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Thermal Inactivation Kinetics of Aflatoxigenic *Aspergillus flavus* and *A. parasiticus* Conidia Isolated from Ethiopian Hot Red Pepper Powder

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ምግብን በሙቀት ኃይል ማብሰል ለዘመናት የቆየ ሥልጣኔ ሲሆን ከጥቅሞቹ አንዱ በምግብ ውስጥ የሚገኙ ምግብ-ወለድ በሽታ አምጭ እና ምግብ-አበላሽ የሆኑ ደቂቅ-ዘአካላትን ለመቀነስ ወይም ለማጥፋት ይረዳል። በምግብ ውስጥ የሚገኙ ደቂቅ-ዘአካላት ግን የሙቀት ኃይልን በመቋቋም ደረጃ የየራሳቸው ባህሪ አላቸው። በዚህ ጥናት በበርበሬ ዱቀት ውስጥ በሚገኙ በበሽታ አምጭነታቸውና በምግብ-አበላሽነታቸው በሚታወቁ ሁለት የሻጋታ ዓይነቶች ላይ የሙቀት ኃይልን የመቋቋም ባህሪያቸው ጥናት ተደርጎባቸዋል። ጥናቱ በ55፣ 65፣ 75፣ 85 እና 95°ሴ ለ30፣ 45፣ 60፣ 75 እና 90 ደቂቃ በኦቶማቲክ ውሃ ማሞቂያ መሣሪያ ውስጥ በማሞቅ ተሰርቷል። በዚህ ጥናት መሠረት አስፔርጅለስ ፓራስቲከስ የሚባለው ሻጋታ አስፔርጅለስ ፊላቩስ ከሚባለው ሻጋታ በላይ የሙቀት ኃይልን የሚቋቋም ሆኖ ተገኝቷል። በተጠቀምነው የሙቀት ኃይል (55 - 95° ሴ) የአስፔርጅለስ ፊላቩስን ቁጥር በ90% ለመቀነስ የወሰደው ሰዓት ከ119.1-14.1 ደቂቃ ሲሆን ለአስፔርጅለስ ፓራስቲከስ ግን ከ147-17.1 ደቂቃ ወስዷል። ምግብ ሲዘጋጅ የሚጨመር የምግብ ጨው በአስፔርጅለስ ፊላቩስ የሙቀት ኃይልን የመቋቋም ባህሪ ላይ ያለው ጫና ከላይ በተገለፁት የሙቀት ኃይል በተለያየ የጨው መጠን (በ2፣ 6 እና 8 በመቶ) ተጠንቶ የጨው መጠን ሲጨምር የአስፔርጅለስ ፊላቩስ የሙቀት ኃይልን የመቋቋም ባህሪ እንዲጨምር አድርጎአል። ከ2-8 በመቶ የጨው መጠን ከላይ በተገለፁት የሙቀት ኃይል የአስፔርጅለስ ፊላቩስን ቁጥር በ90 % ለመቀነስ ከ119.7-188.6፣ 36.1-41.5፣ 29.6-32.9፣ 20.4-21.7 እና 45.6-48.9 ደቂቃ ወስዷል። የዚህ ጥናት ውጤት በድህረ-ምርት የምግብ የቆይታ ጊዜን ለማራዘም በሚደረጉ ሂደቶች ውስጥ እንደ መነሻ መረጃ ሊጠቅም ይችላል። ነገር ግን የጥናቱን ውጤት ለመጠቀም በእያንዳንዱ የምግብ አዘገጃጀትና የምግቡ የይዘት ሁኔታ ላይ በመሞርኮዝ ሰፊና ጥልቅ ጥናት ማካሄድ ይፈልጋል።

Abstract

Thermal food processing is known to inactivate harmful microorganisms in foods. However different microbes have varying degrees of heat resistance. During this study, thermal inactivation characteristics of aflatoxigenic Aspergillus flavus and A. parasiticus conidia isolated and characterized from Ethiopian hot red pepper powder were determined by the survivor curve method in digital water bath to realize their fate and stability under thermal food processing. The experiment was done at 55, 65, 75, 85 and 95 °C with exposure time of 30, 45, 60, 75 and 90 min., and also at different NaCl concentrations for A. flavus. The D-values for A. flavus and A. parasiticus ranged from 119.1-14.1 and 147-17.1 min. respectively with Z-values of 35.2 and 34.4 °C in the written

order. Both fungi were found sensitive to the moist heating following the first-order kinetics model. Aspergillus parasiticus was found resistant than A. flavus. The D-values for A. flavus at 2-8 % NaCl ranged from 119.7-188.6, 36.1-41.5, 29.6-32.9, 20.4-21.7 and 45.6-48.9 min. at 55, 65, 75, 85 and 95 ^{o}C, respectively. The NaCl decreased thermal sensitivity of the conidia and affected the linearity of inactivation. These results may serve as baseline information for postharvest pepper-based food preservation. However, for its practical application, we recommend further detailed studies on respective food processing practices and food matrix.

Keywords: *A. flavus, A. parasiticus,* hot red pepper, thermal inactivation

Introduction

Adequate thermal food processing is a reliable and historical method, and is one of the most important critical control points for inactivating human pathogens and spoilage microorganisms in foods, and it undeniably plays key role in safety (foodborne disease prevention) assurance (Rahman, 2007). Food preservation methods such as microbial growth inhibition, microbial inactivation and avoiding recontamination are currently used by the food industries in order to reduce risks associated with foodborne microbial pathogens (Rahman, 2007). Thermal treatment can be used as a single preserving technique or it can be used as one step in "hurdle technology", where multiple factors or techniques are employed to effect the control of microorganisms in foods (Jay, 2000). However, though heating is well known to destroy microorganisms, different microbial groups are known to have varying degrees of heat resistance which in turn is dependent on the type (strain) of the microbe and also the matrix in which the heating is carried out (Kornacki, 2010; Doyle and Marth, 1975).

Most foodborne fungi are generally supposed to have low level of resistance to wet heating and are generally inactivated by pasteurization temperature of about 70 ^{o}C for 10 min (Kikoku *et al.*,2008). However, several spores of foodborne fungi have been reported tolerating thermal food processing steps and were detected in thermally processed food products in several countries (Ibrahim *et al,* 2014). The main concerns with such heat-resistant foodborne fungi include the deterioration of food (e.g. production of moldy products), production of toxic compounds such as mycotoxins and causing disease. For example, the heat tolerance nature of *A. flavus* was reported to contribute to its pathogenicity to humans and other warm-blooded animals (Yu *et al.,* 2005). A number of *Aspergillus* species, besides causing aflatoxicosis, are well known to cause "aspergillosis", an umbrella term used to describe a wide range of diseases caused by *Aspergillus* species (Yu *et al.*, 2005). Five major clinical forms of aspergillosis are: rhinocerebral (sinuses and brain), pulmonary (lung), gastrointestinal, cutaneous (skin) and disseminated aspergillosis. Of these, rhinocerebral and pulmonary aspergillosis are the most

common types (Bazaz and Denning, 2019; Latge and Steinbach, 2009; Eggimann *et al*., 2006).

Hot red pepper (*Capsicum* spp.), the widely used spice (Nwokem *et al.*, 2010) is known to be susceptible (from farm-to-fork) to contamination with fungi that pose serious public health risks (Costa *et al.*, 2019). In our current study, we have isolated different molds including *Aspergillus* (about log 5 cfu/g) from hot red pepper powder (unpublished data). Pepper is an indispensable ingredient in an Ethiopian daily cuisine and is widely used as paste or sauce and also to modify the color, flavor and aroma of almost every cuisine. Thermal food processing is the most commonly practiced operation in Ethiopian hot red pepper-based food preparations, specially of pepper-based stew. Currently, Ethiopian women entrepreneurs who are engaged in micro and small business enterprises are attempting to package and commercialize Ethiopian pepper-based stew. In spite of the importance of hot red pepper in Ethiopian daily cuisine, its accessibility to be commercialized and contributions in national economy, no work was previously done on fate and stability (during thermal food processing) of such aflatoxigenic *Aspergillus* species. Therefore, this study was conducted to investigate thermal inactivation kinetics of aflatoxigenic *A. flavus* and *A. parasiticus* conidia in order to realize their fate and stability under moist thermal food processing.

Materials and Methods

Fungal test cultures

Assuming thermal food processing as a critical control point (CCP) during Ethiopian hot red pepper-based food preparations, thermal inactivation kinetics: decimal reduction time (*D*-value) and thermal resistance constant (*Z*-value) were determined for aflatoxigenic *A. flavus* and *A. parasiticus* conidia isolated and characterized during this study from Ethiopian hot red pepper powder (unpublished data).

Heating treatments and viability assay

This thermal inactivation kinetic study was performed in a laboratory at five different experimental temperatures (55, 65, 75, 85 and 95 °C) and exposure time (30, 45, 60, 75 and 90 min) in moist heating. In this study, temperatures above the maximum for growth of *Aspergillus* spp. (ca. 45 ^{o}C) (Pitt and Hocks, 2009) were considered since microbes generally lose their viability when they are subjected to temperatures beyond their maximum for growth as key cellular components are destroyed and cannot be replaced (Adams and Moss, 2008). The maximum temperature near to water boiling point was purposely chosen for this moist heating inactivation experiment. The maximum exposure time was chosen based of Ethiopian household level traditional pepper-based stew average cooking time based on

information collected from key informants. The pure culture of *A. flavus* and *A. parasiticus* were cultured on potato dextrose agar plates at 28 ^{o}C for a week to obtain high conidia concentration. The conidia were collected after washing the surface of *A. flavus* and *A. parasiticus* cultures with a sterile physiological saline containing 0.005 % (v/v) Tween 80 and diluted to achieve approximately 10^6 conidia/ml as estimated using a hemocytometer (Fujikawa and Itoh, 1996). For the thermal treatment, 1 ml of the suspension was aseptically inoculated into Erlenmeyer flask containing 99 ml of a sterile physiological saline with 0.005 % (v/v) Tween 80 to obtain a final concentration of about 10^4 conidia/ml. After homogenization, 100 ml of each of the conidial suspension was then separately assembled and introduced into the digital hot water bath preheated and stabilized at the desired experimental temperature. Subsequently, for each temperature, after each exposure time interval, 5 ml of heated conidial suspension was aseptically withdrawn and held in a cold-water bath (17 ± 1 °C) (for about 2-3 min) in sterile vials to quench the thermal treatment. This cooling (17 ± 1 °C) was used to concomitantly quench the thermal treatment and minimize thermal shock effect which otherwise be magnified at about ice cold as more cells (microorganisms) die when the temperature decline above freezing is sudden than when it is slow (Jay, 2000). Then after, the vial was removed and held at room temperature for viability assay (thermal survivors' determination). Viability was determined in duplicate by spread-plate count (0.1 ml plated) using potato dextrose agar. Viability was also determined in the same way from untreated suspension at time zero as a control. All plates were incubated at 28 ^{0}C and counted after three-five days before the mycelia interwoven. All the glass material used were sterilized in an autoclave at 121 °C for 15 min.

Another batch of inactivation experiment was also done for *A. flavus*. During this study, *A. flavus* was found the most abundant of section *Flavi* (unpublished data). *Aspergillus flavus* was also reported famous in the genus *Aspergillus*, the second (next to *A. fumigatus*) most common cause of invasive and non-invasive aspergillosis in humans and animals and the leading causative agent in some geographic areas (Yu *et al*., 2005). As a result of its importance and abundance (during this study), in addition to the moist heating inactivation, effect of different concentrations [2, 6 and 8 % (w/v)] of sodium chloride (NaCl) on its inactivation kinetics was also investigated with combination of the above specified range of temperatures and exposure time.

Determination of D- and Z-values

It is generally accepted that at a constant temperature, thermal inactivation of microbes follows first-order kinetics (Adams and Moss, 2008; Peleg, 2006) and the model based on the first order kinetics is called the first order or log-linear model and the classical equation of exponential inactivation was proposed (Bevilacqua *et al.,* 2015) as follows :

$$N = N_0 e^{-kt} \qquad (1)$$

in the log-linear form equation (1) is given as follows:

$$\log N = \log N_0 - \frac{k_{max} t}{\ln(10)} \qquad (2)$$

where N and N_o are the population at the time t and the initial cell number, respectively; k_{max} is the first order inactivation rate constant; t is the time and ln (10) is natural logarithm of 10.

According to the model of first-order kinetics, a plot of the log of the number of surviving cells at a given temperature against time gives a straight line with negative slope. The reciprocal of the slope is known as the D-value (Adams and Moss, 2008). The D-value (usually in min) is time required for a one log cycle reduction of the microbial population at a designated temperature (T) (Adams and Moss, 2008). It is a measure of an organism's heat resistance in a particular medium at which the inactivation has been monitored (Peleg, 2006) and mathematically, it can be obtained from regression data using the following equation (3).

$$D_T = (t_2 - t_1)/(logN_1 - logN_2) \qquad (3)$$

Where D_T is decimal reduction time (D-value) at temperature (T), N_1 is survivors at time t_1, N_2 is survivors at time t_2.

It can also be calculated as the reciprocal of the first order inactivation rate constant, k_{max}. Alternatively, the relation between the D-value and the inactivation rate constant is given by (Espachs-Barroso *et al.*, 2006) as shown in Equation (4) for first-order reactions:

$$D = \frac{\ln(10)}{K_{max}} \qquad (4)$$

From relationship of Equation (2), at different temperature, D-value would be different. Over the range of temperatures, as temperature increases, the slope of the survivor curve increases and so the D value decreases. From such relationship, plotting log D-value against range of temperatures gives a straight line, which gives another important parameter, thermal resistance constant (Z-value). A Z-value (^oC) is a temperature increase required to cause a one log reduction in D-value (Adams and Moss, 2008). The Z-value relates the resistance of an organism to differing temperatures and reflects the temperature dependence of the D-value. The Z-value is the reciprocal of the slope of plot of log D-value against range of temperatures and can be obtained from the following Equation (5).

$$Z = (T_2 - T_1)/(logD_{T1} - logD_{T2}) \text{ and/or} \qquad Z = -1/(slope) \qquad (5)$$

Where Z is thermal resistance constant (Z-value), T_1 is initial heating temperature considered, T_2 next (final) heating temperature considered, $logD_{T1}$ is Log of D-value for T_1, $logD_{T2}$ is Log of D-value for T_2.

Data analysis

For data analysis, viable cell counts were log transformed (common logarithm) and inactivation data were analyzed by the GlnaFiT tool using log-linear regression (Geeraerd *et al*., 2005). Survival curve plots were all generated by GlnaFiT tool. The *D*- and Z-values were computed using Equations (3, 4) and (5), respectively as indicated above. The factor at which a D-value applies is indicated by a subscript, e.g. $D_{85}{}^{o}{}_{C}$ for temperature.

Results and Discussion

Effect of moist heating on aflatoxigenic *A. flavus* and *A. parasiticus* conidia

The determined D-values are presented in Table 1. Over range of moist heating temperatures, the D-values showed decreasing trends along temperature gradients signifying decreased thermal resistance of the conidia as moist heating temperature increased. For both of the aspergilli, both at 95 ^{o}C and at 90 min of 85 ^{o}C, no reliable count was found probably due to very rapid thermal inactivation. However, the relatively higher D-values at 55 ^{o}C (for both of the aspergilli) indicated that the conidia were relatively resistant to the moist heating at about this range of temperature. The computed D-values also indicated that the two aspergilli differ in their thermal sensitivities. The larger the D-value at a given temperature, the higher the thermal resistance of the microbial population (Heldman and Hartel, 1998). From the D-values. the conidia of *A. parasiticus* were found relatively resistant than the conidia of *A. flavus* (Table 1). Doyle and Marth (1975), reported thermal resistance difference among strains of *A. flavus* and *A. parasiticus*. It is well established that each type of microorganism (virus, bacterial vegetative cell or spore, yeast vegetative cell or spore, fungal cell or spore), as well as each species and strain of the same group of organisms has its own resistance at a particular temperature (T) under defined environmental conditions, and on changing the environment, the microbial resistance changes accordingly (Casolari, 2018).

Table 1. Decimal reduction time (D-value) and thermal resistance constant (Z-value) of *Aspergillus flavus* and *A. parasiticus* isolated from Ethiopian hot red pepper powder

Aspergillus sp.	Temp. (°C)	Time (min)	K_{max}	*D*-values		*Z*-values	
				D_T (min)	(-r) R^2	*Z* (°C)	(-r) R^2
A. flavus	55	30-90	0.019341	119.1	0.9877	35.2	0.8239
	65	30-90	0.087571	26.3	0.9896		
	75	30-90	0.119305	19.3	0.9587		
	85	30-75*	0.154716	14.9	0.9926		
	95	*	*	*	*		
A. parasiticus	55	30-90	0.0155663	147.9	0.9978	34.4	0.7977
	65	30-90	0.08042	28.6	0.9864		
	75	30-90	0.10198	22.6	0.9617		
	85	30-75*	0.134866	17.1	0.9787		
	95	*	*	*	*		

* No reliable count at 95 °C and 85 °C along 30-90 min, and at 90 min exposure time, respectively; K_{max} = first order inactivation rate constant; R^2 = a coefficient of determination, (-r) = a negative coefficient; D_T (min) = D-value at temperature (T) in min.; *Z* (°C) = Z- values in °C.

The Z-values estimated were found to be 35.2 and 34.4 °C for *A. flavus* and *A. parasiticus* conidia, respectively (Table 1) (i.e. the *D*-value varies by a factor of one log cycle if the temperature varies, increase or decrease by 35.2 and 34.4 °C for *A. flavus* and *A. parasiticus* conidia, respectively). Representative plots of log10(N) *versus* moist heating time are shown in Fig.1A for *A. flavus* and Fig.1B for *A. parasiticus* at 85 °C. Similar figures for both aspergilli were also obtained at temperatures of 55, 65 and 75 °C (data not shown). For both of the aspergilli, the coefficient of determination (R^2) of the regression curves at temperature of 55 to 85 °C was found $R^2 > 0.9587$ (Table 1). During this study, the survival curves followed the logarithmic death rate for each of the *Aspergillus* species in line with the assumption of the first order kinetics. In practical sense, a lot of deviations have been observed indicating that inactivation kinetics are not always following first order log-linear relationships. Generally, about four to ten types of survival curves with their own characteristics have been frequently reported (GlnaFit, 2015; Keklik and Demirci, 2014; Kornacki, 2010; Geeraerd *et al.*, 2005). The first order kinetics assumes homogeneity of microbial strain, identical resistance to a lethal agent (thermal treatment in this case) (Keklik and Demirci, 2014) and a single target molecule in each cell whose inactivation causes death (Adams and Moss, 2008). Results of this study indicated that the conidia of *A. flavus* and *A. parasiticus* (from Ethiopian hot red pepper powder) were sensitive to the moist heating temperature following the log-linear thermal inactivation kinetics model, and the observed log-linear trend may be attributed to their homogeneity as the work was done using pure culture.

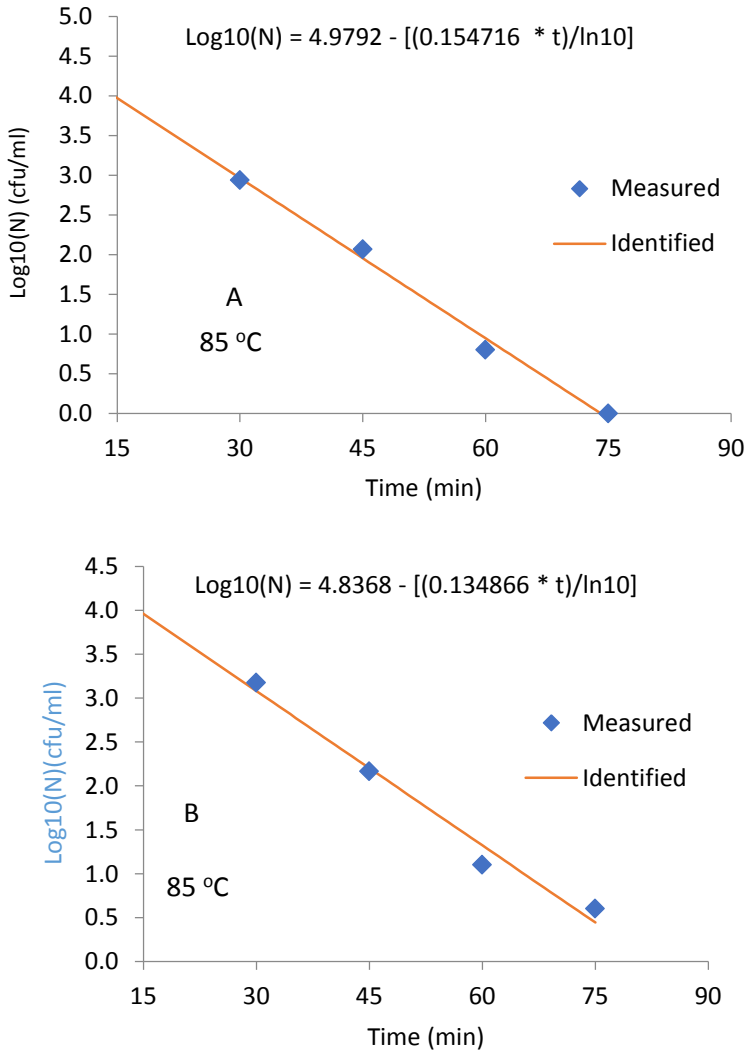


Fig. 1. Plot of log10(N) (cfu/ml) *versus* moist heating time (a decimal reduction curve or survivor curve) for aflatoxigenic *A. flavus* (A) *and A. parasiticus* (B) at 85 °C.

Effect of moist heating and NaCl on *aflatoxigenic A. flavus* conidia

The D-values computed at the presence of NaCl for each particular moist heating temperature showed increasing trends as salt concentration increased (Table 2). Though thermal sensitivity of the conidia was increased as moist heating temperature increased (as already indicated above), the much higher D-values for each corresponding moist heating temperature (and the detection of counts at 95 °C and 90 min of 85 °C) in the presence of salt revealed that NaCl might have decreased thermal sensitivity of the conidia. The salt showed protective effect against thermal inactivation of the conidia and might have also reduced the inactivation effect of the moist heating temperature. Representative plots of log10(N) *versus* moist

heating time are shown in Fig. 2 at 55 ^{o}C/2 % NaCl, 55 to 95 ^{o}C/8 % NaCl. Similar figures were also obtained at 55 ^{o}C/6 % NaCl, 65 to 95 ^{o}C/ 2 and 6 % NaCl (data not shown). The survival curves for each moist heating temperature showed increased deviation from linearity as NaCl concentration increased, and also as moist heating temperature and NaCl concentration increased concomitantly (Fig. 2). Minimum determination coefficient (R^2) of the regression curves was recorded at 8 % NaCl for all moist heating temperature with values of 0.9610, 0.8959, 0.8756, 0.9106 and 0.8672 at 55, 65, 75, 85 and 95 ^{o}C, respectively (Table 2). This result showed that NaCl affected not only the thermal sensitivity of the conidia but also affected the linearity of inactivation as its concentration increased along with the moist heating temperatures. As compared to 65, 75 and 85 ^{o}C, the D-values showed ascending trends at 95 ^{o}C, and this probably indicated the increased thermal tolerance of the conidia as NaCl combines with higher moist heating temperature.

It has been suggested that some salts (solutes) used in the heating menstruum may decrease water activity (a_w) and thereby increase heat resistance of microorganisms (Casolari, 2018), though sensitivity to heat at a reduced a_w depends on the type of microorganism, the solute used, and its concentration (Campos *et al.*, 2015; Jay, 2000).

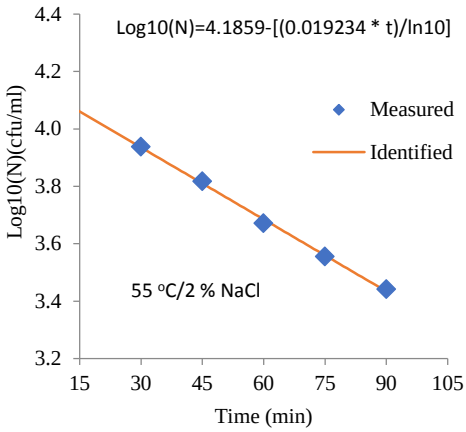
Table 2. The D- and *Z*-values of *A. flavus* at presence of NaCl

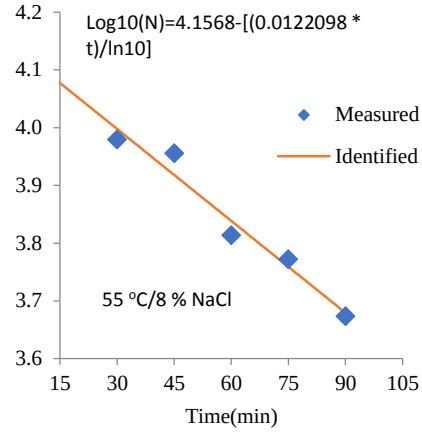
Temp. (^{o}C)	NaCl (%)	Time (min)	K_{max}	*D*-values	
				D_T (min)	(-r) R^2
55	0	30-90	0.019341	119.1**	0.9877
	2		0.019234	119.7	0.9980
	6		0.0159476	144.4	0.9943
	8		0.0122098	188.6	0.9610
65	0	30-90	0.087571	26.3**	0.9896
	2		0.063874	36.1	0.9468
	6		0.061685	37.3	0.9551
	8		0.055452	41.5	0.8959
75	0	30-90	0.119305	19.3**	0.9587
	2		0.077890	29.6	0.9061
	6		0.075366	30.6	0.9128
	8		0.06991	32.9	0.8756
85	0	30-75*	0.154716	14.9**	0.9926
	2		0.112678	20.4	0.9819
	6	30-90	0.1058	21.8	0.9306
	8		0.10612	21.7	0.9106
95	0	*	*	*	*
	2	30-90	0.05053	45.6	0.8767
	6		0.04739	48.6	0.8948
	8		0.047141	48.9	0.8672

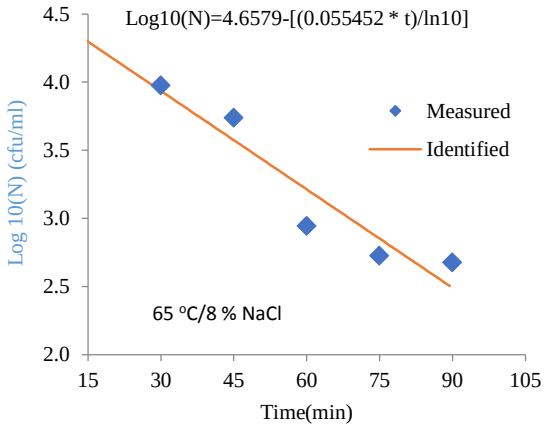
* No reliable count at 95 ^{o}C and 85 ^{o}C along 30-90 min, and at 90 min exposure time, respectively; ** D-values for *A. flavus* at zero percent NaCl from Table 1 for contrast; K_{max} = first order inactivation rate constant; R^2 = a coefficient of determination, (-r) = a negative coefficient; D_T (min) = D-value at temperature (T) in min.

Beuchat (1981), found that the heat tolerance of five, three and one of six strains of yeasts was increased when cells were heated in broth containing 3 %, 6 % and 12 % (a_w= 0.926) of sodium chloride, respectively, while 60 % (a_w= 0.892) of sucrose enhanced heat tolerance of five of the six strains he studied. Corry (1976), found that the heat resistance of *Saccharomyces rouxii* and *Schizosaccharomyces pombe* was enhanced in solutions of sugars and polyols containing 0.1 M phosphate buffer, pH 6.5 at an a_w value of 0.95 with maximum resistance recorded in solutions of sucrose, less in sorbitol and least in solutions of glucose, fructose and glycerol. Though exact relationship between D-value and other factors such as a_w and pH has not been developed (Casolari, 2018), the observed protective nature of NaCl during our study might have also attributed to a_w reduction.

Water activity is usually used as a preservative factor by the addition of salt and sugar (binding water in the food so as to create an unfavorable environment for microbes to grow). As a rule of thumb, lowering a_w extends microorganisms' lag phase of growth and extends the time to toxin production in case of spore formers, decreases microorganisms' growth rate, and reduces the maximum population densities (Adams, 2012). However, a_w can also interact with other factors such as temperature, pH, acid and nutrients and either inhibit or support microbial growth (Adams, 2012). According to Gould (1989), solute effects such as the case of glycerol which readily permeates the membrane of many bacteria and therefore has a lower inhibitory a_w may depend on the ability of the solute to permeate the cell membrane. Corry (1976) also correlated the differences in the order of protectiveness of the solutes he examined to ability of the solutes to penetrate the cell membrane. Gibson (1973), postulated the increased heat resistance exhibited by cells in sucrose solutions of low a_w to be the result of a dehydration of the cell together with a reduction in the pore size of the cell wall.


Log10(N)=4.1859-[(0.019234 * t)/ln10]
Measured
Identified
55 °C/2 % NaCl
Log10(N)(cfu/ml)
Time (min)


Log10(N)=4.1568-[(0.0122098 * t)/ln10]
Measured
Identified
55 °C/8 % NaCl
Time(min)


Log10(N)=4.6579-[(0.055452 * t)/ln10]
Measured
Identified
65 °C/8 % NaCl
Log 10(N) (cfu/ml)
Time(min)

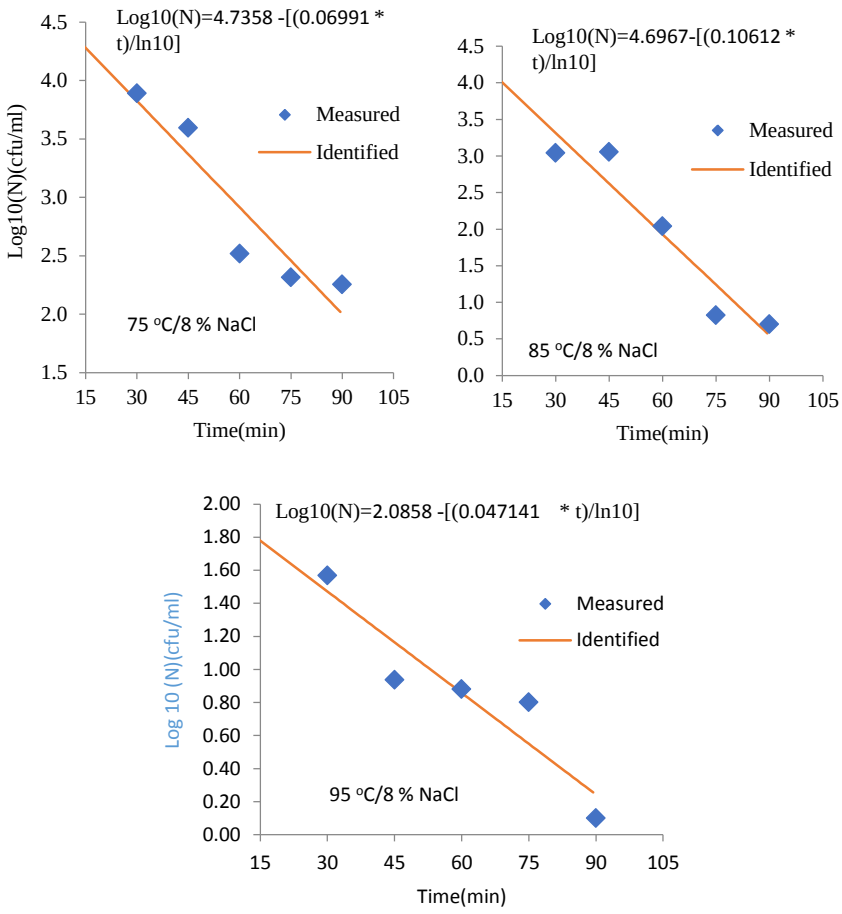


Fig. 2. Plot of log10(N) (cfu/ml) *versus* moist heating time for aflatoxigenic *A. flavus* at specified temperature and NaCl concentration.

The knowledge of D- and Z-values help in the development of microbiologically safe food products and their preservation. As computed in this study, for example, once a D-value at a specific temperature (D_{ref}) and a Z-value have been established, a D-value at any other temperature (T) (in the experimental data range) not considered in the experiment can be estimated using Equation (6) (Horn *et al.*, 2015):

$$log\ D_T = log(D_{ref}) - \left(\frac{T - T_{ref}}{Z}\right) \qquad (6)$$

Where D_{ref} is the known D-value, D_T is the sought D-value, T is the temperature of interest, and T_{ref} is the known temperature which results in D_{ref}.

The results of this study may serve as baseline information for postharvest pepper-based food preservation. However, for its practical application, we recommend further detailed studies on respective food processing practices and food matrix.

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