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D- and *Z*-values of microflora in tuna mince during moist- and dry-heating

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Abstract

Fish and seafood are prone to rapid microbial spoilage, thus adequate care must be taken in drying of fish. The microbial load and its changes during drying and storage are important information in establishing a standard that will ensure food safety. In order to develop drying procedures leading to low safety risk, it is relevant to determine the decimal reduction time (*D*-value) and the thermal resistance constant (*Z*-value) during a heating process to identify the effect of temperature on lethality. In the case of drying, microbial changes occurred due to the effects of heat and concentration process. This study was conducted to investigate the changes of endogenous bacterial counts in minced tuna during dry-heating (convection air-drying) and moist-heating (heating in a closed chamber) as a function of temperature. The *D*-values for total viable counts decreased from 2.52 to 0.26 h for moist-heating and 2.57 to 0.34 h for dry-heating, respectively, when temperature was maintained constant within 60–140°C. In both cases, increasing temperature caused significant decrease in *D*-values ($P < 0.05$), whereas the effect of heating methods was not significant ($P > 0.05$). Thus the heat resistance characteristics of microorganisms in fresh tuna mince was not depended on the changing medium moisture content.

Keywords: Convection drying; Drying stress; Tuna; *D*-value; *Z*-value

1. Introduction

Dried fish is consumed in many countries. In the Sultanate of Oman, shredded dried fish with sliced onion, added spices or herbs, are usually consumed with rice. Some times it is washed or soaked in water, cooked or fried before adding the herbs. Fish and seafood are prone to rapid microbial spoilage, thus adequate care must be taken in drying of fish. Safety and spoilage of processed foods are the major concerns of both manufacturers and consumers. Microbiological standards are usually based on the total number of indicator organisms or number of pathogens (Rillo, Magat, Miguel, & Diloy, 1988). Spoilage of fresh products is due to the proliferation of endogenous microflora. Thus, the total mesophilic count is widely used as an indication of the microbial quality of foods. (Liston & Matches, 1976). Although the flora will be composed of Gram-negative bacteria from the aquatic environment and mainly Gram-positive contaminants, this population will change gradually as drying results in the death of vegetative forms of bacteria (Graikoski, 1973). Although knowledge of the total viable count may be desirable, it is often more useful to obtain an estimate of the numbers of particular component of the total flora such as moulds in cereals, psychrotrophic bacteria in a product to be stored at low temperature, anaerobes in a vacuum-packed food, or yeast in a fruit beverage (Mossel, Corry, Sruijt, & Baird, 1995). The microbial load and its changes during drying and storage are important information for establishing a standard that will ensure food safety. The aim of drying is to ensure

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that the number of microorganisms that survive is reduced to an acceptable level that delays spoilage of the product and probably enhance its safety. To establish the reduction in the number of microorganisms required to achieve this consideration must be given to the initial number of microorganisms of food. Drying also reduces the water activity, thus preserving foods by avoiding microbial growth and chemical reactions causing deterioration. In case of drying (dry-heating) and cooking (moist-heating), microbial lethality needs to be determined by estimating total destruction value (F_0-value). Destruction of microorganisms by heat is one of the hurdles in drying and cooking. In estimating F_0-value for specific processing conditions, it is important to know the D- and Z-values. D-value, the decimal reduction time (h), indicates the time required to kill 90% of bacterial population at a specific temperature. Z-value, the thermal resistance constant (°C), measures the temperature increase required to cause a 90% reduction in the decimal reduction time. These two values offer the basis for calculating process times in food industry. Very limited work has been reported on the effect of drying on the endogenous microorganisms (pathogens and spoiling microflora) present on foods. In the literature, D-values of the endogenous spoilage flora were reported for orange juice (Kopelman & Schyer, 1976), for grape juice (Soffer & Mannheim, 1996) and minced tuna (Rahman, Guizani, Al-Ruzeiki, & Al-Khalasi, 2000) as a function of temperature.

Waliuzzaman, Fletcher, Rahman, and Perera (1999) identified that further research needs to be carried out for a wide variety of highly perishable food products. Rahman et al. (2000) studied the changes of microflora in tuna mince during convection air-drying. They found that microorganisms grow in tuna during drying at low temperatures. The drying temperature of 50°C or below is not lethal to the microflora. The decimal reduction time (D-value) of endogenous flora was determined when drying temperatures were maintained constant within the temperature range of 60–100°C.

The thermal death kinetics (D- and Z-values) may be measured for single specific strains of microorganisms, usually those of highest heat resistance present in a particular food, or occasionally, for the whole natural microbial population associated with that food. It is important to note that that whole foodstuffs are most likely to be heterogeneous and the microbial environment may vary locally with the food in terms of pH, water activity, redox potential and concentration of protein, carbohydrate and fat. Microbial standards for production and distribution of foods are usually based on the total viable counts, the number of indicator organisms or the number of pathogens (Rillo et al., 1988). In the present work, the main focus has been on total viable counts in dried minced tuna. This approach does not solve the problems related to spoilage and safety of this product, but is a contribution to development of specifications for reducing risk.

The objective of this study was to investigate the changes of total bacterial counts in minced tuna during convection air-drying at different temperatures for moist- and dry-heating. The purpose was to identify whether drying produces an extra effect on the microbial death.

2. Materials and methods

Whole fresh tuna (*Thunnus tongol*) (local name, Sahwa) was purchased from the local fish market (Muscat) in the month of May. The mass and length of the whole fish were about 3 kg and 0.45 m, respectively. The fish was washed in tap water and then filleted. The fillets were minced and frozen in blocks (20 cm × 13 cm × 1.7 cm). The blocks were stored at −40°C, thawed at 20°C for 18 h prior to drying in convection dryer. Samples were placed in a closed metal chamber for moist-heating, and samples for dry-heating were placed on a wire mesh tray. The dryer was set at a constant temperature for each experiment. The temperature of drying air was maintained constant in an experimental run within 60–140°C. Samples in closed chamber and on the mesh tray were placed in the drier. Samples were taken out from the drier at different time intervals for the determination of water content and microbial count. The water content and total solids were measured gravimetrically by drying samples (5 replicates) in convection air drier at 105°C for at least 18 h. Protein, fat, and ash were measured according to AOAC (1990). All the compositions were expressed on wet basis (kg/100 kg sample).

Microbial studies were carried out for each temperature tested by taking representative fish samples at regular sampling times of the drying process. Fish samples (5 g) in duplicate were aseptically removed from dried fish and were homogenized in 45 ml of sterile peptone water (0.1 g peptone in 100 ml water) (Oxoid, Basingstoke, Hampshire, UK) in a stomacher Lab blender 400 (Seward Medical, UK). Serial dilutions of the fish homogenates were prepared using the same solution. Each dilution (1 ml) was dispensed and poured in duplicate with *standard plate count agar* for total viable counts (*CM 463*, Oxoid, Basingstone, Hampshire, UK) using aerobic incubation at 32°C for 48 h (FDA, 1992). Results are expressed as cfu/g of fish actual weight. The osmo-tolerant microbes were determined by adding 0.5 g (N_1), 3.0 g (N_2), and 5.0 g (N_3) sodium chloride in 100 ml agar when incubation temperature and time was same as above. The Gram-negative microbes were identified by growing sample in Maconkey broth at 32°C (N_4). The thermo-tolerant microbes

were determined by growing the sample in the agar plate when incubation temperature was at 60°C (N_5).

Statistical analysis (analysis of covariance) was done using SAS to find the effect of heating method and drying temperature on the changes of microflora in tuna mince at 5% significance level (SAS, 1996).

3. Results and discussion

The proximate compositions of fresh tuna meat are given in Table 1. The moisture content of fresh tuna was 70.50 kg per 100 kg sample. Total plate count of fresh tuna meat before mincing and frozen storage was 7.3×10^6 cfu/gm. This number is considered high and is however representative to fish delivered to the consumer or to the food processing plants. It is the result of the temperature abuse during transport at room temperature.

3.1. Effect of drying method on D-value

The isothermal condition with constant moisture content is not possible to maintain during drying since the level of water content decreases with the progress of drying.

The destruction of total aerobic flora was analysed based on the estimation of the decimal reduction time proposed by Bayrock and Ingledew (1997a). The decimal reduction time or *D*-value (time required to reduce the number by 1-log cycle) was obtained from the slope of $\log_{10} N_T$ versus time. The slope was estimated only for the initial linear portion of the plot as used by Rahman et al. (2000). Typical plots of $\log_{10} N_T$ versus time are shown in Figs. 1 and 2 for the moist- and dry-heating conditions, respectively. These plots showed a linear portion. *D*-values were estimated from the slope of the logarithmic survivor versus drying time regression data for the initial portion. The values of *D* are given in Table 2 at different temperatures. The decimal reduction time (*D*-value) varied from 2.52 to 0.26 h and 2.57 to 0.34 h for moist- and dry-heating respectively, when drying temperature was maintained constant within 60–140°C. In this study, temperatures above 60°C were considered since temperature below 60°C is not lethal to the microflora as identified by Rahman et al. (2000).

Table 1
Chemical composition of long tail tuna

Run no.	Proximate composition (kg/100 kg sample)			
	Water	Protein	Fat	Ash
1	71.55	24.13	2.09	1.38
2	70.04	24.20	3.30	1.44
3	70.32	23.23	1.97	1.45
4	70.32	24.48	2.06	1.45
5	70.04	24.39	2.82	1.47
6	70.75	24.32	2.00	1.47
Average	70.50 (0.57)	24.12 (0.46)	2.37 (0.55)	1.44 (0.03)

Note: Values in the parentheses are the standard deviation.

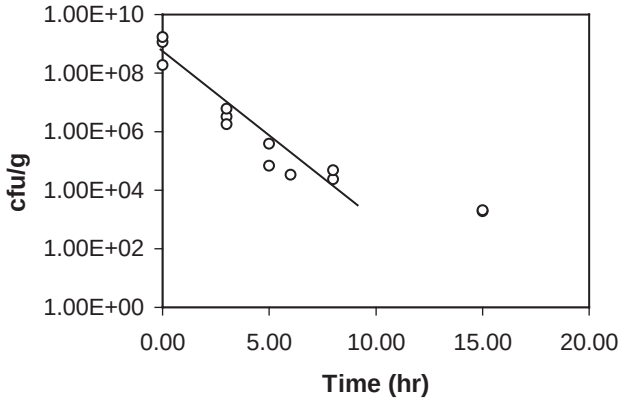

Fig. 1. Plot of $\log N_T$ versus moist-heating time at drying temperature 60°C.

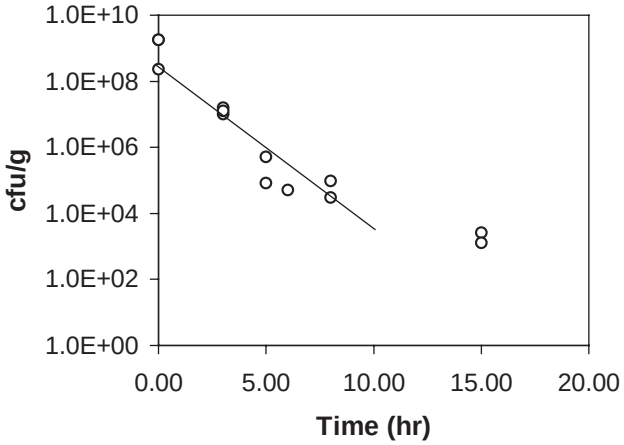

Fig. 2. Plot of $\log N_T$ versus dry-heating time at drying temperature 60°C.

Table 2
Initial *D*-value as a function of processing temperature for moist- and dry-heating

T (°C)	*D*-value (h)	
	Moist-heating	Dry-heating
60	2.52 (0.71)	2.57 (0.30)
70	2.06 (0.27)	2.12 (0.19)
80	2.02 (0.44)	2.62 (0.25)
90	1.72 (0.16)	2.12 (0.28)
100	1.65	1.85
120	0.71	0.84
140	0.26	0.34

Note: Values in the parentheses are the standard deviation of six replicates. Other values are the average of replicates.

These authors found that the *D*-values were 12.66 and 2.63 h at 60°C and 100°C, respectively. These values are significantly different from values found by Rahman et al. (2000). This variation indicates that *D*-values may vary for the same food depending on the source and harvesting time of the raw material. As expected the values *found in the present study* decreased with the increase of temperature, which indicated that increasing temperature increased the lethal effect. In both cases, increasing temperature caused significant decrease in *D*-values ($P<0.05$), whereas the effect of heating methods was not significant ($P>0.05$). Similarly Kim and Bhowmik (1990) found that air temperature was a major parameter affecting the death of lactic acid bacteria during spray drying. Bayrock and Ingledew (1997b) measured the *D*-values for the changing moisture content (i.e. drying) and for moist conditions (i.e. no change of moisture during heating). They estimated the *D*-values from the slope of $\log_{10} N_T$ versus time of drying and found that *D*-values for drying condition were much higher than the values from the moist heat. This indicated that heat resistance of microorganism increased significantly during drying compared to the moist heat conditions. This finding is in contradiction with our results, where the method of drying has no significant influence on *D*-values.

3.2. Effect of temperature

The values of $\log D$ are plotted in Fig. 3 against temperature to estimate the *Z*-value. The *Z*-value is defined as the change of temperature required for 1-log cycle change in *D*-values. The *D*-values below 90°C were found to be *similar* (Table 2). It could be concluded that increasing the temperature from 60°C to 90°C has limited effect on the lethality of microbes, and other criteria (vitamin degradation, maintenance of sensory quality, technical convenience, etc.) could be used to select drying temperature in this area. Fig. 3 showed two linear portions, one for the low temperatures and another for high temperatures. The *Z*-values were found 144°C and 46°C for the temperature range 60–100°C, and 100–140°C, respectively. This indicated that inactivation mechanisms varied at low and high temperature regions. At higher temperature flashing of moisture could damage the cell further. The break of the slope was at around 100°C. The break point observed in this study could be attributed to the destruction of vegetative cells rather than for spores. Vegetative cells are more sensitive to heat when the cell water evaporates while spores are not. This supports our view that *D*- and *Z*-values should not used to guarantee the food safety, as sporulating pathogens, if present, will not be destroyed during the studied drying temperatures. Similar to this, two linear portions were observed by Elizondo and Labuza (1974) and suggested the danger in extrapolating death kinetics to high temperature. They observed the break at 84°C, which is lower than the value found in this study. In case of spray drying of yeast similar order of magnitude was also observed by Elizondo and Labuza (1974) for *Z*-values. In case of *Bacillus subtilis* spores, Molin (1977) found a linear portion within the drying temperatures from 37°C to 190°C, whereas in case of *B. stearothermophilus* spores they found two linear portions in the plot of $\log D$ versus T plot.

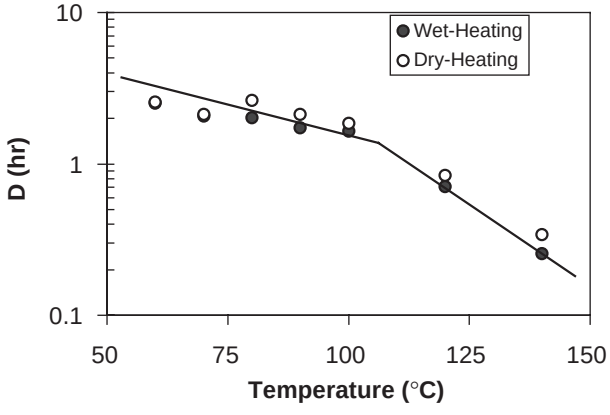

Fig. 3. Plot of $\log D$ versus temperature.

3.3. Effect of moisture content change

In canning it is usual to estimate *D*-value at a specified temperature (isothermal conditions) by maintaining other parameters (such as moisture content) constant. This ideal situation cannot be simulated in the destruction process of microflora during drying. This is due to the change of moisture in the sample during drying process, thus destruction is caused by a combination of temperature and concentration process due to water loss. In addition, the sample temperature during drying is not easy to maintain at constant level since the sample temperature during drying is always lower than the air temperature due to cooling effect of evaporation. *In the case of tuna, change in moisture content during drying showed no significant effect when compared with heating with no moisture loss. In our study all other factors such as pH, additives remained the same in both treatments.* The microbial deactivation kinetics is dependent upon several factors: variety, water content (i.e. water activity), temperature, and composition of a medium (acidity, types of solids, pH, etc.) as well as heating methods (Schaffner & Labuza, 1997; Juneja & Marmer, 1998; Lopez, Martinez, Gonzalez, Martin, & Bernado, 1998). In many studies during isothermal heating, it has been demonstrated that *D*-value decreased (i.e. heat sensitivity increased) as water content (i.e. water activity) increased (Doyle & Marth, 1975; Schelhorn, 1973; Kim et al, 1997; Lopez et al., 1997; Lopez et al., 1998). Opposite results were also observed in the case of

spores (Lopez et al., 1997). *D*-values increased with the increase of water content. For example, *D*-value increased 4.5 times when heat treatment was conducted at water activity, adjusted by glycerol, of 0.99 (high water content) compared to water activity 0.88. Also specific effects of the solute types appeared to influence *D*-values. Glycerol increased the heat sensitivity compared to sodium chloride and sucrose. Lopez et al. (1998) found that solutes of benzoate and potassium sorbate acted as inhibitory while they did not have difference on *D*-values at pH 5.0, 6.0, and 6.5. In case of *Clostridium perfringens*, *D*-values decreased (not inhibitory) when sodium pyrophosphate was added into ground beef and turkey meat (Juneja & Marmer, 1998). Models to predict the *D*-values were also developed as a function of temperature, pH, and water activity for isothermal conditions (Cerf, Davey, & Sadoudi, 1996; Blank, Yang, & Scanlon, 1998; Gaillard, Leguerinel, & Mafart, 1998a b). The purpose of this discussion is to present the complexity of the process even for isothermal condition. It becomes more complicated during drying when both sample temperature and water content varies with time.

3.4. Composition of the indigenous flora

It is important to identify the types and characteristics of the endogenous microbes present in fresh tuna. The microbial analysis of food products yields many diverse types of microorganisms. However, we are more concerned with the predominant types and those which may cause spoilage or be of a health hazard (Banwart, 1989). Heat resistance is also dependent on the composition of the endogenous microflora (Mossel et al., 1995). Initially tuna contained a mixture of different microbes, of which some are more heat and/or osmo-tolerant than other. Gram-positive are often more sensitive than Gram-negatives. Table 3 shows the types of microflora present in the fresh tuna before drying. This shows that most of the microflora present was the osmo-tolerant since at zero time the Gram-negative (N_4) and heat-tolerant (N_5) microbes were 3 orders magnitude lower than N_1 (Table 3). Moreover, addition of salt in the agar can increase the count up to 2 order of magnitude indicating that several of the bacteria in the tuna require salt to grow, and are therefore not detected on the plate count agar that is used for determination of cfu. This result illustrates that the *D*- and *Z*-values estimated in this work is related to aerobic counts obtained on standard PCA, not to the total culture. The salt requiring bacteria were most sensitive to drying (Table 3, N_2 and N_3) and therefore would not affect the measured *D*- and *Z*-values. A *D*-value of salt requiring bacteria would be lower. It is expected that the type of surviving microbes seems to stabilize after some time of starting the drying. Further experiments were conducted to explore more on this aspect by determining the types of microbes count during drying. It shows that the predominant microbes were the moderate-osmo-tolerant since no microbes were detected when the salt concentration was 5% (N_4), and the agar plate was incubated at 60°C (N_5) (Table 3). The evidence of no microbial growth when incubated at 60°C indicated that the dominating microbes grow during drying was heat sensitive. This preliminary study indicates some characteristics of the type of microflora in the fresh and dried tuna mice. More details studies need to be targeted in the future.

Table 3
Types of microbes in fresh and dried tuna mince

Drying time (h)	N_1 (cfu/g)	N_2 (cfu/g)	N_3 (cfu/g)	N_4 (cfu/g)	N_5 (cfu/g)
0	6.2×10^5	1.5×10^6	1.5×10^7	2.4×10^2	1.0×10^2
1	9.6×10^4	9.8×10^4	*n*	2.0×10^2	*n*
2	1.9×10^4	3.5×10^3	*n*	1.5×10^2	*n*
4	1.5×10^4	2.9×10^3	*n*	5.5×10^1	*n*

Note: Drying temperature was 70°C, *n* indicates not detected, N_1 is the count at 0.5 g sodium chloride in 100 ml agar, N_2 is the count at 3 g sodium chloride in100 ml agar, N_3 is the count at 5 g sodium chloride in 100 ml agar, N_4 is the gram-negative microbes as measured on Maconky agar, N_5 is the heat tolerant microbes.

4. Conclusion

The change of total viable counts in minced tuna during moist- and dry-heating as a function of temperature (60–140°C) was studied by estimating their *D*- and *Z*-values. The *D*-values decreased from 2.52 to 0.26 h for moist-heating, and 2.57 to 0.34 h for dry-heating, respectively. In both cases, increasing temperature caused significant decrease in *D*-values, whereas the effect of heating methods was not significant on the lethality of endogenous microflora. The *Z*-values were found 144°C and 46°C for temperature within 60–100°C, and 100–140°C, respectively.

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