (E7) in most of the medium in which the heat treatment was administered at 85° but at higher temperatures used the reverse was observed. The rate of thermal destruction for the *N. fischeri* was very slow at 85°C. D-values for the two isolates of *N. fischeri* (E7 and 3C) at 85°C ranged from 61.9 to 217min. $D_{90^\circ C}$ values of 5.0 to 62.6min. $D_{92.5^\circ C}$ values of 1.1 to 19.7min and $D_{95^\circ C}$ values of <1 to 13.6min depending on the heating medium.

Ascospores of *T. flavus* (2E and 5A) exhibited considerably less heat resistance than those of *N. fischeri.* Complete inactivation was achieved at 95°C in all the media. Ascospores of *T. flavus* had $D_{85^\circ C}$ values, which ranged from 10.6 to 78.9min, $D_{90^\circ C}$ values of <1 to 13.6min and $D_{92.5^\circ C}$ values of <1 to 8.1min. Ascospores of *T. flavus* (2E) were considerably more sensitive to heat than those of *T. flavus* (5A).

The rate of thermal inactivation of *N. fischeri* and *T. flavus* appeared to be slowest in pineapple juice, followed by grape fruit juice. Relating the inactivation rates calculated for the ascospores heated in plain juices with the result of pH and soluble carbohydrate of the juices on Table 14 showed a correlation between D-values and pH and soluble carbohydrate. Pineapple juice that had the highest soluble carbohydrate (111. 2mg/ml) and pH 4.2 had the greatest protective effect against heat on the ascospores. Grape and orange juice had not only lower soluble solids but also lower pHs. The contrast between the D-values of ascospores of test isolates in PBs and plain juices goes to point out that the presence of soluble carbohydrate affected the D-

values since the pH of the buffers was same as that of the juices. The rate of thermal reduction was greater in phosphate buffers than in the plain juices. Ascospores of *N. fischeri* and *T. flavus* were most resistant to heat in juices fortified with sugars. This resistance increased with increasing sugar. A close look at the D-values calculated for the ascospores in juices fortified with sugars showed that sucrose conferred more protection on the ascospores than fructose. Rate of inactivation was most rapid in juices fortified with preservatives, sodium benzoate and citric acid.

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. There was a general low yield of ascospores of *N. fischeri* and *T. flavus* compared to enumeration in the haemocytometer. A similar result 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 (Amaeze and Ugwuanyi, 2011).

Although ascospores of *T. flavus* exhibited considerably more heat resistance than those of *N. fischeri* during the activation studies (at 70°, 75° and 80°) (Amaeze and Ugwuanyi, 2011) ascospores of *T. flavus* were more heat labile to heat at the higher temperatures used for inactivation (85°, 90°, 92.5° and 95°). At 95°C, ascospores did not survive 1min treatment in the entire heating medium unlike ascospores of *N. fischeri.* The relative heat resistance of the *T. flavus* species examined in this study would therefore not be expected to pose the serious inactivation problem associated with *N. fischeri* examined in this study.

Comparison of D-values showed that D-value decreased in the order of juices fortified with 40% sugar >juices fortified 10% sugar > plain juices > juices fortified with preservatives or distilled water or phosphate buffers. This indicates that some components in juices e.g. sugars had a protective effect on ascospores during heating and may have influenced rates of ascospore inactivation. Beuchat and Toledo (1977) reported that ascospores of *B. nivea* were protected against inactivation when heated in fruit products supplemented with sucrose. The mechanism was due in part to establishment of an osmotic pressure differential between the heating medium and ascospores, which favoured heat resistance.

Increased resistance of ascospores in sucrose agrees with Corry (1976) report on osmophilic yeasts. Resistance of *Saccharomyces rouxii* and *Schizosaccharomyces pombe* were maximum in solutions of sucrose, less in sorbitol and least in solutions of glucose, frustose and glycerol. This could be due to differences in ability of solutes to penetrate the cell membrane. Corry (1974) also reported sucrose to be the most protective solute for yeasts during heating. This could be responsible for the somewhat increased resistance of ascospores in pineapple juice as it contains in nature 10.5% of sucrose as opposed to 8.1% of sucrose in orange and 7.3% gluose in grape (Holland *et al.*, 1992). Splittstoesser and churey (1991) have however reported Dvalue in pineapple juice to be same as in grapefruit juice.

Splittstoesser and Churey (1991) reported $D_{85°C}$ of 12, 13 and 12 min in grape fruit, orange and pineapple juices respectively; Tournas and Traxler (1994) reported $D_{85°C}$ of 19.6 to 29.5 min; $D_{90°C}$ of 4.7 to 7.6 min; $D_{95°C}$ of 1.7 to 2.3min in deconized water, pineapple and pineapple juice concentrate. In this study Dvalues as high as 217.5min for *N. fischeri* and 78.9 mins for *T. flavus* at 85°C were obtained. Even though these values are high, there are several other comparable results in literature. Beuchat (1986) reported slightly similar Dvalues especially at 85°C ($D_{85°C}$ for *N. fischeri* ranged from 15.1 to 116min and 20.4 to 68.1mins for *T. flavus* in different fruit juices). Some *Neosartorya* ascospores have extra-ordinary high resistance to heat. Some of them reportedly survived heating at 100°C for 60min or longer when suspended in distilled water (Kavanagh *et al.*, 1963). McEvoy and Stuart (1970) reported a 100% survival after heating in distilled water at 80°C for 20min and 0.002% survival after heating for 15min at 100°C. Ascospores were found to survive for > 300min of heating at 70°C, 75°C and 80°C in various fruit juices (Rajashkhara *et al.*, 2000). This marks the extraordinary heat resistance of the ascospores of the test isolates in this study. There is therefore a possibility the ascospores of HRF isolated in the tropics exhibit unusual resistance to heat and needs to be investigated further.

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

Table 2: 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 3: 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 4: 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 5: 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 6: 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 7: 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 8: 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 9: 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 10: 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 11: 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 12: 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 13: 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

Table 14: pH and soluble carbohydrate content of locally prepared fruit juices

Fruit juice	**pH**	**Soluble carbohydrate (mg/ml at 28^oC)**
Grape	3.6	22.50
Orange	3.6	68.75
Pineapple	4.2	111.25

Table 15: Decimal reduction values (D-values) of *Neosartorya fischeri* (E7) at 85^o, 90^o, 92.5^o and 95^oC in different heating media

Heating Medium	**Dvalues at 85^oC(min)**	**Dvalues at 90^oC(min)**	**Dvalues at 92.5^oC**	**Dvalues at 95^o (min)**
Distilled H_2O	114.2	28.5	19.7	1.1
Acetate buffer (pH 3.6)	79.7	9.9	2.9	<1
Acetate buffer (pH 4.2)	61.9	18.0	5.0	3.9
Orange juice	119.7	34.8	5.3	4.9
Grapefruit juice	144.8	16.7	6.8	6.5
Pineapple juice	217.5	37.5	14.3	5.1
Grapefruit juice + 10% fructose		29.4	12.9	2.9
Grapefruit juice + 40% fructose		40.2	16.3	10.7
Grapefruit juice + 10% sucrose		21.2	16.3	4.8
Grapefruit juice + 40% sucrose		28.5	8.4	10.7

Orange juice + 10% fructose		20.5	10.7	5.3
Orange juice + 40% fructose		45.0	10.1	9.6
Orange juice + 10% sucrose		33.2	5.5	6.2
Orange juice + 40% sucrose		41.6	8.4	10.0
Pineapple juice + 10% fructose		50.8	6.9	12.3
Pineapple juice + 40% fructose		62.6	17.6	13.6
Pineapple juice + 10% sucrose		46.0	13.9	9.4
Pineapple juice + 40% sucrose		46.2	16.3	6.6
Grapefruit juice + 0.1% citric acid			5.1	
Grapefruit juice + 0.1% sodium benzoate			<1	

Table 16: Decimal reduction values of *Neosartorya fischeri* ascospores (3C) at 85°, 90°, 92.5° and 95°C in different heating medium

Heating Medium	**Dvalue at 85°C (min)**	**Dvalues at 90°C (min)**	**Dvalues at 92.5°C**	**Dvalues at 95° (min)**
Distilled H_2O	197.4	28.8	18.4	10.2
Acetate buffer (pH 3.6)	85.9	5.5	3.1	<1
Acetate buffer (pH 4.2)	106.8	5.0	2.2	<1
Orange juice	169.6	18.0	5.4	3.0
Grapefruit juice	205.2	9.9	6.1	2.3
Pineapple juice	216.4	23.4	10.3	1.6
Grapefruit juice + 10% fructose		27.4	19.5	4.2
Grapefruit juice + 40% fructose		45.1	7.7	8.8
Grapefruit juice + 10% sucrose		20.1	6.3	2.6
Grapefruit juice + 40% sucrose		28.8	2.2	7.3
Orange juice + 10% fructose		10.9	2.6	2.3
Orange juice + 40% fructose		40.2	8.9	6.3
Orange juice + 10% sucrose		21.2	1.2	4.2
Orange juice + 40% sucrose		29.4	1.1	11.3
Pineapple juice + 10% fructose		10.0	1.7	2.9
Pineapple juice + 40% fructose		32.5	15.6	5.8
Pineapple juice + 10% sucrose		36.4	5.2	5.1
Pineapple juice + 40% sucrose		51.7	15.5	5.5
Grapefruit juice + 0.1% citric acid			6.0	
Grapefruit juice + 0.1% sodium benzoate			<1	

Table 17: Decimal reduction values of *Talaromyces flavus* ascospores (2E) at 85°, 90° and 92.5° C in different heating medium

Heating Medium	**Dvalue at 85°C (min)**	**Dvalues at 90°C (min)**	**Dvalues at 92.5°C**
Distilled H_2O	31.4	5.6	<1
Acetate buffer (pH 3.6)	12.3	2.7	<1
Acetate buffer (pH 4.2)	11.6	4.0	<1
Orange	19.9	3.7	<1
Grapefruit	20.7	5.6	<1
Pineapple	41.6	7.2	<1
Orange juice + 0.1% citric acid	12.0	3.5	<1
Orangejuice+0.1%sodium benzoate	11.0	3.3	<1
Grapefruit juice 0.1% citric acid	12.4	5.2	<1
Grapefruitjuice+0.1%sodium benzoate	12.1	<1	<1
Pineapple juice +0.1%citric acid	12.1	<1	<1
Pineapple juice+0.1%sodium benzoate	12.1	<1	<1
Orange juice + 10% fructose	26.6	6.6	<1
Orange juice + 40% fructose	31.9	6.7	<1

Orange juice + 10% sucrose	15.3	6.8	<1
Orange juice + 40% sucrose	26.4	8.4	<1
Pineapple juice + 10% fructose	15.0	8.2	<1
Pineapple juice + 40% fructose	23.3	13.6	<1
Pineapple juice + 10% sucrose	15.6	13.0	<1
Pineapple juice + 40% sucrose	26.8	10.3	7.2
Grapefruit juice + 10% fructose	20.8	7.2	<1
Grapefruit juice + 40% fructose	20.8	8.8	3.5
Grape fruit juice+ 10% sucrose	31.3	8.5	<1
Grapefruit juice + 40% sucrose	35.7	13.6	<1

Table 18: Decimal reduction values (D-values) of *Talaromyces flavus* ascospores (5A) at 85°, 90° and 92.5°C in different heating medium

Heating Medium	**Dvalues at 85°C (min)**	**Dvalues at 90°C (min)**	**Dvalues at 92.5°C**
Distilled H_2O	36.4	5.6	2.7
Acetate buffer (pH 3.6)	18.0	2.3	<1
Acetate buffer (pH 4.2)	10.6	4.2	<1
Orange juice	34.3	4.6	5.0
Grapefruit juice	24.6	4.5	2.3
Pineapple juice	28.7	12.6	5.3
Orange juice + 0.1%citric acid	12.7	3.3	<1
Orangejuice+0.1%sodium benzoate	12.3	2.6	<1
Grapefruit juice 0.1%citric acid	20.9	4.6	<1
Grapefruit juice+0.1%sodium benzoate	17.6	3.0	<1
Pineapple juice +0.1%citric acid	28.5	1.5	<1
Pineapple juice+0.1%sodium benzoate	30.1	6.3	6.0
Orange juice + 10% fructose	20.2	8.4	7.5
Orange juice + 40% fructose	26.6	10.5	7.1
Orange juice + 10% sucrose	15.4	6.7	7.4
Orange juice + 40% sucrose	28.7	7.9	6.3
Pineapple juice + 10% fructose	64.5	11.4	7.1
Pineapple juice + 40% fructose	82.5	7.4	7.1
Pineapple juice + 10% sucrose	42.3	13.2	6.2
Pineapple juice + 40% sucrose	78.9	10.0	3.6
Grapefruit juice + 10% fructose	41.0	12.3	7.1
Grape fruit juice+ 40% fructose	52.7	10.5	5.9
Grapefruit juice + 10% sucrose	50.9	10.2	8.1
Grape fruit juice+ 40% sucrose	65.5		

The poor recovery of ascospores raised a question as to the validity of heat resistance determinations that has been conducted in different media; water, buffers juices and juices fortified with sugars and preservatives. If only a fraction of the dormant spores were being cultured, it was possible that the Dvalues that were obtained do not reflect the resistance of the spore population in general. Splittstoesser *et al.* (1993) have investigated this and reported that although a large proportion of the ascospores population may not be cultured, this does not necessarily introduce a significant error into heat-resistance measurement.

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